European Commission

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

ISOFLUCYPRAM

Volume 3 – B.9 (PPP) – Isoflucypram EC 50

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

Version History

When	What
March 2019	Initial DAR

Table of contents

B.9. ECOTOXICOLOGY DATA AND ASSESSMENT OF RISKS FOR NON-TARGET SPECIES 4

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES 4

B.9.1.1. Effects on birds 4
B.9.1.2. Effects on terrestrial vertebrates other than birds 5

B.9.2. RISK ASSESSMENT FOR BIRDS AND OTHER TERRESTRIAL VERTEBRATES 7

B.9.2.1. Risk assessment for birds 7
B.9.2.2. Risk assessment for terrestrial vertebrates other than birds 14

B.9.3. EFFECTS ON AQUATIC ORGANISMS 18

B.9.3.1. Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes 18
B.9.3.2. Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms 32
B.9.3.3. Further testing on aquatic organisms 32

B.9.4. RISK ASSESSMENT FOR AQUATIC ORGANISMS 32

B.9.5. EFFECTS ON ARTHROPODS 53

B.9.5.1. Effects on bees 53
B.9.5.2. Effects on non-target arthropods other than bees 53

B.9.6. RISK ASSESSMENT FOR ARTHROPODS 124

B.9.6.1. Risk assessment for bees 124
B.9.6.2. Risk assessment for non-target arthropods other than bees 124

B.9.7. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA 144

B.9.7.1. Earthworms 144
B.9.7.2. Effects on non-target soil meso- and macrofauna (other than earthworms) 148

B.9.8. RISK ASSESSMENT FOR NON-TARGET SOIL MESO- AND MACROFAUNA 157

B.9.9. EFFECTS ON SOIL NITROGEN TRANSFORMATION 163

B.9.10. RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION 167

B.9.11. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS 168

B.9.11.1. Testing on non-target plants 168
B.9.11.2. Extended laboratory studies on non-target plants 182
B.9.11.3. Semi-field and field tests on non-target plants 182

B.9.12. RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS 182

B.9.13. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA) 183

B.9.14. RISK ASSESSMENT FOR OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA) 183

B.9.15. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT 183

B.9.16. RISK ASSESSMENT FOR BIOLOGICAL METHODS FOR SEWAGE TREATMENT 184

B.9.17. REFERENCES RELIED ON 184

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

Use pattern considered in this risk assessment

For information, the proposed use, for the formulation 'isoflucypram EC 50', assessed in the following risk assessments is summarised in Table B.9-1.

Table B.9-1: Intended application pattern

Crop	Growth stage (range)	Number of applications	Application interval [days]	Maximum label rate (range) [L prod./ha]	Maximum application rate, individual treatment (ranges) [kg a.s./ha] Isoflucypram
Cereals (Wheat, rye, triticale, barley, oats)	BBCH 30-69	1	-	1.5	0.075

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

The acute oral and reproductive studies conducted by the applicant testing the active substance, isoflucypram, are presented in Table B.9.1.1-1 below, and the appropriate endpoints have been considered for use in the risk assessment (selected endpoints highlighted in bold). **The two submitted short-term dietary toxicity studies have not been evaluated by the RMS or relied on to inform an EU regulatory decision. as short-term dietary studies are not a data requirement under EU Commissioning Regulation 283/2013.**

Acute endpoints: While both studies were considered valid, the endpoint from the test on the species bobwhite quail was used for the risk assessment as this is the standard bird species for European registration.

Long-term endpoint: One mallard duck and two bobwhite quail long-term effects studies were performed. The bobwhite quail study ([redacted] 2017) was not considered reliable to derive an endpoint, therefore another bobwhite quail study was conducted [redacted] 2018). However, the endpoint from the mallard duck study was more critical, therefore this will be used.

Table B.9.1.1-1: Ecotoxicological endpoints for birds exposed to isoflucypram (as a.s.): Acute and reproductive studies. Endpoints in bold text were used in the avian risk assessment

Test substance	Risk assessment	Species	Endpoint	Reference
Isoflucypram	Acute	*Colinus virginianus*, Bobwhite quail,	LD_{50} > 2000 mg as/kg bw Extrapolated **to 3776 mg a.s./kg bw** as there was no mortalities at the limit dose	[redacted] 2015; M-535551-01-1 KCA 8.1.1.1/01
		Serinus canaria, Wild canary	LD_{50} > 2000 mg as/kg bw Extrapolated to 3776 mg a.s./kg bw as there was no mortalities at the limit dose	[redacted] 2016; M-547051-01-1 KCA 8.1.1.1/02
	Long-term	*Anas platyrhynchos*, Mallard duck	NOEC 1000 ppm **NOEL 60 mg a.s./kg bw/d**	[redacted] 2017; M-597500-01-1 KCA 8.1.1.3/01
		Colinus virginianus, Bobwhite quail,	Endpoint not considered reliable, see study summary for information.	[redacted] 2017; M-597500-01-1 KCA 8.1.1.3/01
		Colinus virginianus, Bobwhite quail,	NOEL 174 mg as/ kg bw [b)]	[redacted] 2018; M-611590-01-1 KCA 8.1.1.3/02

a) This study has not had a comprehensive evaluation by the RMS. However, it has been checked to ensure there is no greater toxicity compared to the [redacted] (2017) Mallard duck study.

Metabolites of isoflucypram

Discussion of plant metabolites is included in section B.9.2.1 in relation to both birds and mammals.

B.9.1.2. Effects on terrestrial vertebrates other than birds

Acute endpoint

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

Table B.9.1.2-1 summarises the acute oral toxicity study considered when setting the relevant endpoint for use in risk assessment of isoflucypram.

Table B.9.1-2: Acute mammalian endpoint for isoflucypram

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

Long-term endpoint

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

Table B.9.1-3 summarises the long-term toxicity studies considered when setting the relevant endpoint for use in risk assessment of isoflucypram. See the footnotes to the table for discussion of the appropriate endpoint for use in the risk assessment.

Table B.9.1-3: Information relevant to identifying the ecotoxicologically relevant long-term endpoints for mammals

Endpoint	NOAEL (mg a.s./kg bw.d)	Reference
Body weight change[1], behavioural effects and systemic toxicity[2]	Rats: 83.3/25.6 [3)] mg/kg bw/d in M/F; ↓ body-weight gain & final body weight, ↑ absolute & relative liver weight & ↑ relative thyroid weight [5)] (LOAEL of 22.8 mg/kg bw/d-lowest dose tested- hyaline droplets in kidneys in male rats, indicative of chronic progressive nephropathy – a male rat specific effect which can lead to mortality over longer periods)	Repeated-dose 28-day oral toxicity study in rodents (OECD 407) KCA 5.3.1/.1; [redacted], 2017
	Rats; 18.4/ 21.9 mg/kg bw/d in M/F; ↑ relative liver and thyroid weight [5)] (NOAEL of 6.34 [4)] mg/kg bw/d hyaline droplets in kidneys in male rats, indicative of chronic progressive nephropathy – a male rat specific effect which can lead to mortality over longer periods at the LOAEL of 18.4 mg/kg bw/d)	Sub-chronic oral toxicity study-rodent 90-day study; (OECD 408) KCA 5.3.2/01; [redacted] 2006
	Mice; 32/149 mg/kg bw/d in M/F; Hepatocellular necrotic foci, single cell necrosis, ↑ relative liver weight [5)]	Repeated dose 28-day oral toxicity study in rodents (OECD 407) KCA 5.3.1/02; [redacted] 2012
	Mice; 51/59.8 mg/kg bw/d in M/F; ↑ relative liver weight [5)]	Sub-chronic oral toxicity study-rodent 90-day study; (OECD 408); KCA 5.3.2/02; [redacted] 2013
	Rats; 11.27-13.91/11.28-14.62 mg/kg bw/d in M/F; ↑relative liver weight and ↑ relative thyroid weight [5)]	Multigeneration study (OECD 416); KCA 5.6.1/01; [redacted] 2018
	Rats; 125 mg/kg bw/d; ↑ abs liver weight (16%), enlarged liver [5)]	Developmental toxicity study (OECD 414); KCA 5.6.2/01; [redacted] 2017b
	Rabbits; 70 mg/kg bw/d; ↓ BWG, ↓ FC, 2 does aborted, ↑ abs liver weight [5)]	Developmental toxicity study (OECD 414); KCA 5.6.2/92; [redacted] 2017
	Rats; 6.27 [6)] /8.54 mg/kg bw/d in M/F; Hair loss (F)↓ body weight gain (F)↑ abs liver weight (F), minimal thyroid histopath changes (M & F) **9.68 mg/kg bw/d in F based on decreased body weight gain (days 1-344).**	24-month toxicity and carcinogenicity dietary study [7)], OECD 453, KCA 5.5/01, [redacted] 2018
	Mice; 29/38.1 mg/kg bw/d in M/F; ↑ mortality rate (F), ↓ body weight and body weight gain (M & F), ↑ liver weight [5)] and	18-month dietary carcinogenicity study, KCA 5.5/02, [redacted] 2017

	histopathological correlates (M & F), ↑ kidney weight and histopathological correlates (M)	

The endpoint used in the risk assessment is indicated by **bold text.**

[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] Although the decrease in body weight gain is a relevant effect to set an NOEAL endpoint in ecotoxicology, it is not the lowest NOAEL for this effect. Therefore, this endpoint has not been used in the risk assessment.

[4] Although this NOAEL is the lowest, it is noted that this effect is isolated to one organ and is rat-specific. Therefore, it is not considered appropriate for use to set the mammalian NOAEL for use in the ecotoxicology risk assessment.

[5] An increase in liver weight or thyroid weight indicates toxicity. However, these early signs of toxicity are not considered enough to set a NOAEL for use in risk assessment, unless they are coupled with whole-body effects such as decreased body weight gain.

[6] Although the male NOAEL is lower than the female, it is based on different toxic effects. Only the female NOAEL is based on a decreased body weight gain effect.

[7] Although this study is a carcinogenicity dietary study (OECD 453) rather than OECD 407, 408, 416 or 414, the design of the study (e.g. the dosing of the rats) is comparable. Therefore, any effects that are seen in this long-term study should not be ignored. In this study, a 10% decrease in body weight gain occurred over the first 344 days in female rats. As this is a relevant toxic effect and is also the lowest NOAEL, 9.68 mg a.s./kg bw has been used in the long-term mammalian risk assessment.

The endpoint selected in bold in Table B.9.1-3 (NOAEL of 9.68 mg/kg bw/d) is the lowest statistically-defined endpoint which is linked to a relevant parameter for mammalian populations (10% decreased body weight gain of adult rats, when compared to control body weight gain at the LOAEL). This also corresponded with a statisitcially significant ($p<0.05$) decrease in female bodyweight on days 316 and 344 at the LOAEL.

As there are no other equivalent (in terms of guideline followed) studies with which this endpoint could be further considered for a potential combining of dose groups tested, the NOAEL is set using this study at 8.54 mg/kg bw/d.

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

B.9.2.1. Risk assessment for birds

The proposed GAP table for 'Isoflucypram EC 50' for consideration in the risk assessment is provided in Table B.9.2.1-1

Table B.9.2.1-1: GAP table for 'Isoflucypram EC 50'

Crop	Timing of application (range)	Number of applications	Application interval [days]	Maximum label rate (range) [L prod./ha]	Maximum application rate, individual treatment (ranges) [kg a.s./ha] Isoflucypram
Cereals (Wheat, rye, triticale, barley, oats)	BBCH 30-69	1	-	1.5	0.075

The following risk assessment has been conducted according to the EFSA (2009) 'Guidance Document on Risk Assessment for Birds & Mammals'.

The relevant endpoints used in the screening assessment and tier 1 risk assessments for acute and long-term/reproductive risk are presented in Table B.9.2.1-2 below.

Table B.9.2.1-2: Endpoints used in Tier 1 risk assessment

Test substance	Exposure	Species	Endpoint		Reference
Isoflucypram	Acute risk assessment endpoint	Acute, oral Bobwhite quail	LD_{50}	3776 mg a.s./kg bw (extrapolated value)	[redacted] (2015)
	Reproductive risk assessment endpoint	Mallard duck	NOEL	60 mg a.s./kg bw/d	[redacted] (2017)

Screening assessment

An initial screening assessment was conducted to assess the acute dietary and long-term/reproductive risk of isoflucypram to birds considering a worst-case scenario indicator species. The DDD (daily dietary dose), based on the predicted daily exposure of birds to residues of the active substance following the proposed representative use, was calculated using shortcut values (SV) obtained from EFSA guidelines (2009) based on application to cereals. The relevant indicator species and short cut values for the representative use of isoflucypram in cereals are shown in Table B.9.2.1-3 below.

Table B.9.2.1-3: Shortcut values for avian indicator species.

Crop	Indicator species	Shortcut value for acute assessment	Shortcut value for reproductive assessment
Cereals	Small omnivorous bird	158.8	64.8

Acute dietary screening assessment for birds

The DDD for acute dietary risk was calculated as follows:

$DDD_{\text{single application}}$ = application rate [kg/ha] x shortcut value [1]

[1] Based on a small omnivorous bird (see Table B.9.2.1-3 for shortcut values based on specific species-crop combinations from EFSA 2009 guidelines)

The toxicity exposure ratio (TER) for the acute dietary screening assessment was calculated as follows:

$TER = LD_{50}/DDD$

A summary of the acute screening assessment is presented in Table B.9.2.1-4 below.

Table B.9.2.1-4: Acute dietary screening assessment for birds

Crop	Indicator species	DDD		DDD mg a.s./kg bw/d	LD_{50} [mg a.s./kg bw]	TER_A	Trigger
		Application rate [kg a.s./ha]	SV				
Cereals	Small omnivorous bird	0.075	158.8	11.91	3776	317	10

Conclusion:

The TER value calculated was > 10 therefore the risk is acceptable at the screening stage.

Long-term/reproductive risk screening assessment

The DDD for long-term/reproductive risk was calculated as follows:

DDD = application rate x shortcut value [1] x TWA[2]

[1] Based on a small omnivorous bird (see Table B.9.2.1-3 for shortcut values based on specific species-crop combinations from EFSA 2009 guidelines)

[2] TWA = time weighted average

The TER value for the long-term/reproductive screening assessment was calculated as follows:

TER = NOEL/DDD

A summary of the long-term/reproductive screening assessment is presented in Table B.9.2.1-5 below:

Table B.9.2.1-5: Long-term/reproductive screening assessment for birds

Crop	Indicator species	DDD			DDD mg a.s./kg bw/d	NOEL mg a.s./kg bw/d	TER_{LT}	Trigger
		Application rate [kg a.s./ha]	SV	f_{TWA}				
Cereals	Small omnivorous bird	0.075	64.8	0.53	2.58	60	23.3	5

Conclusion:
As the TER value calculated was > 5, the long-term/reproductive risk to birds at the screening stage is acceptable and no further consideration of the risk is required.

Risk assessment for birds drinking contaminated water

In the EFSA GD (2009), section 5.5, step 1 the following guidance is given on the selection of relevant scenarios for assessing the risk of pesticides via drinking water to birds and mammals:

Leaf scenario: Birds taking water that is collected in leaf whorls after application of a pesticide to a crop and subsequent rainfall or irrigation. For the crop under assessment in this evaluation (cereals) the leaf scenario is not considered relevant and is thus not considered further in the risk assessment.

Puddle scenario. Birds and mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. The risk for birds from drinking water in puddles for acute and reproductive risk is addressed in Table B.9.2.1-6.

For the calculation of the effective application rate (AR_{eff}) a worst-case scenario was considered assuming a full application rate with no degradation as follows:

AR_{eff} = application rate (g a.s./ha) x no. of applications = 75 x 1 = 75

Acute exposure ratio = AR_{eff}/acute toxicity endpoint = 75/3776 = 0.02

Long-term exposure ratio = AR_{eff}/long-term/reproductive toxicity endpoint = 75/60 = 1.25

Table B9.2.1-6: Evaluation of potential concern for exposure of birds drinking water (escape clause)

Crop	Koc [L/kg]	AR_{eff} [g as/ha]	Endpoint [mg as/ kg bw]	Exposure ratio	"Escape clause" No concern if ratio	Conclusion
Isoflucypram						
Cereals	1346.6 [a)]	75	3776	0.02	≤ 3000	No concern
Cereals	1346.6 [a)]	75	60	1.25	≤ 3000	No concern

[a)] Based on a geomean of values, presented in Environmental Fate and Behaviour section B.8.

Conclusion:
According to EFSA GD (2009) no specific calculations of exposure and TER are necessary when the ratio of effective application rate to the relevant endpoint does not exceed 3000 for substances with a Koc ≥ 500 L/kg. Therefore, in this case, there is an acceptable risk to birds following exposure to isoflucypram via the puddle scenario and no further consideration of the risk is required.

Risk assessment of secondary poisoning

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

Isoflucypram has a log P_{OW} of 4, indicating a risk of bioaccumulation and, hence, secondary poisoning. Therefore, further consideration of the risk via secondary poisoning is required.

Risk assessment for earthworm-eating birds via secondary poisoning

The assessment has been conducted using PEC_{soil} values based on the peak PEC_{soil} accumulation value as confirmed by the Environmental fate and Behaviour evaluator. These values are presented below for information:

Table B.9.2.1-7: Maximum PEC values for isoflucypram

Substance	$PEC_{soil(accumulation)}$ value for proposed GAPs (mg/kg)
Isoflucypram	0.0616

Table B.9.2.1-8: Assessment of the risk for earthworm-eating birds due to exposure to isoflucypram via bioaccumulation in earthworms (secondary poisoning) for the intended use

Parameter	**Isoflucypram**	**Reference**
$PEC_{soil,\ accumulation}$ [mg/kg soil]	0.0616	Table B.8.2.2-1.
K_{ow}	10000	-
K_{oc} (geometric mean)	1346.6	Based on a geomean of values, presented in Environmental Fate and Behaviour section B.8.
f_{oc} (default)	0.02	EFSA/2009/1438
BCF 1)	120.84/26.932 = 4.46	--
PEC_{worm} [mg/kg] 2)	0.275	--
Daily dose [mg/kg bw/d] 3)	0.288	--
NO(A)EL [mg/kg bw.d/d]	60	See Table B.9.2.1-2
TER_{LT} 4)	208	--

1) Bioconcentration factor (BCF) = (0.84 + 0.012 x Kow) / foc x Koc
2) PECworm = PECsoil x BCF
3) Daily dose = 1.05 x PECworm
4) TERlt = NO(A)EL / Daily dose.

As shown in Table B.9.2.1-8 the TER_{LT} for isoflucypram exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to earthworm-eating birds via secondary poisoning. No further consideration is required.

Risk assessment for fish-eating birds via secondary poisoning

The assessment has been conducted using PEC_{sw} values based on the peak PEC_{sw} value as confirmed by the Environmental fate and Behaviour evaluator. These values are presented below for information:

Table B.9.2.1-9: Maximum PEC values for isoflucypram

Substance	Maximum PEC_{SW} value for proposed GAPs (µg/L)
Isoflucypram	9.63

Table B.9.2.1-10: Assessment of the risk for fish-eating birds due to exposure to isoflucypram via bioaccumulation in fish (secondary poisoning) for the intended use

Parameter	Isoflucypram	Reference
$PEC_{sw,}$ [mg/L]	0.00963	FOCUS Step 1 value. See Table B.8.5.2-1.
BCF fish (max. worst case)	370	[redacted] 2017; M-610008-01-1 KCA 8.2.2.3/01
PEC_{fish} [mg/kg] [1)]	3.56	--
Daily dose [mg/kg bw/d] [2)]	0.57	--
NO(A)EL [mg/kg bw/d]	60	See Table B.9.2.1-2
TER_{LT} [3)]	105	--

1) PECfish = PECsw, (spray drift, 1m) x BCF
2) Daily dose = 0.159 x PECfish
3) TERlt = NO(A)EL / Daily dose.

As shown in table B.9.2.1-10 the TER_{LT} for isoflucypram exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to fish-eating birds via secondary poisoning.

Acute oral toxicity from the formulated product

No additional studies are available or required as the toxicity can be derived from the studies on the active substance.

Metabolites of isoflucypram

Within the available studies in Section B.7.2. isoflucypram was found to metabolise in cereal crops to BCS-CN88460-propanol (M01), BCS-CN88460-propanol-Glyc (M18), BCS-CN88460-propanol-Glyc-MA (M21), BCS-CN88460-desmethyl-propanol (M06), BCS-CN88460-desmethyl-propanol-Glyc-MA (M41), BCS-CN88460-hydroxyphenyl-Gluc-MA (M23), BCS-CN88460-hydroxyphenyl-Glyc-MA (M24) and BCS-CN88460-2-propanol-Glyc-MA (M22). However, only BCS-CN88460-propanol-Glyc-MA (M21) was identified as a potentially-relevant plant metabolite in Section B.7.2 as it occurred at >10% total radioactive residue (TRR). The risk from M21 is not considered to be covered by the active substance risk assessment because it was not identified in mammalian or bird studies used in the active substance risk assessment. Therefore, the risk of M21 to birds and mammals is discussed in this section.

Exposure - Residue level

The following residue levels of metabolite M21 were found in the study conducted in hay (see Table B.9.2.1-10 or Section B.7.2.1.3 (AS)):

Table B.9.2.1-10: residue levels of ecotoxicologically-relevant metabolites of isoflucypram.

	Percentage of total a.s. applied (%TRR)	Equivalent to residue level (mg substance/kg)
Total residues	100.0	4.032
BCS-CN88460 (isoflucypram) parent compound at time of peak metabolite formation (see below)	50.0	2.016
BCS-CN88460-propanol-Glyc-MA (M21)	10.3	0.414

The application rate of the residue study conducted in hay is 2 x 65 g a.s./ha; whereas the application rate of the GAP for 'Isoflucypram' is 1 x 75 g a.s./ha. Therefore, the proposed GAP for the parent (0.075 kg a.s./ha) was multiplied by the maximum formation fraction of the metabolite (0.103) to calculate an effective applied rate of metabolite residue of **0.0077 kg/ha**. This rate is then used in a Tier 1 risk assessment to calculate a daily dietary

dose (DDD), which is then used to calculate a TER. As there is no toxicity data on this metabolite, 10 x the toxicity of the parent active substance is assumed.

As the active substance was present at 50% of its initial applied residue at the same time as M21 in the hay residue study, a combined risk assessment is also provided (the RMS notes that this is not a standard recommendation in EFSA 2009 but has provided the combined risk assessment for information purposes as there is potential for exposure to both the active substance and M21 cocurrently). Note that due to evidence suggesting that metabolite M21 cleaves to M01 in the stomach of mammals and that M01 is covered by the risk from the parent isoflucypram (due to structural similarieties, see B.6.8.1.1_CA), the metabolite risk assessment has been removed from the mammal section of this assessment. However, due to uncertainties about extrapolation of this theory to birds, the M21 risk assessment has been retained for birds, with the conservative assumption of 10x toxicity of the parent isoflucypram. The rate of the active substance that was present in combination with the metabolite is calculated by multiplying the GAP rate in the test (0.075 kg a.s./ha) by the fraction that was present at the time of the peak metabolite formation (0.5) to calculate a residue of **0.0375 kg a.s./ha**. This is then used to calculate the DDD for the active substance.

Risk to birds from metabolites of isoflucypram

Selection of suitable generic focal species

The small omnivorous bird in cereal crops is used as the indicator species in the screening risk assessment. The metabolite M21 is present in hay following a spray application (see Table B.9.2.1-11). The generic focal species small omnivorous bird "lark" at BBCH 30-39 is therefore considered to be most appropriate (and worst-case shortcut value) for extrapolation from residues observed in hay. The shortcut value of the small omnivorous bird 'lark' at BBCH 30 – 39 in cereals is 12.0 for acute and 5.4 for long term RUDs.

Daily dietary dose

The daily dietary dose (DDD) of M21 is derived as follows:

DDD_{acute} = 'peak' formation rate x shortcut value

DDD_{acute} = 0.0077 x 12.0 = 0.0924 mg/kg bw/d

$DDD_{long\text{-}term}$ = 'peak' formation rate x shortcut value x TWA.

Note: A TWA of 1 is used here, as the degradation profile of the metabolite is unknown, and extrapolation of 10 days based on an active substance dataset is uncertain.

$DDD_{long\text{-}term}$ = 0.0077 x 5.4 = 0.0416 mg/kg bw/d

The DDD of isoflucypram is derived as follows:

DDD_{acute} = 0.0375 x 12.0 = 0.45

$DDD_{long\text{-}term}$ = 0.0375 x 5.4 x 0.53 (TWA) = 0.107 mg/kg bw/d.

Risk assessment

A risk assessment was conducted for both the metabolite and active substance. This can be found in Table B.9.2.1-11. However, as the metabolite and active substance occurred at these residue levels simultaneously, a combined risk assessment was also conducted. This can be found at Table B.9.2.1-14.

Table B.9.2.1-11: Tier 1 acute and long-term risk assessment from exposure to metabolite M21 and active substance, isoflucypram.

Acute or long-term	Portion	DDD	Toxicity	TER
Acute	M21	0.0924	378	4091
	a.s.	0.45	3776	8391
Long term	M21	0.0416	6	144
	a.s.	0.107	60	561

As the active substance was present at 50% at the same time as M21 in the hay residue study, a combined risk assessment is also provided. Calculation of an acute $LD_{50\ (mix)}$ value and long-term $NOEL_{(mix)}$ value used the following equation from Appendix B of EFSA 2009[1]

$$LD_{50}(\text{mix}) = \left(\sum_i \frac{X(\text{a.s.}_i)}{LD_{50}(\text{a.s.}_i)} \right)^{-1}$$

With:

$X(\text{a.s.}_i)$ = fraction of active substance [i] in the mixture;
(please note that the sum $\sum X(\text{a.s.}_i)$ must be 1)

$LD_{50}(\text{a.s.}_i)$ = acute toxicity value for active substance [i]

X(a.s.i) was calculated by dividing the residue of each portion with the sum of the two relevant portions as shown in Table B.9.2.1-12. These fractions were then used in $LD_{50(mix)}$ and $NOEL_{(mix)}$ equations in Table B.9.2.1-13. Note that the NOEL(mix) calculation follows the same process as the $LD_{50(mix)}$ equation. However, the $NOEL_{(mix)}$ is not currently an EU-harmonised approach.

Table B.9.2.1-12: Calculation of fraction of residue to use in $LD_{50(mix)}$ and $NOEL_{(mix)}$ equations

Sum of relevant residue portions (mg/kg)	Residues	Residue portion	Fraction of residue portion in the mixture (a.s.i)
2.016 + 0.414 = 2.43	M21	0.414	0.17037
	a.s.	2.016	0.82963

Table B.9.2.1-13: Calculation of $LD_{50(mix)}$ and $NOEL_{(mix)}$ endpoints

Acute or long term	Sum of residues of isoflucypram and M21 (mg/kg a.s.)	Residues	Proportion of total residues above 10% of applied active substance ($X(\text{a.s.}_i)$)	Endpoint (mg a.s./kg bw)	X(asi)/Endpoint	$LD_{50}/NOEL_{mix}$ (mg a.s./kg bw) Sum of X(asi) / Endpoint $^{-1}$
Acute	2.43	M21	0.17037	378 [a]	0.000451	2016
		a.s.	0.82963	3776	0.000045	
Long term		M21	0.17037	6 [a]	0.0286	24
		a.s.	0.82963	60	0.0138	

[a] *Based on a conservative estimate of 10 x toxicity of the active substance.*

The risk assessments considering the acute and long-term risk to birds are presented in Table B.9.2.1-14.

Table B.9.2.1-14: Tier 1 acute and long-term risk assessment for birds from combined exposure to isoflucypram and M21.

Acute or long term	Substance	Generic focal species	DDD (mg/kg bw/d)	$LD_{50\ mix}$ or $NOEL_{mix}$ (mg/kg bw) [1]	TER	Annex VI Trigger
Acute	Combined risk assessment: M21 + isoflucypram	small omnivorous bird 'lark'	0.0924 + 0.45 = 0.542	2016	3720	10

[1] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA; Appendix B. EFSA Journal 2009; 7(12):1438. [5 pp.]. doi:10.2903/j.efsa.2009.1438. Available online: www.efsa.europa.eu

Long term		BBCH 30 – 39	0.0416 + 0.107 = 0.1489	24	161	5

[1] *These endpoints are theoretical and are calculated by the RMS using the Finney Equation, see above for explanation.*

The TERs are above the trigger values for M21 separately and combined with isoflucypram, for both acute and long-term risk assessments. **Therefore an acceptable risk to birds from the metabolites of isoflucypram can be concluded based on the proposed use pattern of isoflucypram and no further consideration is required.**

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

The proposed GAP table for 'Isoflucypram EC 50' for consideration in the risk assessment is provided in Table B.9.2.2-1

Table B.9.2.2-1: Proposed GAP table for 'Isoflucypram EC 50'

Crop	Timing of application (range)	Number of applications	Application interval [days]	Maximum label rate (range) [L prod./ha]	Maximum application rate, individual treatment (ranges) [kg a.s./ha] Isoflucypram
Cereals (Wheat, rye, triticale, barley, oats)	BBCH 30-69	1	-	1.5	0.075

The following risk assessment has been conducted according to the EFSA (2009) 'Guidance Document on Risk Assessment for Birds & Mammals'.

The relevant endpoints used in the screening assessment and tier 1 risk assessments for acute and long-term/reproductive risk are presented in Table B.9.2.2-2 below.

Table B.9.2.2-2: Endpoints used in Tier 1 risk assessment

Test substance	Exposure	Species	Endpoint		Reference
Isoflucypram	Acute risk assessment endpoint	Rat (gavage) (up and down procedure; OECD 425)	LD_{50}	>2000 mg a.s./kg bw	[redacted] (2014a) B.6.2.1
	Reproductive risk assessment endpoint	Rat (24-month dietary)	NOAEL	9.68 mg a.s./kg bw	[redacted] (2018) B.6.5.1

Screening assessment

An initial screening assessment was conducted to assess the acute dietary and long-term/reproductive risk of isoflucypram to mammals considering a worst-case scenario indicator species. The DDD (daily dietary dose), based on the predicted daily exposure of birds to residues of the active substance following the proposed representative use, was calculated using shortcut values (SV) obtained from EFSA guidelines (2009) based on application to cereals. The relevant indicator species and short cut values for the representative use of isoflucypram in cereals are shown in Table B.9.2.2-3 below.

Table B.9.2.2-3: Shortcut values for mammalian indicator species.

Crop	Indicator species	Shortcut value for acute assessment	Shortcut value for reproductive assessment
Cereals	Small herbivorous mammal	118.4	48.3

Acute dietary screening assessment for mammals

The DDD for acute dietary risk was calculated as follows:

DDD $_{\text{single application}}$ = application rate [kg/ha] x shortcut value [1]

[1] Based on a small herbivorous mammal (see Table B.9.2.2-3 for shortcut values based on specific species-crop combinations from EFSA 2009 guidelines)

The toxicity exposure ratio (TER) for the acute dietary screening assessment was calculated as follows:

TER = LD_{50}/DDD

A summary of the acute screening assessment is presented in Table B.9.2.2-4 below.

Table B.9.2.2-4: Acute dietary screening assessment for mammals

Crop	Indicator species	DDD		DDD mg a.s./kg bw/d	LD_{50} [mg a.s./kg bw]	TER_A	Trigger
		Application rate [kg a.s./ha]	SV				
Cereals	Small herbivorous	0.075	118.4	8.88	>2000	225	10

Conclusion:

The TER value calculated was > 10 therefore the risk is acceptable at the screening stage.

Long-term/reproductive risk screening assessment

The DDD for long-term/reproductive risk was calculated as follows:

DDD = application rate x shortcut value [1] x TWA[2]

[1] Based on a small herbivorous mammal (see Table B.9.2.2-3 for shortcut values based on specific species-crop combinations from EFSA 2009 guidelines)

[2] TWA = time weighted average

The TER value for the long-term/reproductive screening assessment was calculated as follows:

TER = NOEAL/DDD

Screening assessment

A summary of the long-term/reproductive screening assessment is presented in Table B.9.2.2-5 below:

Table B.9.2.2-5: Long-term/reproductive screening assessment for mammals

Crop	Indicator species	DDD			DDD mg a.s./kg bw/d	NOEL mg a.s./kg bw/d	TER_{LT}	Trigger
		Application rate [kg a.s./ha]	SV	f_{TWA}				
Cereals	Small herbivorous mammal	0.075	48.3	0.53	1.92	9.68	5.0	5

Conclusion:
As the TER value calculated was ≥ 5, the long-term/reproductive risk to mammals is resolved at the screening stage. Therefore, further consideration of the risk is not required at Tier 1.

Risk assessment for mammals drinking contaminated water

In the EFSA GD (2009), section 5.5, step 1 the following guidance is given on the selection of relevant scenarios for assessing the risk of pesticides via drinking water to birds and mammals:

Puddle scenario. Birds and mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. The risk to mammals from drinking water in puddles for acute and reproductive risk is addressed in Table B.9.2.2-7.

For the calculation of the effective application rate (AR_{eff}) a worst-case scenario was considered assuming a full application rate with no degradation as follows:

AR_{eff} = application rate (g a.s./ha) x no. of applications = 75 x 1 = 75

Acute exposure ratio = AR_{eff}/acute toxicity endpoint = 75/ 2000 = 0.038

Long-term exposure ratio = AR_{eff}/long-term/reproductive toxicity endpoint = 75/ 9.68 = 7.7

Table B.9.2.2-7: Evaluation of potential concern for exposure of mammals drinking water (escape clause)

Crop	Koc [L/kg]	AR_{eff} [g as/ha]	Endpoint [mg as/ kg bw]	Exposure ratio	"Escape clause" No concern if ratio	Conclusion
Cereals	1346.6 a)	75	>2000	0.038	≤ 3000	No concern
Cereals	1346.6 a)	75	9.68	7.7	≤ 3000	No concern

a) Based on a geomean of values, presented in Environmental Fate and Behaviour section B.8.

Conclusion:
According to EFSA GD (2009) no specific calculations of exposure and TER are necessary when the ratio of effective application rate to the relevant endpoint does not exceed 3000 for substances with a Koc ≥ 500 L/kg. Therefore, in this case, there is an acceptable risk to mammals following exposure to isoflucypram via the puddle scenario and no further consideration of the risk is required.

Risk assessment of secondary poisoning

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

Isoflucypram has a log P_{OW} of 4, indicating a risk of bioaccumulation and, hence, secondary poisoning. Therefore, further consideration of the risk via secondary poisoning is required.

Risk assessment for earthworm-eating mammals via secondary poisoning
The assessment has been conducted using PEC_{soil} values based on the peak PEC_{soil} accumulation value as confirmed by the Environmental fate and Behaviour evaluator. These values are presented below for information:

Table B.9.2.2-8: Maximum PEC values for isoflucypram

Substance	$PEC_{soil(accumulation)}$ (mg/kg) value for proposed GAPs
Isoflucypram	0.0616

Table B.9.2.2-9: Assessment of the risk for earthworm-eating mammals due to exposure to isoflucypram via bioaccumulation in earthworms (secondary poisoning) for the intended use

Parameter	**Isoflucypram**	**Reference**
$PEC_{soil,\ accumulation}$ [mg/kg soil]	0.0616	Table B.8.2.2-1
K_{ow}	10000	-
K_{oc} (geometric mean)	1346.6	Based on a geomean of values, presented in Environmental Fate and Behaviour section B.8.
f_{oc} (default)	0.02	EFSA/2009/1438
BCF 1)	4.46	--
PEC_{worm} [mg/kg] 2)	0.275	--
Daily dose [mg/kg bw/d] 3)	0.352	--

Parameter	Isoflucypram	Reference
NO(A)EL [mg/kg bw/d]	9.68	See Table B.9.2.2-2
TER_{LT} 4)	27.5	--

1) Bioconcentration factor (BCF) = (0.84 + 0.012 x Kow) / foc x Koc
2) PECworm = PECsoil x BCF
3) Daily dose = 1.28 x PECworm
4) TERlt = NO(A)EL / Daily dose.

As shown in the above table the TER_{LT} for isoflucypram exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to earthworm-eating mammals via secondary poisoning. No further consideration is required.

Risk assessment for earthworm-eating mammals via secondary poisoning
The assessment has been conducted using PEC_{sw} values based on the peak PEC_{sw} value as confirmed by the Environmental fate and Behaviour evaluator. These values are presented below for information:

Table B.9.2.2-10: Maximum PEC values for isoflucypram

Substance	Maximum PEC_{SW} value for proposed GAPs (µg/L)
Isoflucypram	9.63

Table B.9.2.2-11: Assessment of the risk for fish-eating mammals due to exposure to isoflucypram via bioaccumulation in fish (secondary poisoning) for the intended use

Parameter	Isoflucypram	Reference
$PEC_{sw,}$ [mg/L]	0.00963	FOCUS Step 1 value. See Table B.8.5.2-1.
BCF fish (max. worst case)	370	[redacted] 2017; M-610008-01-1 KCA 8.2.2.3/01
PEC_{fish} [mg/kg] 1)	3.56	--
Daily dose [mg/kg bw/d] 2)	0.506	--
NO(A)EL [mg/kg bw/d]	9.68	See Table B.9.2.2-2
TER_{LT} 3)	19.1	--

1) PECfish = PECsw, (spray drift, 1m) x BCF
2) Daily dose = 0.142 x PECfish
3) TERlt = NO(A)EL / Daily dose.

As shown in table B.9.2.2-11 the TER_{LT} for isoflucypram exceeds the relevant trigger of 5 for acceptability of effects, indicating an acceptable risk to fish-eating mammals via secondary poisoning.

Acute oral toxicity from the formulated product

No additional studies are available or required as the toxicity can be derived from the studies on the active substance and the co-formulants.

Metabolite toxicity

Discussion of plant metabolites is included in section B.9.2.1 in relation to both birds and mammals, including how daily dietary doses of M21 and isoflucypram are calculated for use in the metabolite risk assessment. Note that due to evidence suggesting that metabolite M21 cleaves to M01 in the stomach of mammals and that M01 is covered by the risk from the parent isoflucypram (due to structural similarieties, see B.6_CA), the metabolite risk assessment has been removed from the mammal section of this assessment as it not considered necessary.

$$LD_{50}(mix) = \left(\sum_i \frac{X(a.s._i)}{LD_{50}(a.s._i)} \right)^{-1}$$

With:

$X(a.s._i)$ = fraction of active substance [*i*] in the mixture;
(please note that the sum $\sum X(a.s._i)$ must be 1)

$LD_{50}(a.s._i)$ = acute toxicity value for active substance [*i*]

B.9.3. EFFECTS ON AQUATIC ORGANISMS

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

For the reason of planned global registration more than the European data requirements had to be fulfilled. Especially for the United States additional studies are needed for a submission. As these data exist they have to be part of the submitted dossier and therefore have been submitted by the notifier for regulatory review.

Previous evaluation:	New data, submitted for purpose of review

Report:	KCP 10.2.1/01; [redacted] 2017;
Title:	BCS-CN88460 EC 50 G acute toxicity to rainbow trout (Oncorhynchus mykiss) under static conditions - Final report
Report No.:	E 203 05016-7
Guideline(s):	EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985) OCSPP 850.1075 (Public Draft, 1996) OECD No. 203 (rev.1992) JMAFF, 12 Nousan No. 8147 (2000)
GLP/GEP:	**yes**

Material and methods

Test material	BCS-CN88460 EC 50 G lot/batch 2016-001002 Specification 102000031262 analyzed content of active substance: 51.45 g/L (5.28% w/w) Density : 0.975 g/mL
Test species	Rainbow trout (*Oncorhynchus mykiss*) [redacted]
Acclimation	At least 14 days, fed daily with commercial trout food under 16/8 hour light/dark period Health during acclimation: less than 5% mortality during 14 day acclimation period and 48 hours before the test. No treatment for disease.
Organism age/size at study initiation	Mean length: 4.4 ± 0.9 cm Mean body weight: 0.9 ± 0.4 g
Preparation of test solutions :	Prior to the test the stock solution was prepared by solving 227 mg of the test substance into 1000mL test water. The stock solution was prepared immediately prior to the test and was well agitated before further use.

	The corresponding amounts of the aqueous stock solution of the test material were dispersed in the aquaria prior to the test
Test solutions	Nominal concentrations: 0.178, 0.355, 0.710, 1.42 and 2.84 mg formulation/L based on a range-finding test. Corresponding geometric mean measured concentrations: not relevant Controls: water and solvent controls Evidence of undissolved material: not mentioned
Replication	No. of vessels per concentration (replicates): 1 No. of vessels per control (replicates): 1 No. of vessels per solvent control (replicates): 1
Organisms per replicate	No. of organisms per vessel: 10
Exposure	Static conditions Total exposure duration: 96 hours
Test Vessel Loading	0.23 g fish/L test medium
Feeding during test	No food 48 hours before and during study
Test water :	Reconstituted water was used during the acclimation period and for the test. It was prepared by adding salt stock solutions to demineralized water (conductivity < 10.0 µS / cm). The water was aerated to reach oxygen saturation.
Test conditions	Temperature: 13.4 - 14.4°C Photoperiod: 16 hours light / 8 hours dark Light intensity: not specified pH: 6.9 - 7.4 Water hardness: 40 - 60 mg $CaCO_3$/L Dissolved oxygen: 94 -108% saturation Conductivity: < 10 µS/cm
Test vessels :	Glass aquaria (size of 38 x 32 x 36 cm - h x w x d). The test volumes for each treatment were 40 L.
Parameters Measured / Observations	Fish were observed for mortalities and signs of intoxication four hours after the start of the exposure and then once a day (day 1 - 4). Dissolved oxygen, water temperature and pH values were determined daily in each aquarium, water temperature was additionally measured in the control aquarium and recorded hourly
Chemical analysis	BCS-CN88460 was analyzed in all test levels at test initiation (0 hours), day 2, and day 4 of the exposure period to confirm nominal concentrations. Additionally, samples were taken and analyzed from the 2.84 mg form./L test solution after 24 hours since all fish at this treatment level were dead at this assessment date. Samples were analyzed by HPLC-MS/MS for determination of BCS-CN88460 in test water.
Data analysis	Depending on the suitability of the data set, LC_{50} values and the 95%-confidence intervals were calculated for each 24 hour interval using computer software ToxRat, which estimated the LC_{50} using one of three statistical techniques: moving average, logit analysis or probit analysis. The appropriate method was determined according to the data characteristics. All values calculated with Microsoft® Excel were shown as rounded values.

Results

Validity criteria

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

All validity criteria were met

Analytical results:

The chemical analysis of BCS-CN88460 resulted in recoveries of 122 to 128% of nominal at test initiation in the freshly prepared test media. In the aged test media recoveries ranged from 85 to 114%. Therefore, the analytical recoveries over the whole testing period of 96 hours ranged between 85% and 128% of nominal.
Since the analytical results confirm a correct dosing of the test item at test initiation and day 4 recoveries maintained measured concentrations sufficiently, all results were related to nominal test concentrations of the formulated product.

Table B9.3.1-1: Nominal and measured concentrations of BCS-CN88460

Nominal conc. form. [mg/L]	**Nominal conc. BCS-CN88460 [mg/L]**	**Measured concentration of BCS-CN88460 [mg/L)**				**Measured concentration of BCS-CN88460 [%]**			
		Day 0	**Day 1**	**Day 2**	**Day 4**	**Day 0**	**Day 1**	**Day 2**	**Day 4**
0.178	0.00938	0.0117	-	0.00917	0.00802	124	-	97.6	85.3
0.355	0.0188	0.0240	-	0.0192	0.0166	128	-	102	88.6
0.710	0.0375	0.0463	-	0.0388	0.0335	124	-	103	89.4
1.42	0.0750	0.0922	-	0.0768	0.0784	123	-	102	105
2.84	0.1500	00183	0.171**	-*	-*	122	114	-*	-*

* No measurement (all fish dead)
**Sampled due to 100% mortality on day 1

Biological results:

Lethal effects were observed in the two highest concentration of 1.42 and 2.84 mg form./L. All fish were dead within 24 hours at the highest test concentrations of 2.84 mg form./L. At 1.42 mg form./L one fish died within 24 hours of exposure and five further dead fish were observed at 1.42 mg form./L within 48 hours of exposure. After 72 hours at 1.42 mg form./L nine fish were dead. No further mortalities were observed during the test.
At 1.42 mg form./L severe sub-lethal effects were observed in all fish after 4 hours of exposure. At test termination (96 hours) the remaining fish at the 1.42 mg form./L test level showed sub-lethal effects such as fish mainly on the bottom, fish lying on side or back on the bottom and showed reduced activity.

Table B9.3.1-2: **Cumulative mortality and behavioural observations during the test period**

Exposure time (hours)	**4**		**24**		**48**		**96**	
Test conc. form. [mg/L]	**No. dead (%)**	**Observ.**	**No. dead (%)**	**Observ.**	**No. dead (%)**	**Observ.**	**No. dead (%)**	**Observ.**
Control	0	10N	0	10N	0	10N	0	10N
0.178	0	10N	0	10N	0	10N	0	10N
0.355	0	10N	0	10N	0	10N	0	10N
0.710	0	3N 7OB	0	2N, 8BO, AT	0	6N, 4OB, AT, AK	0	7N, 3OB, AK
1.42	0	10OB, AK, OM	10	3OB, SR, AP, AT, OM 3BO, SR. AT, AK, AP, SD 3AT, AK	60	3OB, AT, SR, AK 1BO, SR, AT, AK	90	1BO, SR, AP, AT
2.84	0	3OB, AT, AK, SD 7BO, SR, AT, AK, AP, SD	100	-	100	-	100	-

Abbreviations of behavioral observations:
AK: strongly extended gills
AP: reduced activity; apathy
AT: labored respiration
BO: fish mainly on the bottom
HF: brighter coloration
N: no signs of sublethal effects
OB: fish mainly at the water surface
OM: open mouth, gag-bit
SD: displayed mucous evacuation of the intestine
SR: fish lying on side or back on the bottom

Conclusion

T he endpoints based on nominal concentrations are:

LC_{50} 96 hours (95% C.I.):	1.29 mg form./L (1.19 – 1.41 mg form./L)
NOEC: highest concentration without adverse effects	0.355 mg form./L

RMS comments

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

The following was noted by the RMS:

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

It is noted that the test item concentration was >20% higher than nominal at test initiation; as such the test concentrations should have been reported as geometric mean measured rather than based on nominal concentrations. However as the endpoint is more conservative by considering the nominal test concentrations, the RMS considers this acceptable.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.0625 µg/L (see section 3CA B5.1.2.6.1 for further details).

This study is valid and the endpoints confirmed as:

LC_{50} 96 hours (95% C.I.): 1.29 mg formulation/L (1.19 – 1.41 mg formulation/L) equivalent to 0.068 mg a.s./L

NOEC: 0.355 mg formulation/L equivalent to 0.018 mg a.s./L

Previous evaluation:	New data, submitted for purpose of review

Report:	KCP 10.2.1/02; Kuhl, K.; 2017;
Title:	Acute toxicity of BCS-CN88460 EC 50 G to the waterflea Daphnia magna in a static laboratory test system
Report No.:	EBLNN499
Guideline(s):	EU Directive 91/414/EEC Regulation1107/2009 (Europe) OECD Test Guideline 202 US EPA OCSPP 850.1010
Guideline deviation(s):	none
GLP/GEP:	yes

Material and methods

Test material	BCS-CN88460 EC 50 G lot/batch 2016-001002 Specification 102000031262 Content active substance 5.28% w/w
Test species	Water flea (*Daphnia magna*) Preparation of test organisms: Breeding stock were maintained in a cimate-controlled environment under study conditions and were fed three times per week with living cells of *Desmodesmus subspicatus* in aqueous suspension. There were no males, ephippia or dead animals present in the cultures within 48 hours before the start of the exposure. There was also no disease treatment in the used breeding batch.
Organism age/size at study initiation	First instar neonates, less than 24 hours old, third or later brood of coeval parent daphnids.
Test solutions	Nominal concentrations: 0.194, 0.427, 0.939, 2.07, 4.55 and 10.0 mg form./L Control: water control Preparation of test solution: the primary stock solution was made by mixing 100.2 mg of formulation with test water (Elendt M7) up to 1000 mL and by stirring with a magnetic stirrer for 1 minute. The exposure concentrations were prepared as sub-dilutions of the primary stock solution (not serial dilutions).
Replication	No of test concentrations: 6 (+ water control) No. of vessels per concentration (replicates): 6 No. of vessels per control (replicates): 6

Organisms per replicate	No. of organisms per vessel: 5
Exposure	Static; total exposure duration: 48 hours

Feeding during test	None
Test conditions	Temperature: 20.2 - 21.1°C Photoperiod: 16 hours light / 8 hours dark Light intensity: max. 1200 lux Light colour-temp.: 5400 K (cool white) pH: 7.7 – 7.9. Water hardness: 231.4 mg $CaCO_3$/L Dissolved oxygen: 8.5 - 8.8 mg/L (94 - 97% saturation) Conductivity: 597 µS/cm Alkalinity: 53 mg/L $CaCO_3$/L
Parameters Measured / Observations	Macroscopic visual counting of mobile daphnids. Visual comparison of untreated control animals and treated animals, performed after 24 and 48 hours of exposure. Measurement of pH-value; Measurement of dissolved oxygen, both determined for all freshly prepared solutions (batch sample) and again in the aged solutions (composite replicates) at the end of exposure. Water temperatures within the test system were recorded at start and end of exposure from one vessel of the untreated control group and of the highest treatment group. Verification of sensitivity was performed using acute non-GLP reference testing of $K_2Cr_2O_7$, p.a. grade (test concentrations: 0.56, 0.75, 1.00, 1.33 and 1.78 mg/L). The current 24h EC_{50} of 0.86 mg/L met the range of 0.79 – 0.93 mg/L, as defined by OECD inter-laboratory ringtesting (published with OECD Guideline 202 (2004).
Chemical analysis	Stock solution: an unicate sample of the stock solution (100 mg form./L) was taken and handled as the test media samples. Freshly prepared test media: Sampling immediately before distribution to the test vessels, from batch preparation for each treatment and control group. Aged test media: Sampling immediately after termination of exposure as composite from all replicates of a treatment group and control group. All samples were measured by HPLC-MS/MS.
Data analysis	Logit analysis, fitted by an iterative weighed linear regression according to the Maximum Likelihood principle. For calculations Tox-Rat-Professional and Excel 2010 were used.

Results

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

Validity criteria	**Required**	**Obtained**
Immobilisation in control during test	≤ 10%	3.3%
Dissolved oxygen concentration at the end of the test	≥ 3 mg/L	≥ 8.6 mg/L

All of these criteria were met.

Analytical results:
The accompanying chemical analysis of BCS-CN88460 in the freshly prepared test solutions at test initiation revealed measured contents between 105% and 114% of the aspired nominal concentrations (see Table B9.3.1-3). The corresponding concentrations of the aged test solutions at the end of the 48 hours exposure period ranged between 108% and 112% of nominal. No contaminations of BCS-CN88460 were detected in samples from untreated water control. As these measured concentrations ranged well within the recommended range of 80 – 120% of nominal, all reported results are based on nominal concentrations of BCS-CN88460 EC 50 G in the test solutions.

Table B9.3.1-3: Measured concentrations of BCS-CN88460 in the exposure solutions

Nominal test concentration		Analysed concentrations of the freshly prepared solutions *		Analysed concentrations of the aged solutions after 48 hours *	
mg form./L	**mg a.s./L**	**mg a.s./L**	**% of nominal**	**mg a.s./L**	**% of nominal**
0.194	0.0102	0.0115	112	0.0114	112
0.427	0.0225	0.0253	113	0.0250	111
0.939	0.0496	0.0563	114	0.0544	110
2.07	0.109	0.120	110	0.121	111
4.55	0.240	0.262	109	0.259	108
10.0	0.528	0.555	105	0.573	109

* mean value of two measurements

Biological results:

Table B9.3.1-4 shows the effect of the test item on the mobility and morbidity of *D.magna* after 24 and 48 hour exposure to the formulation.

Table B9.3.1-4: Effects of Isoflucypram EC50 (50 g/l) on *D.magna*

Nominal test concentration (mg form./L)	Exposed daphnids (=100%)	Immobilised daphnids			
		24 h		48 h	
		n	**%**	**n**	**%**
control	30	0	0	1	3.3
0.194	30	0	0	0	0
0.427	30	0	0	1	3.3[C]
0.939	30	0	0	1	3.3
2.07	30	5	16.7[A]	7	23.3[D]
4.55	30	16	53.3[B]	30	100
10.0	30	30	100	30	100

[A] 2 daphnids were mainly moving along the bottom of the test vessel (not considered in the % of immobilized daphnids)
[B] 8 dapnids were mainly moving along the bottom of the test vessel and 11 daphnids showed a clear decrease in frequency of antennae movements (not considered in the % of immobilized daphnids)
[C] 1 dapnid trapped on the water surface with a clear decrease in frequency of antennae movements (not considered in the % of immobilized daphnids)
[D] 9 daphnids were mainly moving along the bottom of the test vessel and showed a clear decrease in frequency of antennae movements (not considered in the % of immobilized daphnids)

Conclusion
The study meets the validity criteria and the endpoint based on nominal concentration is:

EC_{50} 48 hours (95% C.I.):	2.22 mg form./L (2.12 - 2.33 mg form./L) (Equivalent to 0.117 mg a.s./L, 95% CI: 0.112 – 0.123 mg a.s./L)

RMS Comments

This study has been conducted to OECD 202 (2004) guidelines and is considered valid.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.0625 µg/L (see section 3CA B5.1.2.6.1 for further details).

The endpoint considered suitable for use in the risk assessment is: **EC_{50} 2.22 mg formulation/L (equivalent to 0.117 mg a.s./L).**

Previous evaluation:	New data, submitted for purpose of review

Report:	KCP 10.2.1/03; Kuhl, K.; 2017;
Title:	Pseudokirchneriella subcapitata growth inhibition test - BCS-CN88460 EC50 G
Report No.:	EBLNN500
Guideline(s):	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; U.S. EPA Pesticide Assessment Guidelines, Subdivision J, §122-2, 123-2; OCSPP Guideline 850.4500 (January 2012)
GLP/GEP:	Yes

Material and methods

Test material	BCS-CN88460 EC 50 G Supplier Batch ID: 2016-001002 Specification: 102000031262 5.28% w/w BCS-CN88460
Test species	Freshwater green algae (*Pseudokirchneriella subcapitata)* Strain SAG 61.81 Origin: Collection of Algal Cultures, Inst. for Plant Physiology, University of Göttingen, Nikolausberger Weg 18, 37077 Göttingen, Germany
Reference item:	Sensitivity of test organisms was tested twice per year using potassium dichromate.
Culturing conditions	400 µL of a 7-10 days old stock culture was transferred into a 300 mL cotton plugged Erlenmeyer flask containing 100 mL of nutrient medium every 7-10 days. Stock cultures of algae were kept at 22 ± 2 °C with 24 hours light (4500 – 7000 lux). Test vessels were placed on a tablet rotating 100 rpm to prevent sedimentation of the cells. All operations were conducted under sterile conditions to handle an axenic[2] algae culture. Pre-cultures were prepared from stock cultures 3 days before the start of the test using OECD medium. Nutrient medium was prepared 4 days prior to the test using Milli-Q water and sterilised using memebrane filtration.
Organism age/size at study initiation	Pre cultures were prepared from stock cultures 3 days before the start of the test using OECD medium.
Preparation of test solutions:	Prior to the test, the stock solution was prepared by dissolving 12.10 mg of the test substance and 1200 g nutrient medium by stirring for 5 minutes. 375 of the stock solution was transferred to a dilution series to obtain the concentration levels used in the study. Evidence of undissolved material: No precipitations observed.
Test solutions	Nominal concentrations: 0.0954, 0.305, 0.977, 3.13 and 10.0 mg/L based on an initial range-finding test. Controls: Water control
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4 One additional replicate per test level and control containing the test concentration without algae used for observation of the test substance and used to calibrate the photometer.
Exposure	Static Total exposure duration: 96 hours
Initial cells density	10^4 cells/mL in each test group

[2] Axenic cultures are cultures of a single species.

Test conditions	Temperature: 22.5 – 23.0°C Photoperiod: continuous light Light intensity at surface of test vessels: 4440 to 4670 lux pH of controls: 7.7 – 9.4 pH of test solutions: 7.7 – 9.3 Water hardness: not specified Conductivity: not specified Growth medium same as culture medium: Yes Type of light: artificial (Cool white fluorescent lamps) Agitation: Test vessels were placed on a tablet rotating 100 rpm to prevent sedimentation of the cells without additional aeration.

Test vessels :	300mL Erlenmeyer flaks containing 150mL of test medium.
Parameters Measured / Observations	Temperature was determined by a continuous measurement in one additional incubated glass vessel filled with the same amount of de-ionised water as in the test vessels. The pH was measured at the start of the study and additionally after 72 and after 96 hours in all test levels and the controls. Light was measured once during the test using a luxmeter. Morphological examination of cells using a microscope were made after 0, 24, 48, 72 and 96 hours. Cell numbers per volume (as a surrogate for biomass per volume) were estimated photometricallly after 24, 48, 72 and 96 hours.
Sampling for chemical analysis	For the verification of the test item concentrations, duplicate water samples of 10 mL were taken from the bulk solution at test start and from the corresponding aged media (pooled replicates) after 72 and 96 hours of exposure from each test concentration and the control. The a.s. content was determined at least in one of the duplicate samples using HPLC-MS/MS and the given results are expressed at the average of the two measurements.
Data analysis	EC_x values (e.g. x = 50) and confidence intervals were calculated for the standard exposure period, using a commercial program (ToxRatPro 3.2.1).

Results

Validity criteria

Validity criteria acc. to OECD TG 201	**Required**	**Obtained**
1) The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	16	71.4
2) The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	< 35%	72h: 12.7% 96h: 16.6%
3) The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%.	< 7%	72h: 1.1% 96h: 0.62%

All validity criteria were met for the study

Analytical results:

Measured concentrations of the samples ranged from 87 to 123% of nominal concentrations. Therefore results of the study were based on nominal concentrations. No residues of BCS-CN88460 were found in the control above the limit of quantification (LOQ = 0.000625 mg a.s./L).

Table B9.3.1-5: Analytical measurements of BCS-CN88460 EC 50 G

	0 hour		**72h**		**96h**	
Nominal Concentration (mg formulation/L)	**Measured concentration (mg a.s./L)***	**% Nominal***	**Measured concentration (mg a.s./L)***	**% Nominal***	**Measured concentration (mg a.s./L)***	**96-hour % Nominal***
0.0954	0.00479	95	0.00459	91	0.00437	87
0.305	0.0166	103	0.0163	101	0.0180	112
0.977	0.0573	111	0.0509	99	0.0499	97
3.13	0.178	108	0.176	107	0.182	110
10.0	0.587	111	0.590	112	0.648	123

* Mean of two determinations

Reference item

An E_rC_{50} value of 1.15 mg/L (95% confidence intervals 1.05 – 1.27 mg/L) was confirmed from a study conducted with potassium dichromate following a request for additional information during evaluation.

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

Table B9.3.1-6 shows the effect of the test item on growth rate after 72 and 96 hours

Table B9.3.1-6: Growth rate of *P.subcapitata* after exposure to BCS-CN88460 EC 50 G

	0 - 72 hours		**0 - 96 hours**	
Nominal concentrations (mg/L)	**Mean cell number after 72 h (cells/mL)**	**Inhibition of average specific growth rate (%)**	**Mean cell number after 72 h (cells/mL)**	**Inhibition of average specific growth rate (%)**
Water Control	714000	-	2133000	-
0.0954	711000	0.1	2121000	0.1
0.305	664000	1.7	2006000	1.1
0.977	588000	4.5	1864000	2.5
3.13	115000	45.2*	490000	28.9
10.0	8000	122.9*	20000	87.3*

*Statistically significantly different from the control $p < 0.05$

Table B9.3.1-7 shows the effect of the test item on yield after 72 and 96 hours

Table B9.3.1-7: Yield of *P.subcapitata* after exposure to BCS-CN88460 EC 50 G

	72 hours		**96 hours**	
Nominal concentrations (mg/L)	**Yield (cells × 1000/mL)**	**Inhibition of yield (%)**	**Yield (cells × 1000/mL)**	**Inhibition of yield (%)**
Water Control	70.4	-	212.3	-
0.0954	70.1	0.4	211.1	0.6
0.305	65.4	7.1*	199.6	6.0
0.977	57.8	17.9*	185.4	12.7*
3.13	10.5	85.1*	48.0	77.4*
10.0	-0.2	100.3*	1.0	99.5*

*Statistically significantly different from the control $p < 0.05$

Table B9.3.1-8 shows the effect of the test item on biomass after 72 and 96 hours

Table B9.3.1-8: Area under the growth curve for *P.subcapitata* after exposure to BCS-CN88460 EC 50 G

	72 hours		**96 hours**	
Nominal concentrations (mg/L)	**Area under the growth curve (biomass integral)**	**Inhibition of Biomass integral (%)**	**Area under the growth curve (biomass integral)**	**Inhibition of Biomass integral (%)**
Water Control	1277.3	0.0	4668.9	-
0.0954	1237.1	3.1	4611.5	1.2
0.305	1194.8	6.5	4375.3	6.3
0.977	1038.6	18.7*	3957.1	15.2*
3.13	211.9	83.4*	912.9	80.4*
10.0	13.9	98.9*	23.4	99.5*

*Statistically significantly different from the control $p < 0.05$

Conclusion
The endpoints based on nominal concentrations are:

E_rC_{50} 72 hours (95% C.I.):	**3.39 mg/L (2.77 – 4.20 mg/L)**
E_rC_{20} 72 hours (95% C.I.)	1.88 mg/L (1.10 to 2.38 mg/L)
E_rC_{10} 72 hours (95% C.I.)	1.38 mg/L (0.63 to 1.89 mg/L)
NOE_rC 72 hours: highest concentration without adverse effects	0.977 mg/L
E_yC_{50} 72 hours (95% C.I.):	**1.68 mg/L (1.59 – 1.79 mg/L)**
E_yC_{20} 72 hours (95% C.I.):	1.01 mg/L (0.93 – 1.09 mg/L)
E_yC_{10} 72 hours (95% C.I.):	0.78 mg/L (0.70 – 0.85 mg/L)
NOE_rC 72 hours: highest concentration without adverse effects	0.095 mg/L
E_bC_{50} 72 hours (95% C.I.):	**1.70 mg/L (1.60 – 1.81 mg/L)**
E_bC_{20} 72 hours (95% C.I.):	0.995 mg/L (0.913 – 1.072 mg/L)
E_bC_{10} 72 hours (95% C.I.):	0.752 mg/L (0.673 – 0.825 mg/L)
NOE_rC 72 hours: highest concentration without adverse effects	0.305 mg/L
E_rC_{50} 96 hours (95% C.I.):	**4.58 mg/L (4.35 – 4.82 mg/L)**
E_rC_{20} 96 hours (95% C.I.)	2.56 mg/L (2.37 to 2.74 mg/L)
E_rC_{10} 96 hours (95% C.I.)	1.88 mg a.s./L (1.71 to 2.06 mg a.s./L)
LOE_rC 96 hours: Lowest concentration with an effect	3.13 mg/L
NOE_rC 96 hours: highest concentration without adverse effects	0.977 mg/ L
E_yC_{50} 96 hours (95% C.I.):	**1.97 mg/L (1.83 – 2.11 mg/L)**
E_yC_{20} 96 hours (95% C.I.):	1.170 mg/L (1.044 – 1.285 mg/L)
E_yC_{10} 96 hours (95% C.I.):	0.891 mg/L (0.770 – 1.002 mg/L)
NOE_yC 96 hours: highest concentration without adverse effects	0.305 mg/L
E_bC_{50} 96 hours (95% C.I.):	**1.84 mg/L (1.73 – 1.95 mg/L)**
E_bC_{20} 96 hours (95% C.I.):	1.09 mg/L (0.99 – 1.18 mg/L)
E_bC_{10} 96 hours (95% C.I.):	0.83 mg/L (0.74 – 0.91 mg/L)

NOE_bC 96 hours: highest concentration without adverse effects	0.305 mg/ L

RMS comments
This study was conducted according to OCSPP Guideline 850.4500 and OECD 201(2011).
The following was noted by the RMS :

It is stated that the sensitivity of the test organisms was tested twice per year ; however no results were provided to determine if this is within the acceptable range in OECD 201 (2011) guidelines (E_rC_{50} of 0.60 – 1.03 mg/L). Therefore it cannot be confirmed if the test organisms were sufficiently sensitive. Following a request for additional information during evaluation, the applicant provided an E_rC_{50} value of 1.15 mg/L from a positive control study with potassium dichromate. This is within the acceptable range according to ISO 8692 (2012) (0.92 – 1.46 mg/Land therefore the test organisms are sufficiently sensitive.

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

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.0625 µg/L (see section 3CA B5.1.2.6.1 for further details).

This study is considered valid and the endpoint confirmed as relevant for the risk assessment is as follows:

72 hour E_rC_{50}: 3.39 mg formulation/L (equivalent to 0.179 mg a.s./L)

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

No further data submitted.

B.9.3.3. Further testing on aquatic organisms

No further data submitted.

B.9.4. RISK ASSESSMENT FOR AQUATIC ORGANISMS

The risk assessment is based on the current guidance: EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 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.

The toxicity data from studies conducted with the active substance, formulated product and relevant metabolite are presented in Table 9.4-1 below. These studies have been evaluated, assessed and considered suitable for use within risk assessments. The study summaries and evaluation of these studies are presented in III CA B.9.4 and III CP B9.4.

Commission Regulation (EU) 283/2013 and 284/2013 require estimates of ECx (e.g. EC_{10}, EC_{20}) together with the NOEC for chronic studies. If appropriate, an ECx is given in the study summaries (chapter III CA B.9.4) or a respective justification. Where it is possible for both the NOEC and reliable EC_{10} to be calculated for chronic studies, the EC_{10} value has been used in preference as per the latest aquatic guidance document (EFSA Journal 2013;11(7):3290).

Table B9.4-1: Aquatic endpoints for isoflucypram, its metabolite and representative formulation isoflycypram EC 50

Test system	Test substance	Endpoint		Reference
Acute toxicity to fish				
Static, 96 hour *Pimephales promelas*	Isoflucypram	96 h LC_{50}	0.0861 mg a.s./L (nom)	[redacted] 2018; M-542897-02-1 KCA 8.2.1/01
Static, 96 hour *Oncorhynchus mykiss*	Isoflucypram	96 h LC_{50}	0.098 mg a.s./L (gmm)	[redacted] 2015; EBLNN024 KCA 8.2.1/02
Static, 96 hour *Cyprinodon variegatus*	Isoflucypram	96 h LC_{50}	0.544 mg a.s./L (mm)	[redacted]; 2015; EBLNN023; KCA 8.2.1/03
Static, 96 hour *Oncorhynchus mykiss*	Isoflucypram EC50	96 h LC_{50}	1.29 mg/L (nom) (**~0.068 mg a.s./L**)[A]	[redacted] 2017; M-595274-01-1 KCP 10.2.1/01
Fish, acute *Oncorhynchus mykiss*, *Pimephales promelas*, *Cyprinodon variegatus*	Isoflucypram	96 h LC_{50}	**0.156 mg a.s./L**[B]	Geometric mean (EFSA Journal 2013;11(7):3290)
Static, 96 hour *Oncorhynchus mykiss*	BCS-CN88460-carboxylic acid (M12)	96 h LC_{50}	**> 33.5 mg p.m./L** (gmm)	[redacted] 2017; M-587655-01-1 KCA 8.2.1/04
Long-term toxicity to fish				
Flow-through, ELS *Pimephales promelas*	Isoflucypram	33 d NOEC (larval survival)	**0.01328 mg a.s./L** (mm)	[redacted] 2017; M-580247-01-1 KCA 8.2.2.1/01
Flow-through, ELS *Cyprinodon variegatus*	Isoflucypram	35 d NOEC (fry survival)	0.025 mg a.s./L	[redacted] 2016; M-575119-01-1 KCA 8.2.2.1/02
Bioconcentration in fish				
BCF study flow through *Lepomis macrochirus*	Isoflucypram	BCF	**370** (kinetic BCF lipid normalized and growth corrected)	[redacted]; 2017; M-610008-01-1 KCA 8.2.2.3/01
Acute toxicity to aquatic invertebrates				
Static, 48 hour *Daphnia magna*	Isoflucypram	48 h EC_{50}	0.201 mg a.s./L (gmm)	Kuhl, K.; 2016; M-574184-01-1 KCA 8.2.4.1/01
Static renewal, 96 hour *Americamysis bahia*	Isoflucypram	96 h EC_{50}	0.27 mg a.s./L (mm)	Brougher, D. S.; Siddiqui, A. I.; Gallagher, S. P.; 2016; 149A-257B; KCA 8.2.4.2/01;
Flow-through, 96 hour *Crassostrea virginica*	Isoflucypram	96 h EC_{50} (shell deposition)	0.170 mg a.s./L (mm)	Brougher, D. S.; Siddiqui, A. I.; Gallagher, S. P.; 2016; 149A-258; KCA 8.2.5.2/03;
Static, 48 hour *Daphnia magna*	Isoflucypram EC50	48 h EC_{50}	2.22 mg/L (nom) (**~0.117 mg a.s./L**)[A]	Kuhl, K.; 2017; M-607779-01-1 KCP 10.2.1/02

Test system	Test substance	Endpoint		Reference
Invertebrate, acute *Daphnia magna Americamysis bahia*	Isoflucypram	EC_{50}	**0.203 mg a.s./L**[B]	Geometric mean (EFSA Journal 2013; 11 (7) 2013;11(7):3290)
Static, 48 hour *Daphnia magna*	BCS-CN88460-carboxylic acid (M12)	48 h EC_{50}	**> 24 mg p.m./L** (nom)	Riebschlaeger, T; 2016; M-573296-01-1 KCA 8.2.4.1/02
Long-term toxicity to invertebrates				
Static renewal, 21 days *Daphnia magna*	Isoflucypram	21 d EC_{10} (dry body weight) 21 d NOEC	0.0584 mg a.s./L (mm) 0.072 mg a.s./L (mm)	Bruns, E.; 2017; M-593961-01-1 KCA 8.2.5.1/01
Flow-through, 28 days *Americamysis bahia*	Isoflucypram	28 d NOEC (14 – 28 day adult mortality)	**0.020 mg a.s./L (mm)**	Milligan, A. L.; Siddiqui, A. I.; Gallagher, S. P.; Krueger, H. O.; 2016; 149A-256; KCA 8.2.5.2/01
Toxicity to sediment dwelling invertebrates				
Static renewal, 61 days *Chironomus dilutus*	Isoflucypram	61 d NOEC	**100 mg a.s./kg sediment (nom)**	Bradley, M. J.; 2017; M-596883-01-1 KCA 8.2.5.4/01
Toxicity to algae				
Static, 96 hours *Navicula pelliculosa*	Isoflucypram	72h-E_rC_{50} 72 h-E_yC_{50}	> 2.0 mg a.s./L (gmm) > 2.0 mg a.s./L (gmm)	Arnie, J. R.; Siddiqui, A. I.; Porch, J. R.; Martin, K. H.; 2017; M-604809-01-1 KCA 8.2.6.2/03
Static, 96 hours *Pseudokirchneriella subcapitata*	Isoflucypram	72h-E_rC_{50} 72 h-E_yC_{50}	>2.02 mg a.s./L (gmm) >2.02 mg a.s./L (gmm)	Kuhl, K.; EBLNN050; 2017; KCA 8.2.6.1/01
Static, 96 hours *Anabaena flos-aquae*	Isoflucypram	96h-E_rC_{50} 96 h-E_yC_{50}	3.7 mg a.s./L (gmm) 2.9 mg a.s./L (gmm)	Arnie, J. R.; Siddiqui, A. I.; Porch, J. R.; 149P-111; Martin, K. H.; 2017; KCA 8.2.6.2/01
Static, 96 hours *Skeletonema costatum*	Isoflucypram	96h-E_rC_{50} 96h-E_yC_{50}	>2.538 mg a.s./L (gmm) 1.5 mg a.s./L (gmm)	Arnie, J. R.; Siddiqui, A. I.; Porch, J. R.; Martin, K. H.; 2017; 149P-11; KCA 8.2.6.2/02
Static, 96 hour *Pseudokirchneriella subcapitata*	Isoflucypram EC50	72h E_rC_{50}	3.39 mg/L (nom) (**~0.179 mg a.s./L**)[A]	Kuhl, K.; 2017; M-600970-01-1 KCP 10.2.1/03
Static, 96 hour *Pseudokirchneriella subcapitata*	BCS-CN88460-carboxylic acid (M12)	72h-E_rC_{50}	**> 35.1 mg p.m./L** (gmm)	Kuhl, K.; 2017; M-587659-01-1 KCA 8.2.6.1/02
Toxicity to aquatic macrophytes				
Semi-static, 7 days *Lemna gibba*	Isoflucypram	7d-E_rC_{50}	**> 2.48 mg a.s./L** (gmm)	Kuhl, K.; 2017; M-593965-01-1 KCA 8.2.7/01

Bold: endpoints used in risk assessment

Nom = nominal concentrations, mm = mean measured concentration, gmm = geometric mean measured concentration

[A] Endpoints in the study report were reported based on the formulation only. For this table the endpoint is converted to mg a.s./L based on the reported content of isoflucypram of 5.28%.

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

Selection of endpoints for Tier 1 risk assessment for the active substance isoflucypram

The following endpoints were selected for use in the tier 1 risk assessment for isoflucypram:

Acute toxicity to fish: Four acute toxicity studies were conducted with fish; 3 with the active substance (*Pimephales promelas*, *Oncorhynchus mykiss* and *Cyprinodon variegatus*) and one with the representative formulated product (*Oncorhynchus mykiss*). All studies were deemed valid for use for use in the risk assessment; however it is noted that the most sensitive species tested with the active substance (*P.promelas*) is not the species tested with the representative formulation (*O.mykiss*), which adds some uncertainty to whether the derived formulation endpoint is sufficiently protective. As the acute toxicity endpoints for *O.mykiss* testing the active substance and the formulated product differ only by a factor of 1.44, the RMS considers this acceptable. The acute *O.mykiss* study conducted with the representative formulation gives the lowest endpoint when expressed based on the active substance content. The difference between the lowest active substance endpoint (0.0861 mg a.s./L) and the formulation endpoint (0.068 mg a.s./L) is a factor of 1.27. As such, it is not considered necessary to conduct a separate formulation risk assessment and the RMS considers the formulation data to be representative of the toxicity of the active substance alone. Therefore in accordance with EFSA (2013), the LC_{50} value of **0.068 mg a.s./L** will be used in the tier 1 risk assessment, which will address the acute risk to fish from exposure to both the a.s. and the intact formulated product.

Long-term toxicity to fish: A long-term toxicity early life stage (ELS) study was conducted with the most sensitive fish species, *P.promelas* according to the acute toxicity data on the active substance. A further ELS study was submitted testing *C.variegatus;* which had a higher endpoint than that conducted with *P.promelas.*Therefore the lowest endpoint from the study conducted with *P.promelas* was used in the tier 1 risk assessment.

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

A NOEC was derived from the *P.promelas* study; however it was not possible to derive EC_{10} values as effects were only observed at the highest test concentration. The endpoint for use in the risk assessment based on larval survival at 33 days in *P.promelas* is a **NOEC = 0.01328 mg a.s./L**.

Acute toxicity to aquatic invertebrates: Three acute toxicity studies were conducted with aquatic invertebrates; two with the active substance (*Daphnia magna* and *Americamysis bahia*) and one with the representative formulation (*D.magna*). All studies were deemed valid for regulatory use. The acute study conducted with *D.magna* testing the formulated product gives the lowest endpoint when expressed based on the active substance content. The difference between the active substance (0.201 mg a.s./L) and formulation endpoints is a factor of 1.7. As such, it is not considered necessary to conduct a separate formulation risk assessment and the RMS considers the formulation data to be representative of the toxicity of the active substance alone. Therefore in accordance with EFSA (2013), the EC_{50} value of **0.117 mg a.s./L** will be used in the risk assessment, which will address the acute risk to invertebrates from exposure to both the a.s. and the intact formulated product.

Long-term toxicity to aquatic invertebrates: Two long-term studies were conducted with aquatic invertebrates (*D.magna* and *A.bahia*) testing the active substance. Both studies were considered valid for regulatory use. The most sensitive endpoint was a NOEC based on 14 -28 adult mortality in the saltwater crustacean *A. bahia*. The study met all validity criteria; however there were some issues with the derivation of endpoints and for several of the measured endpoints it was not possible to derive a robust L/EC_{10} value for statistical reasons. Statistically significant reductions in 14 – 28 adult survival were observed at 79 µg a.s./L; the applicant stated that this result

was anomalous as there were no statistically significant effects at the next concentration of 146 µg a.s./L; however it is unclear from the data which of these results is anomalous as the other results appear to follow a dose-response (see table 9.4-2 below). Furthermore, 14 – 28 day adult survival in replicate A in the 179 µg a.s./L treatment group was 90.9%, whereas in replicates B – D it was 50 – 66.7%, indicating that replicate A was potentially an outlier. As no reliable LC_{10} value could be derived and a 14.2% reduction in survival was observed at 37 µg a.s./L, the RMS considered it appropriate to set the NOEC at 20 µg a.s./L in order to be protective of potentially biologically-relevant effects.

The NOEC value of **0.020 mg a.s./L** will therefore be used in the risk assessment.

Table B9.4-2 : Summary of survival of G1adult saltwater mysids exposed to BCS-CN88460

Mean measured conc. (µg a.s./L	**Adult survival day 14 - 28**			
	No. alive at pairing[1]	**No. surviving**	**% survival**	**% change in survival in comparison to pooled control[2]**
Control	49	38	77.6	-
Solvent control	50	38	76.0	-
Pooled control	99	76	76.8	-
20	48	34	70.8	7.8
37	44	29	65.9	14.2
79	46	25*	54.3*	29.3*
146	42	28	66.7	13.2
299	50	9*	18.0*	76.6*

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

[1] The number alive at pairing may be less than the number surviving to Day 13 due to the fact that extra females that cannot be used to form pairs and any immature mysids are discarded at the time of pairing on Day 13.

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

Toxicity to algae: Four algal studies were conducted with the active substance (*Navicula pelliculosa*, *Pseudokirchneriella subcapitata*, *Anabaena flos-aquae* and *Skeletonema costatum*). All studies were deemed valid for regulatory use; however, for the study with *P.subcapitata* the toxic reference result was higher than the recommended range, for *A. flos-aquae* no toxic reference item was tested and for *N.pelliculosa* and *S.costatum* the toxic reference item was tested below the recommended range and the resulting E_rC_{50} for the toxic reference item was an unbound greater-than value. This adds some uncertainty regarding the sensitivity of the test systems. The study conducted with *N.pelliculosa* had the lowest toxicity endpoint (> 2.0 mg a.s./L), noting the uncertainty regarding the toxic reference item result. The remaining studies were within a similar range at >2.538, >2.02 and 3.7 mg a.s./L for *S.costatum P.subcapitata* and *A.flos-aquae* respectively.

The study testing the formulation was conducted with *P.subcapitata*, which although not the most sensitive species tested with the active substance, the endpoint was the highest concentration tested and both active substance study endpoints are close together (>2.02 vs > 2.0 mg a.s./L for *P.subcapitata* and *N.pelliculosa* respectively). The algal study testing the formulated product has a lower endpoint (0.179 mg a.s./L) than the active substance endpoint for algae when considering the active substance content. Given the margin of endpoint difference from all active substance algal studies, the RMS considers that using the formulation endpoint (expressed in terms of active substance) will cover the risk to algae from the active substance, and depending on the margin of safety, should also account for the risk from the representative formulation. As such, the RMS considers the derived endpoint of **E_rC_{50} = 0.179 mg a.s./L** acceptable for use in the risk assessment.

Toxicity to aquatic macrophytes: One study was submitted with an aquatic macrophyte species (*Lemna gibba*) testing the active substance. The study was deemed valid for regulatory use and the endpoint confirmed for use in the risk assessment is **E_rC_{50}: > 2.48 mg a.s./L.**

Toxicity to sediment dwelling organisms: Three studies were submitted testing the toxicity of the active substance to sediment dwelling organisms (*Chironomus dilutus, Leptocheirus plumulosus* and *Hyalella Azteca*) via spiked sediment studies. Only the study testing *C.dilutus* was deemed valid for regulatory purposes. Potentially biologically relevant effects (46- 56 % reduction in mean offspring number in comparison to the control) were noted in the in the solvent control group for *H.azteca*¸ which were not statistically significant, indicating that the test system was not suitable for sensitively detecting effects and the solvent was having a negative effect on performance of the test organisms. For the study testing *L.plumulosus* statistical significance

could not be detected despite a reduction of 20-25% in number of offspring per surviving female at the two lowest test concentrations. As such, the RMS considers this study to be insensitive to detecting potentially biologically significant effects. Furthermore, if these effects were to be considered biologically relevant, it would not be possible to set a NOEC from this study.

Regarding the study testing *C.dilutus*, there were some uncertainties with this study: The test was not conducted according to standard test guidelines for a sediment dweller (OECD 218); however validity criteria were checked against the validity criteria in OECD 218 as this is the only suitable study design for a sediment dweller according to current data requirements. Validity criteria were met with the exception of temperature which varied by 2°C rather than the 1°C specified in the test guidelines. In general, the controls met the validity criteria, indicating that this did not have a significant effect on control performance; however the time to emergence of males in the solvent control was 19.3 days, which is below the 20 – 65 days specified in the validity criteria of OECD 218 (2004). However, as there was no statistically significant difference in emergence rate of males between the solvent and negative control for comparison to the test groups, this is not considered to invalidate the test results. There were >10% effects on mean ash-free dry weight at several of the test concentrations, which did not follow a dose response and following a request for additional information the applicant provided historical results that demonstrated that this variability was apparent in other runs of the test, indicating that this is not a clear effect of the test item and is instead related to biological variability. As such, the RMS considers that reliance on this parameter to set an endpoint from this study is likely to be inaccurate and endpoints should be based on parameters based on reproductive output and emergence instead. The endpoint confirmed for use in the risk assessment is: **NOEC: 100 mg a.s./kg sediment.**

As per the EFSA Aquatic Guidance Document (EFSA Journal 2013;11(7):3290), assessment of the risk from the active substance will be undertaken using the appropriate PEC value for the proposed use of isoflucypram. RAC values will be compared to the relevant PEC in a tiered process for both acute and long-term risk to aquatic organisms. The RACs to be used in the risk assessments are presented in Table 9.4-3 below.

The most sensitive endpoint for each species has been used in calculating the RAC.

Table B9.4-3: Regulatory acceptable concentrations (RAC) for isoflucypram for each organism group

Species	Fish		Aquatic invertebrates		Sediment dwellers	Algae	Aquatic macrophytes
	O.mykiss	*P.promelas*	*D.magna*	*A.bahia*	*C.dilutus*[2]	*P.subcapitata*	*L.gibba*
Endpoint (µg/L or µg/kg sediment)	LC50	NOEC	EC50	NOEC	NOEC	ErC50	ErC50
	68	13.28	117	20	100000	179	>2480
AF	100	10	100	10	10	10	10
RAC (µg/L or µg/kg sediment)	0.68[1]	1.328	1.17[1]	2.0	10000	17.9[1]	248.0

[1]Based on the formulation endpoint expressed as active substance content
[2] Spiked sediment study; value is expressed as µg/kg sediment

Predicted environmental concentrations used in the risk assessment

Predicted environmental concentrations of isoflucypram in surface water and sediment were calculated according to FOCUS Steps 1-3 for the use in cereals (see section B8 and table 9.4-4 and 9.4-5 below). Predicted Exposure Concentrations (PECs) used for the risk assessment have been established by the Environmental Fate Evaluator.

Accumulation in the sediment is predicted for the active substance isoflucypram therefore further consideration of the risk to sediment dwelling organisms is required.

Table B9.4-4: Initial max PEC_{sw} values for Isoflucypram – FOCUS Steps 1 and 2

Compound	FOCUS Scenario	Cereals* 1 × 75 g a.s./ha, BBCH 30-69			
		Early (March – May)	Late (June – September)	Early (March – May)	Late (June – September)
		PECsw, max [µg/L]		PECsed, max [µg/kg]	
Isoflucypram	STEP 1 - North	9.63	9.63	123.53	123.53
	STEP 1 - South	9.63	9.63	123.53	123.53
	STEP 2 – North	1.73	1.73	22.36	22.36
	STEP 2 - South	3.15	2.44	41.43	31.90

*Worst case PEC_{sw} values considering all scenarios relevant for use in winter and spring cereals.

Table B9.4-5: Initial max PECsw values for Isoflucypram– FOCUS Steps 3

Compound	FOCUS Scenario	1 × 75 g a.s./ha, BBCH 30-69			
		Winter cereals		Spring cereals	
		Early (March – May)	Late (June – September)	Early (March – May)	Late (June – September)
		$PEC_{sw,max}$ [µg/L]		$PEC_{sw,max}$ [µg/L]	
Isoflucypram	D1 ditch	1.387	0.6537	1.181	0.6891
	D1 stream	0.869	0.4216	0.7401	0.4226
	D2 ditch	1.369	0.8686	-	-
	D2 stream	0.8551	0.5859	-	-
	D3 ditch	0.4745	0.4755	0.4746	0.4750
	D4 pond	0.1005	0.0529	0.1224	0.0699
	D4 stream	0.3652	0.4103	0.3880	0.4090
	D5 pond	0.1182	0.0600	0.1113	0.0635
	D5 stream	0.3798	0.4426	0.3992	0.4142
	D6 ditch	0.7443	0.4768	-	-
	R1 pond	0.0425	0.0531	-	-
	R1 stream	0.3124	0.3133	-	-
	R3 stream	0.4415	0.4415	-	-
	R4 stream	0.4364	0.3921	0.4179	0.4361

Tier 1 risk assessment for isoflucypram

The tier 1 isk assessment for isoflucypram is presented in the following tables for early and late application to Winter cereals (Tables 9.4-6 and 9.4-) and early and late application to Spring cereals (Tables 9.4-8 and 9.4-9).

Table B9.4-6: Tier 1 risk assessment for isoflucypram – use in winter cereals – Early application (March – May)

Scenario	PECsw global max (µg L)	Fish acute	Fish chronic	Aquatic invertebrates	Aquatic invertebrates chronic	Algae	Higher plant		PECsed global max (µg/kg)	Sed. dweller prolonged
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*	*Americamysis bahia*	*Pseudokirchneriella subcapitata*	*Lemna gibba*			*Chironomus riparius*
		RAC (LC_{50})	RAC (NOEC)	RAC (EC_{50})	RAC (NOEC)	RAC (E_rC_{50})	RAC (ErC_{50})			RAC (NOEC)
		0.68	1.328	1.17	2.0	17.9	248.0			10000 µg a.s./kg sediment)
FOCUS Step 1					PEC/RAC ratio			FOCUS Step 1		PEC/RAC ratio
	9.63	**14.16**	**7.25**	**8.23**	**4.82**	0.54	0.04		123.53	0.01
FOCUS Step 2					PEC/RAC ratio			PEC/RAC ratio		PEC/RAC ratio
North Europe	1.73	**2.54**	**1.30**	**1.48**	0.87	0.10	0.01	North Europe	22.36	<0.01
South Europe	3.15	**4.63**	**2.37**	**2.69**	**1.58**	0.18	0.01	South Europe	41.43	<0.01
FOCUS Step 3					PEC/RAC ratio			PEC/RAC ratio		
D1 ditch	1.387	**2.04**	**1.04**	**1.19**	0.69	-	-			-
D1 stream	0.869	**1.28**	0.65	0.74	0.43	-	-			-
D2 ditch	1.369	**2.01**	**1.03**	**1.17**	0.68	-	-			-
D2 stream	0.8551	**1.26**	0.64	0.73	0.43	-	-			-
D3 ditch	0.4745	0.70	0.36	0.41	0.24	-	-			-
D4 pond	0.1005	0.15	0.08	0.09	0.05	-	-			-
D4 stream	0.3652	0.54	0.28	0.31	0.18	-	-			-
D5 pond	0.1182	0.17	0.09	0.10	0.06	-	-			-
D5 stream	0.3798	0.56	0.29	0.32	0.19	-	-			-
D6 ditch	0.7443	1.09	0.56	0.64	0.37	-	-			-
R1 pond	0.0425	0.06	0.03	0.04	0.02	-	-			-
R1 stream	0.3124	0.46	0.24	0.27	0.16	-	-			-
R3 stream	0.4415	0.65	0.33	0.38	0.22	-	-			-
R4 stream	0.4364	0.64	0.33	0.37	0.32	-	-			-

Values in **bold** are > 1 and therefore high risk

Table B9.4-7: Tier 1 risk assessment for isoflucypram – use in winter cereals – Late application (June – September)

Scenario	PECsw global max (µg L)	Fish acute	Fish chronic	Aquatic invertebrates	Aquatic invertebrates chronic	Algae	Higher plant		PECsed global max (µg/kg)	Sed. dweller prolonged
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*	*Americamysis bahia*	*Pseudokirchneriella subcapitata*	*Lemna gibba*			*Chironomus riparius*
		RAC (LC_{50})	RAC (NOEC)	RAC (EC_{50})	RAC (NOEC)	RAC (E_rC_{50})	RAC (ErC_{50})			RAC (NOEC)
		0.68	1.328	1.17	2.0	17.9	248.0			10000 µg a.s./kg sediment)
FOCUS Step 1					PEC/RAC ratio			FOCUS Step 1		PEC/RAC ratio
	9.63	**14.16**	**7.25**	**8.23**	**4.82**	0.54	0.04		123.53	0.01
FOCUS Step 2					PEC/RAC ratio			FOCUS Step 2		PEC/RAC ratio
North Europe	1.73	**2.54**	**1.30**	**1.48**	0.87	0.10	0.01	North Europe	22.36	<0.01
South Europe	2.44	**3.59**	**1.84**	**2.09**	**1.22**	0.14	0.01	South Europe	31.90	<0.01
FOCUS Step 3					PEC/RAC ratio					PEC/RAC ratio
D1 ditch	0.6537	0.96	0.49	0.56	0.33	-	-			-
D1 stream	0.4216	0.62	0.32	0.36	0.21	-	-			-
D2 ditch	0.8686	**1.28**	0.65	0.74	0.43	-	-			-
D2 stream	0.5859	0.86	0.44	0.50	0.29	-	-			-
D3 ditch	0.4755	0.70	0.36	0.41	0.24	-	-			-
D4 pond	0.0529	0.08	0.04	0.05	0.03	-	-			-
D4 stream	0.4103	0.60	0.31	0.35	0.21	-	-			-
D5 pond	0.0600	0.09	0.05	0.05	0.03	-	-			-
D5 stream	0.4426	0.65	0.33	0.38	0.22	-	-			-
D6 ditch	0.4768	0.70	0.36	0.41	0.24	-	-			-
R1 pond	0.0531	0.08	0.04	0.05	0.03	-	-			-
R1 stream	0.3133	0.46	0.24	0.27	0.16	-	-			-
R3 stream	0.4415	0.65	0.33	0.38	0.22	-	-			-
R4 stream	0.3921	0.58	0.30	0.34	0.29	-	-			-

Values in **bold** are > 1 and therefore high risk

Table B9.4-8: Tier 1 risk assessment for isoflucypram – use in spring cereals – Early Application (March – May)

Scenario	PECsw global max (µg L)	Fish acute	Fish chronic	Aquatic invertebrates	Aquatic invertebrates chronic	Algae	Higher plant		Sed. dweller prolonged
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*	*Americamysis bahia*	*Pseudokirchneriella subcapitata*	*Lemna gibba*	PECsed global max (µg/kg)	*Chironomus riparius*
		RAC (LC_{50})	RAC (NOEC)	RAC (EC_{50})	RAC (NOEC)	RAC (E_rC_{50})	RAC (ErC_{50})		RAC (NOEC)
		0.68	1.328	1.17	2.0	17.9	248.0		10000 µg a.s./kg sediment)
FOCUS Step 1					PEC/RAC ratio			FOCUS Step 1	PEC/RAC ratio
	9.63	**14.16**	**7.25**	**8.23**	**4.82**	0.54	0.04	123.53	0.01
FOCUS Step 2					PEC/RAC ratio			FOCUS Step 2	PEC/RAC ratio
North Europe	1.73	**2.54**	**1.30**	**1.48**	0.87	0.10	0.01	North Europe 22.36	<0.01
South Europe	3.15	**4.63**	**2.37**	**2.69**	**1.58**	0.18	0.01	South Europe 41.43	<0.01
FOCUS Step 3					PEC/RAC ratio				PEC/RAC ratio
D1 ditch	1.181	**1.74**	0.89	**1.01**	0.59	-	-		-
D1 stream	0.7401	**1.09**	0.56	0.63	0.37	-	-		-
D3 ditch	0.4746	0.70	0.36	0.41	0.24	-	-		-
D4 pond	0.1224	0.18	0.09	0.10	0.06	-	-		-
D4 stream	0.3880	0.57	0.29	0.33	0.19	-	-		-
D5 pond	0.1113	0.16	0.08	0.10	0.06	-	-		-
D5 stream	0.3992	0.59	0.30	0.34	0.20	-	-		-
R4 stream	0.4179	0.61	0.31	0.36	0.21	-	-		-

Values in **bold** are > 1 and therefore high risk

Table B9.4-9: Tier 1 risk assessment for isoflucypram – use in spring cereals – Late Application (June – September)

Scenario	PECsw global max (µg L)	Fish acute	Fish chronic	Aquatic invertebrates	Aquatic invertebrates chronic	Algae	Higher plant		Sed. dweller prolonged
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*	*Americamysis bahia*	*Pseudokirchneriella subcapitata*	*Lemna gibba*	PECsed global max (µg/kg)	*Chironomus riparius*
		RAC (LC_{50})	RAC (NOEC)	RAC (EC_{50})	RAC (NOEC)	RAC (E_rC_{50})	RAC (ErC_{50})		RAC (NOEC)
		0.68	1.328	1.17	2.0	17.9	248.0		10000 µg a.s./kg sediment)
FOCUS Step 1					PEC/RAC ratio			FOCUS Step 1	PEC/RAC ratio
	9.63	**14.16**	**7.25**	**8.23**	**4.82**	0.54	0.04	123.53	0.01
FOCUS Step 2					PEC/RAC ratio			FOCUS Step 2	PEC/RAC ratio
North Europe	1.73	**2.54**	**1.30**	**1.48**	0.87	0.10	0.01	North Europe 22.36	<0.01
South Europe	2.44	**3.59**	**1.84**	**2.09**	**1.22**	0.14	0.01	South Europe 31.90	<0.01
FOCUS Step 3					PEC/RAC ratio			FOCUS Step 3	PEC/RAC ratio
D1 ditch	0.6891	**1.01**	0.52	0.59	0.34	-	-		-
D1 stream	0.4226	0.62	0.32	0.36	0.21	-	-		-
D3 ditch	0.4750	0.70	0.36	0.41	0.24	-	-		-
D4 pond	0.0699	0.10	0.05	0.06	0.03	-	-		-
D4 stream	0.4090	0.60	0.31	0.35	0.20	-	-		-
D5 pond	0.0635	0.09	0.05	0.05	0.03	-	-		-
D5 stream	0.4142	0.61	0.31	0.35	0.21	-	-		-
R4 stream	0.4361	0.64	0.33	0.37	0.22	-	-		-

Values in **bold** are > 1 and therefore high risk

Conclusion at Tier 1: For Winter cereals (early application) there is an unresolved acute and chronic risk to fish and an unresolved acute risk to aquatic invertebrates at FOCUS Step 3; for late application in Winter cereals there is an unresolved acute risk to fish at FOCUS Step 3.

For Spring cereals (early application) there is an unresolved acute risk to fish and an unresolved acute risk to aquatic invertebrates at FOCUS Step 3; for late application in Winter cereals there is an unresolved acute risk to fish at FOCUS Step 3.

Therefore refinement of the endpoints following a geometric mean approach has been considered below at tier 2.

The margin of safety seen in the FOCUS step 2 risk assessment with regards to algae is deemed sufficient to conclude a low risk for both the active substance and representative formulation (on which the RAC is based), when considering the slight under-sensitivity seen in the source study to a reference chemical.

Selection of endpoints for Tier 2

As the acute risk to aquatic invertebrates and the acute and chronic risk to fish was not resolved at FOCUS Step 3, refinement of the endpoints considering a tier 2a geomean approach has been conducted.

In Table B9.4-10 below, the observed endpoints for the active substance and the product are compared to each other. The difference observed for fish with respect to active substance and the product is 1.2 overall (i.e. considering the most sensitive species tested with each the a.s. and the formulation) and 1.4 based on data from the studies with the rainbow trout only. The difference between the active substance and the product based endpoint for *Daphnia magna* is 1.7 only.

Therefore it can be stated that the toxicity of the formulation and the active substance are comparable.

Table B9.4-10: Endpoints for fish acute and daphnia acute (Factors between a.s. and product)

Test substance	**Species (Scientific name)**	**Endpoint [mg a.s./L]**	**Factor between a.s. and product**
Isoflucypram a.s.	*Pimephales promelas*	0.081	1.2
Isoflucypram a.s.	*Oncorhynchus mykiss*	0.098	1.4
Isoflucypram EC 50	*Oncorhynchus mykiss*	0.068	-
Geometric mean:	***Oncorhynchus mykiss***	**0.082**	-
Isoflucypram a.s.	*Daphnia magna*	0.201	1.7
Isoflucypram EC 50	*Daphnia magna*	0.117	-
Geometric mean:	***Daphnia magna***	**0.153**	-

As such, it is possible to consider data from both the active substance and formulated product when calculating the geometric mean. In the case where there is a formulation study and active substance study with the same species, a geometric mean of the two endpoints has been conducted first, before inclusion into an overall geometric mean considering the other tested species. This is presented below in Table B9.4-11.

Fish acute: If more species are tested as required according to the data requirements in Commission Regulation (EU) No 283/2013 a Tier 2A approach may be considered. In case of isoflucypram the data requirements were exceeded for acute testing of fish. Data for the following fish species are available: *Oncorhynchus mykiss*, *Pimephales promelas* and *Cyprinodon variegatus*.

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

As two data points are available for *Oncorhynchus mykiss* (product and active substance), the geomean derived from the respective two endpoints is used.

The following LC_{50} values for the three tested species are available:

Table B9.4-11 Consideration of endpoints for acute fish geometric mean calculation

Species	**Species (Scientific name)**	**96h LC_{50} (mg a.s. /L)**
Rainbow trout	*Oncorhynchus mykiss*	0.068[1]
Rainbow trout	*Oncorhynchus mykiss*	0.098[2]
Geometric mean of *Oncorhynchus mykiss* studies		**0.082**
Fathead minnow	*Pimephales promelas*	**0.081[2]**
Sheepshead minnow	*Cyprinodon variegtaus*	**0.544[2]**
Geometric mean:		0.156

[1]Formulation endpoint expressed as active substance content
[2]Active substance endpoints
Bold values used in the calculation of a geometric mean

The difference between the derived geometric mean value and the lowest acute fish endpoint is a factor of 2.25, therefore the geometric mean of 0.156 mg a.s./L is considered to be suitably protective.

The above presented information demonstrates that the Tier 2A fish acute 96 h LC_{50} of 0.156 mg a.s./L and the resulting **Tier 2A RAC of 0.00156 mg/L** is protective and can therefore be used within the aquatic risk assessment of the product Isoflucypram EC50. Please note that this approach may be suitable only for this formulation and may not be usable as Tier 2 refinement options for other formulations in national/zonal registrations.

Aquatic invertebrate acute: If more species are tested than required according to the data requirements in Commission Regulation (EU) 283/2013 then it may be appropriate to use a Tier 2a approach. Data for two different crustacean species, the waterflea (*Daphnia magna*) and the mysid shrimp (*Americamysis bahia*) are provided. According to the toxicity data, aquatic invertebrates are not the most sensitive organism tested; furthermore the derived NOEC values are not < 0.01 mg a.s./L and as such do not trigger the toxicity criterion (according to classifying a substance as PBT). Therefore isoflucypram is not considered to have insecticidal activity and the data available at tier 1 exceed the data requirements and can thus be considered in a higher tier refinement. The available data are not sufficient to use Tier 2B, the Species Sensitivity Distribution but allow the use of Tier 2A, the geomean assessment. As two data points are available for *Daphnia magna* (product and active substance), the respective geomean derived from the two endpoints is used.

The following EC_{50} values for the two tested species are available in table B9.4-12:

Table B9.4-12: Endpoints for aquatic invertebrates

Species	**Species (Scientific name)**	**EC_{50} [mg a.s. /L]**
Water flea	*Daphnia magna*	0.117
Water flea	*Daphnia magna*	0.201
Geometric mean of *Daphnia magna* studies		**0.153**
Mysid shrimp	*Americamysis bahia*	**0.270**
Geometric mean:		0.203

[1]Formulation endpoint expressed as active substance content
[2]Active substance endpoints
Bold values used in the calculation of a geometric mean

The difference between the derived geometric mean value and the lowest acute aquatic invertebrate endpoint is a factor of 1.74, therefore the geometric mean of 0.203mg a.s./L is considered to be suitably protective of all species tested.

The above presented information demonstrates that the Tier 2A aquatic invertebrate EC_{50} of 0.203 mg a.s./L and the resulting **Tier 2A RAC of 0.00203 mg/L** is protective and can therefore be used within the aquatic risk assessment of the product Isoflucypram EC50.

Fish chronic: Regarding refinement of the chronic fish endpoint, it was not possible to consider the study conducted with *C.variegatus* in a tier 2a geomean refinement as the solvent used in this study was triethylene glycol (TEG), whereas in the study conducted with *P.promelas* dimethyl formamide (DMF) was used. It is stated

in the study report for the study conducted with *C.variegatus* that several tests conducted using DMF were discontinued due to toxicity in the solvent control; therefore in the definitive test TEG was used. As such, the RMS does not consider these studies to be sufficiently comparable for consideration in a geometric mean refinement and the tier 1 RAC of 13.28 µg a.s./L is used in the risk assessment.

The risk assessment considering the Tier 2A geomean RACs for the acute risk to fish and aquatic invertebrates is presented below for both Winter and Spring cereals, early and late application in the following tables:

Table B9.4-13: Tier 2 risk assessment for isoflucypram – use in winter cereals – Early Application (March – May)

Scenario	PECsw global max (µg L)	Fish acute	Fish chronic	Aquatic invertebrates
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*
		RAC (LC_{50})	RAC (NOEC)	RAC (EC_{50})
		1.56	1.328	2.03
FOCUS Step 1			PEC/RAC ratio	
	9.63	**6.17**	**7.25**	**4.74**
FOCUS Step 2			PEC/RAC ratio	
North Europe	1.73	**1.11**	**1.30**	0.85
South Europe	3.15	**2.02**	**2.37**	**1.55**
FOCUS Step 3			PEC/RAC ratio	
D1 ditch	1.387	0.89	**1.04**	0.68
D1 stream	0.869	0.56	0.65	0.43
D2 ditch	1.369	0.88	**1.03**	0.67
D2 stream	0.8551	0.55	0.64	0.42
D3 ditch	0.4745	0.30	0.36	0.23
D4 pond	0.1005	0.06	0.08	0.05
D4 stream	0.3652	0.23	0.28	0.18
D5 pond	0.1182	0.08	0.09	0.06
D5 stream	0.3798	0.24	0.29	0.19
D6 ditch	0.7443	0.48	0.56	0.37
R1 pond	0.0425	0.03	0.03	0.02
R1 stream	0.3124	0.20	0.24	0.15
R3 stream	0.4415	0.28	0.33	0.22
R4 stream	0.4364	0.28	0.33	0.21

Values in **bold** are > 1 and therefore high risk

Table B9.4-14: Tier 2 risk assessment for isoflucypram – use in winter cereals – Late application (June – September)

Scenario	PECsw global max (µg L)	Fish acute
		Geomean of 3 fish species
		RAC (LC_{50})
		1.56
FOCUS Step 1		PEC/RAC ratio
	9.63	**6.17**
FOCUS Step 2		PEC/RAC ratio
North Europe	1.73	**1.11**
South Europe	2.44	**1.56**
FOCUS Step 3		PEC/RAC ratio

Scenario	PECsw global max (µg L)	Fish acute
		Geomean of 3 fish species
		RAC (LC_{50})
		1.56
D1 ditch	0.6537	0.42
D1 stream	0.4216	0.27
D2 ditch	0.8686	0.56
D2 stream	0.5859	0.38
D3 ditch	0.4755	0.30
D4 pond	0.0529	0.03
D4 stream	0.4103	0.26
D5 pond	0.06	0.04
D5 stream	0.4426	0.28
D6 ditch	0.4768	0.31
R1 pond	0.0531	0.03
R1 stream	0.3133	0.20
R3 stream	0.4415	0.28
R4 stream	0.3921	0.25

Values in **bold** are > 1 and therefore high risk

Table B9.4-15: Tier 2 risk assessment for isoflucypram – use in spring cereals – Early application (March – May)

Scenario	PECsw global max (µg L)	Fish acute	Aquatic invertebrates
		Geomean of 3 fish species	*Daphnia magna*
		RAC (LC_{50})	RAC (EC_{50})
		1.56	2.03
FOCUS Step 1		PEC/RAC ratio	
	9.63	**6.17**	**4.74**
FOCUS Step 2		PEC/RAC ratio	
North Europe	1.73	**1.11**	0.85
South Europe	3.15	**2.02**	**1.55**
FOCUS Step 3		PEC/RAC ratio	
D1 ditch	1.181	0.76	0.49
D1 stream	0.7401	0.47	0.31
D3 ditch	0.4746	0.30	0.23
D4 pond	0.1224	0.08	0.04
D4 stream	0.388	0.25	0.20
D5 pond	0.1113	0.07	0.04
D5 stream	0.3992	0.26	0.20
R4 stream	0.4179	0.27	0.19

Values in **bold** are > 1 and therefore high risk

Table B9.4-16: Tier 2 risk assessment for isoflucypram – use in spring cereals – Late application (June – September)

Scenario	PECsw global max (µg L)	Fish acute Geomean of 3 fish species RAC (LC_{50}) 1.56
FOCUS Step 1		PEC/RAC ratio
	9.63	**6.17**
FOCUS Step 2		PEC/RAC ratio
North Europe	1.73	**1.11**
South Europe	2.44	**1.56**
FOCUS Step 3		PEC/RAC ratio
D1 ditch	0.6891	0.44
D1 stream	0.4226	0.27
D3 ditch	0.475	0.30
D4 pond	0.0699	0.04
D4 stream	0.409	0.26
D5 pond	0.0635	0.04
D5 stream	0.4142	0.27
R4 stream	0.4361	0.28

Values in **bold** are > 1 and therefore high risk

Overall conclusion for isoflucypram: For early application of isoflucypram in Winter cereals, there is an unresolved chronic risk to fish for the D1 and D2 ditch scenarios; for late application in Winter cereals the acute risk to fish is resolved at FOCUS Step 3 following consideration of a tier 2a geometric mean.
For early application of isoflucypram in Spring cereals, the acute risk to fish and aquatic invertebrates is resolved at FOCUS Step 3 following consideration of a tier 2a geometric mean. The acute risk to fish from late application of isoflucypram in Spring cereals is also resolved following consideration of a tier 2a geomean.
As the main entry route for the D1 and D2 scenarios is driven by drainage, it is not possible to mitigate the risk under current EU models. **As such, member states may wish to further consider the risk to these aquatic organism groups at national registration.**

Metabolites of isoflucypram

According to Section III CA B.8.2.1 only one major metabolite of isoflucypram has been identified as occurring in the water and sediment phase; BCS-CN88460-carboxylic acid (M12). Discussion of the endpoints for the metabolite risk assessment is presented below.

Selection of endpoints for Tier 1 risk assessment for BCS-CN88460-carboxylic acid (M12)

The risk from the metabolite BCS-CN88460-carboxylic acid (M12) is considered below following the EFSA AGD (2013) stepwise approach:

- A complete acute experimental data set is available for the metabolite BCS-CN88460-carboxylic acid (M12).
- Based on the acute data (> 10 times less toxic on a molar basis than the parent) it can be concluded that BCS-CN88460-carboxylic acid (M12) has lost its toxophore.
- Due to its limited formation in aquatic systems, no reliable degradation half-lives for BCS-CN88460-carboxylic acid can be derived. As a conservative approach it is assumed that the trigger for chronic risk assessment (DT_{90} > 1d) is met for BCS-CN88460-carboxylic acid (M12)

According to the AGD stepwise approach, the parent chronic endpoints can be used in the metabolite risk assessment as surrogate values for all Tier 1 taxonomic groups. Thus the chronic risk assessment for the metabolite BCS-CN88460-carboxylic acid (M12) is based on parent endpoints.

The following endpoints were selected for use in the tier 1 risk assessment:

Acute toxicity to fish: One study testing the metabolite BCS-CN88460-carboxylic acid (M12) with *Oncorhynchus mykiss* was submitted. The study was deemed valid for regulatory use and the endpoint for use in the risk assessment confirmed as **33.5 mg p.m./L.**

Long-term toxicity to fish: Parental toxicity of the active substance was assumed as detailed above. The endpoint confirmed for use in the metabolite risk assessment is **NOEC = 0.01328 mg a.s./L**.

Acute toxicity to aquatic invertebrates: One study testing the metabolite BCS-CN88460-carboxylic acid (M12) with *Daphnia magna* was submitted. The study was deemed valid for regulatory use and the endpoint for use in the risk assessment confirmed as **24 mg p.m./L.**

Long-term toxicity to aquatic invertebrates: Parental toxicity of the active substance was assumed as detailed above. The endpoint confirmed for use in the metabolite risk assessment is **NOEC = 0.020 mg a.s./L**.

Toxicity to algae: One study testing the metabolite BCS-CN88460-carboxylic acid (M12) with *Pseudokirchneriella subcapitata* was submitted. The study was deemed valid for regulatory use and the endpoint for use in the risk assessment confirmed as **>35.1mg p.m./L.**

Toxicity to aquatic macrophytes: Parental toxicity of the active substance was assumed as detailed above. The endpoint confirmed for use in the metabolite risk assessment is **E_rC_{50}: > 2.48 mg a.s./L.**

Toxicity to sediment dwelling organisms: Parental toxicity of the active substance was assumed as detailed above. The endpoint confirmed for use in the metabolite risk assessment is: **NOEC: 100 mg a.s./kg sediment.**

The regulatory acceptable concentrations confirmed for use in the metabolite assessment are detailed in table 9.4-17 below:

Table B9.4-17: Regulatory acceptable concentrations (RAC) for BCS-CN88460-carboxylic acid (M12) for each organism group

Species	Fish		Aquatic invertebrates		Sediment dwellers	Algae	Aquatic macrophytes
	O.mykiss	*P.promelas*	*D.magna*	*A.bahia*	*C.riparius*[1]	*P.subcapitata*	*L.gibba*
Endpoint (µg/L or µg/kg sediment)	LC50	NOEC	EC50	NOEC	NOEC	ErC50	ErC50
	33500	13.28	24000	20	100000	35100	2480
AF	100	10	100	10	10	10	10
RAC (µg/L or µg/kg sediment)	335	1.328[1]	240	2.0[1]	10000[1, 2]	3510	248.0[1]

[1] Based on parental toxicity
[2] Spiked sediment study; value is expressed as µg/kg sediment

Predicted environmental concentrations used in the risk assessment

Predicted environmental concentrations of BCS-CN88460-carboxylic acid (M12) in surface water and sediment were calculated according to FOCUS Steps 1-3 for the use in cereals (see section B8 and table B9.4-18 and B9.4-19 below). Predicted Exposure Concentrations (PECs) used for the risk assessment have been established by the Environmental Fate Evaluator.

Accumulation in the sediment is predicted for the metabolite BCS-CN88460-carboxylic acid (M12) therefore further consideration of the risk to sediment dwelling organisms is required.

The worst-case values for Winter and Spring cereals have been used in each case (early application) as this covers the risk from late application.

Table B9.4-18: Initial max PEC_{sw} values for BCS-CN88460-carboxylic acid (M12) – FOCUS Steps 1 and 2

Compound	**FOCUS Scenario**	**Cereals* 1 × 75 g a.s./ha, BBCH 30-69**	
		PECsw, max [µg/L]	**PECsed, max [µg/kg]**
BCS-CN88460-carboxylic acid (M12)	STEP 1	4.20	1.56
	STEP 2 – North	0.70	0.26
	STEP 2 - South	1.35	0.50

*Worst-case values considered (early application to Winter and Spring cereals, average crop cover). PEC values for Winter and Spring cereals are the same at FOCUS Steps 1 and 2.

Table B9.4-19: Initial max PECsw values for BCS-CN88460-carboxylic acid (M12) – FOCUS Step 3

Compound	**FOCUS Scenario**	**1 × 75 g a.s./ha, BBCH 30-69**	
		Winter cereals*	**Spring cereals***
BCS-N88460-carboxylic acid (M12)	D1 ditch	0.4044	0.3904
	D1 stream	0.2584	0.2486
	D2 ditch	0.487	-
	D2 stream	0.3164	-
	D3 ditch	0.1650	0.2270
	D4 pond	0.4367	0.4145
	D4 stream	0.2194	0.1973
	D5 pond	0.3007	0.2859
	D5 stream	0.1334	0.1329
	D6 ditch	0.1417	-
	R1 pond	0.0187	-
	R1 stream	0.0081	-
	R3 stream	0.03184	-
	R4 stream	0.0159	0.0072

*Worst-case values considered (early application to Winter and Spring cereals, average crop cover)

Tier 1 risk assessment for BCS-CN88460-carboxylic acid (M12)

The tier 1 isk assessment for BCS-CN88460-carboxylic acid (M12) is presented in the following tables for early application to Winter cereals (Table 9.4-20) and early application to Spring cereals (Table 9.4-21).

Table B9.4-20: Tier 1 risk assessment for BCS-CN88460-carboxylic acid (M12) – use in Winter cereals – Early application

Scenario	PECsw global max (µg L)	Fish acute	Fish chronic	Aquatic invertebrates	Aquatic invertebrates chronic	Algae	Higher plant	PECsed global max (µg/kg)	Sed. dweller prolonged
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*	*Americamysis bahia*	*Pseudokirchneriella subcapitata*	*Lemna gibba*		*Chironomus riparius*
		RAC (LC_{50})	RAC (NOEC)	RAC (EC_{50})	RAC (NOEC)	RAC (E_rC_{50})	RAC (ErC_{50})		RAC (NOEC)
		335	1.328	240	2.0	3510	248.0		10000 µg a.s./kg sediment)
FOCUS Step 1					PEC/RAC ratio			FOCUS Step 1	PEC/RAC ratio
	4.2	0.01	**3.16**	0.02	**2.10**	<0.01	0.02	1.56	<0.01
FOCUS Step 2					PEC/RAC ratio			FOCUS Step 2	PEC/RAC ratio
North Europe	0.70	<0.01	0.53	<0.01	0.35	-	-	North Europe	0.26	<0.01
South Europe	1.35	<0.01	**1.02**	0.01	0.68	-	-	South Europe	0.50	<0.01
FOCUS Step 3					PEC/RAC ratio				PEC/RAC ratio

Scenario	PECsw global max (µg L)	Fish acute	Fish chronic	Aquatic invertebrates	Aquatic invertebrates chronic	Algae	Higher plant		PECsed global max (µg/kg)	Sed. dweller prolonged
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*	*Americamysis bahia*	*Pseudokirchneriella subcapitata*	*Lemna gibba*			*Chironomus riparius*
		RAC (LC_{50})	RAC (NOEC)	RAC (EC_{50})	RAC (NOEC)	RAC (E_rC_{50})	RAC (ErC_{50})			RAC (NOEC)
		335	1.328	240	2.0	3510	248.0			10000 µg a.s./kg sediment)
D1 ditch	0.4044	-	0.30	-	-	-	-	-	-	-
D1 stream	0.2584	-	0.19	-	-	-	-	-	-	-
D2 ditch	0.487	-	0.37	-	-	-	-	-	-	-
D2 stream	0.3164	-	0.24	-	-	-	-	-	-	-
D3 ditch	0.1650	-	0.12	-	-	-	-	-	-	-
D4 pond	0.4367	-	0.33	-	-	-	-	-	-	-
D4 stream	0.2194	-	0.17	-	-	-	-	-	-	-
D5 pond	0.3007	-	0.23	-	-	-	-	-	-	-
D5 stream	0.1334	-	0.10	-	-	-	-	-	-	-
D6 ditch	0.1417		0.11	-	-	-	-	-	-	-
R1 pond	0.0187		0.01	-	-	-	-	-	-	-
R1 stream	0.0081		0.01	-	-	-	-	-	-	-
R3 stream	0.03184		0.02	-	-	-	-	-	-	-
R4 stream	0.0159		0.01	-	-	-	-	-	-	-

Values in **bold** are > 1 and therefore high risk

Table B9.4-21: Tier 1 risk assessment for BCS-CN88460-carboxylic acid (M12) – use in spring cereals – Early application

Scenario	PECsw global max (µg L)	Fish acute	Fish chronic	Aquatic invertebrates	Aquatic invertebrates chronic	Algae	Higher plant		PECsed global max (µg/kg)	Sed. dweller prolonged
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*	*Americamysis bahia*	*Pseudokirchneriella subcapitata*	*Lemna gibba*			*Chironomus riparius*
		RAC (LC_{50})	RAC (NOEC)	RAC (EC_{50})	RAC (NOEC)	RAC (E_rC_{50})	RAC (ErC_{50})			RAC (NOEC)
		335	1.328	240	2.0	3510	248.0			10000 µg a.s./kg sediment)
FOCUS Step 1					PEC/RAC ratio			FOCUS Step 1		PEC/RAC ratio
	4.2	0.01	**3.16**	0.02	**2.10**	<0.01	0.02		1.56	<0.01
FOCUS Step 2					PEC/RAC ratio			FOCUS Step 2		PEC/RAC ratio
North Europe	0.70	<0.01	0.53	<0.01	0.35	-	-	North Europe	0.26	<0.01
South Europe	1.35	<0.01	**1.02**	0.01	0.68	-	-	South Europe	0.50	<0.01
FOCUS Step 3					PEC/RAC ratio					PEC/RAC ratio
D1 ditch	0.3904	-	0.29	-	-	-	-	-	-	-
D1 stream	0.2486	-	0.19	-	-	-	-	-	-	-
D3 ditch	0.2270	-	0.17	-	-	-	-	-	-	-
D4 pond	0.4145	-	0.31	-	-	-	-	-	-	-
D4 stream	0.1973	-	0.15	-	-	-	-	-	-	-
D5 pond	0.2859	-	0.22	-	-	-	-	-	-	-
D5 stream	0.1329	-	0.10	-	-	-	-	-	-	-
R4 stream	0.0072	-	0.01	-	-	-	-	-	-	-

Values in **bold** are > 1 and therefore high risk

Overall conclusion for BCS-CN88460-carboxylic acid (M12): An acceptable risk to all aquatic organisms from the metabolite BCS-CN88460-carboxylic acid (M12) was demonstrated at FOCUS Step 3 following application of Isoflucypram in Winter and Spring cereals. No further consideration is required.

B.9.5. EFFECTS ON ARTHROPODS

B.9.5.1. Effects on bees

Previous evaluation:	New data, submitted for purpose of review

Report:	KCP 10.3.1.1.1/01; Ehmke, A.; 2016
Title:	BCS-CN88460 EC 50 (50.0 g/L): Effects (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory - Final report -
Report No.:	113451035
Guideline(s):	OECD 213 and 214 (1998); US EPA OCSPP 850.3020, 850.supp.
GLP/GEP:	Yes

Material and methods:

Test item:	BCS-CN88460 EC 50 (50 g/L): 50.46 g/L, 5.18% w/w; supplier batch no.: 2016-001002, Specification No.: 102000031262, Sample Description: TOX20246-00.
Reference item :	BAS 152 11 I (active ingredient 420.3 g/L dimethoate, Batch no.: FRE-001226)
Test organism:	Female worker honeybees (*Apis mellifera*), obtained from a healthy and queen-right colony, bred by IBACON. Collected with glass or plastic tubes, from the outer honeycombs (away from the brood) without the use of smoke and without anaesthetics, collected on the morning of use.
Test duration:	*Oral test:* 72 hours *Contact test:* 96 hours Due to increasing mortality between 24/48 and 48/72 hours the contact test was prolonged for further 48 hours up to 96 hours. Due to increasing mortality between 24 and 48 hours the oral test was prolonged for further 24 hours up to 72 hours.
Test concentrations :	*Oral test:* Control: 50 % w/v sucrose solution. Treatment groups: 200.0, 90.9, 41.3, 18.8 and 8.5µg a.s./bee (nominal); 90.7, 81.1, 39.8, 19.9 and 9.1 a.s./bee (measured). Reference item: 0.30, 0.15, 0.08 and 0.05 µg dimethoate per bee (nominal); 0.33, 0.17, 0.08 and 0.06 µg dimethoate per bee (measured). *Contact test:* Control: tap water with 0.5 % Adhäsit. Treatment groups: 80.0, 36.4, 16.5, 7.5, 3.4 and 1.6 µg a.s./bee. Reference item: 0.30, 0.20, 0.15 and 0.10 µg dimethoate per bee.
Application of the test item:	*Oral test:* The test item and reference item were applied in 50 % w/v sucrose solution, which was used as carrier (food) in the oral test. For the control pure 50 % w/v sucrose solution was offered to the bees. The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake ranged from 1 hour 35 minutes to 6 hours for the test item treatments). After a maximum of 6 hours, the syringes containing the treated food were removed, weighed and replaced by ones containing fresh,

	untreated food. Bees were starved for 20 minutes prior to application of the test item in all treatment groups. *Contact test:* The test item was applied as one 5 µL droplet of BCS-CN88460 EC 50 (50 g/L) dissolved in tap water with 0.5% Adhasit, placed on the dorsal bee thorax using a calibrated pipette. The reference item was applied as one 5 µL droplet of dimethoate dissolved in tap water containing 0.5 % Adhäsit. For the controls, one 5 µL droplet of tap water containing 0.5 % Adhäsit was used.. Bees were anaesthetised with CO_2 for 20 seconds prior to application.
No. of individuals :	10
No. of replicates	3 per treatment group, control and reference item
Test units :	Stainless steel cages with 8 cm × 6 cm × 4 cm (length × height × width) with removal glass sheets, ventilation holes and lined with filter paper.
Test conditions :	Temperature: 23.8 – 25.4 °C Relative humidity 59.2 – 64.1% Light: Darkness (except during observation) Ventilation: Yes
Feeding:	50 % w/v sucrose solution (500 g/L tap water) (provided as "household sugar") ad libitum; was given directly after treatment. This was done with syringes that were inserted into the cages via an opening in the top of the test units and from which bees accessed the food directly. Fresh 50 % w/v sucrose solution was supplied after 48 hours.
Observations:	Mortality and behavioural abnormalities were recorded after 4, 24 and 48 and 72 hours (oral and contact test) and 96 hours (contact test).
Statistical analysis:	Results obtained with the bees treated with the test item and the reference item were compared to those obtained with the control in both the contact and oral tests. The contact and oral $LD_{50}{}_{+20}{}_{+10}$ values of the test item were estimated with Probit Analysis. The contact and oral LD50 values of the reference item were estimated with Probit Analysis. The LD_{50} calculation was carried out taking into account the mortality data corrected by control mortality using Abbott's formula. The NOED was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. The software used to perform the statistical analysis was ToxRat® Professional, Version 3.2.1, ToxRat® Solutions GmbH.

Results:

Validity criteria:

Validity criteria according to OECD 213 and 214	Obtained in this study
Control mortality should not exceed 10% at test end	Contact test Control: 6.7% Oral test Control: 3.3%
LD_{50} of the reference item should be in the specified range (contact test: 0.10 – 0.30 µg a.s./bee, oral test: 0.10 – 0.35 µg a.s./bee)	Contact test 0.22 µg a.s./bee Oral test 0.15 µg a.s./bee

All validity criteria of the test were met.

Biological findings:

Oral Test:
The oral toxicity test was prolonged for further 24 hours up to 72 hours due to increasing mortality between 24/48 hours. The maximum nominal dose levels of the test item (200.0, 90.9 and 41.3 µg a.s./bee) could not be achieved, because the bees did not ingest the full volume of treated sugar solution even when offered over a period of six hours. Actual oral doses of 90.7, 81.1, 39.8, 19.9 and 9.1 µg a.s./bee resulted in dose dependent corrected mortality of 100.0, 37.9, 10.3, 0.0 and 0.0 % at the end of the test (72 hours after application). Also 3.3 % mortality occurred in the control group (sucrose 50 % w/v solution = 500 g sucrose/L tap water).

Table B9.5.1-1: Mortality and behavioural abnormalities of the bees in the oral toxicity test

Dosage	**After 4 hours**		**After 24 hours**		**After 48 hours**		**After 72 hours**	
	Mortality	**Behav. abnorm.**	**Mortality**	**Behav. abnorm.**	**Mortality**	**Behav. abnorm.**	**Mortality**	**Behav. abnorm.**
	Mean %	**Mean %**	**Mean %**	**Mean %**	**Mean %**	**Mean %***	**Mean %**	**Mean %***
Test item								
µg a.s./bee								
90.7	0	70	83.3**	16.7	100**	0	100**	0
81.1	0	26.7	33.3**	6.7	37.9**	0	37.9**	0
39.8	0	20	13.3	0	10.3	0	10.3	0
19.9	0	0	3.3	0	0	0	0	0
9.1	0	0	0	0	0	0	0	0
Water	0	0	0	0	0	0	0	0
Reference item								
µg a.s./bee								
0.33	0	86.7	96.7	0	100	0	100	0
0.17	0	46.7	66.7	0	72.4	0	75.8	0
0.08	0	16.7	3.3	0	6.9	0	10.3	0
0.06	0	6.7	0	0	0	0	0	0

Results are average from three replicates (ten bees each) per dosage/control
Behav. abnorm. = Behavioural abnormalities
**Statistically significantly different from the control (Fisher's Exact test)

Contact Test:

The contact toxicity test was prolonged for further 48 hours up to 96 hours due to increasing mortality between 24/48 and 48/72 hours. Dose levels of 80.0, 36.4, 16.5, 7.5, 3.4 and 1.6 µg a.s./bee led to a corrected mortality of 100.0, 78.6, 53.6, 25.0, 3.6 and 7.1 % at test termination (96 hours), respectively. 6.7 % mortality occurred in the control group (water + 0.5 % Adhasit).

Table B9.5.1-2: Mortality and behavioural abnormalities of the bees in the contact toxicity test

Dosage	**After 4 hours**		**After 24 hours**		**After 48 hours**		**After 72 hours**		**After 96 hours**	
	Mort-ality	**Behav. abnorm.**	**Mort-ality**	**Behav. abnorm.**	**Mort-ality**	**Behav. abnorm.**	**Mort-ality**	**Behav. abnorm.**	**Mort-ality**	**Behav. abnorm.**
	Mean %	**Mean %**	**Mean %**	**Mean %**	**Mean %**	**Mean %***	**Mean %**	**Mean %***	**Mean %**	**Mean %**
Test item										
µg a.s./bee										
80	16.7	83.3	96.7**	3.3	100**	0	100**	0	100**	0
36.4	0	100	20.0**	80.0	23.3**	76.7	48.3**	50.0	78.6**	3.3
16.5	0	80.0	26.7**	30.0	46.7**	6.7	51.7**	3.3	53.6**	0
7.5	0	40.0	3.3	10.0	20.0**	3.3	27.6**	3.3	25.0**	0
3.4	0	3.3	3.3	3.3	10.0	0	6.9	0	3.6	0
1.6	0	0	0	0	3.3	0	10.3	0	7.1	0
Water	0	0	0	0	0	0	0	0	0	0
Reference item										
µg a.s./bee										
0.30	20.0	20.0	73.3	0	76.7	0	76.7	0	76.7	0
0.20	3.3	0	50.0	6.7	56.7	0	63.3	3.3	66.7	0
0.15	0	0	16.7	3.3	26.7	0	30.0	0	30.0	0
0.10	0	0	3.3	0	10.0	0	16.7	0	16.7	0

Results are averages from three replicates (ten bees each) per dosage/control
Behav. abnorm. = Behavioural abnormalities, Water = CO_2/water-treated control
**Statistically significantly different from the control (Fisher's Exact test)

Conclusion

The following endpoints were derived from this study:

Test Item	**BCS-CN88460 EC 50 (50.0 g/L)**	
Test object	***Apis mellifera L.***	
Exposure	**Contact (solution in Adhasit (0.5 %)/water)**	**Oral (50 % w/v sucrose solution)**
Dose rate [µg a.s./bee]	80.0, 36.4, 16.5, 7.5, 3.4 and 1.6	90.7, 81.1, 39.8, 19.9 and 9.1
LD_{50} µg a.s./bee (95% confidence intervals)	24 hours: 36.8 (11.3 – 6449.2) 48 hours: 25.7 (4.6 – n.d.) 72 hours: 17.6 (6.5 – 73.9) 96 hours: 14.1 (11.0 – 18.2)	24 hours: 74.8 (42.6 – 131.3) 48 hours: 69.1 (42.3 – 112.9) 72 hours: 69.1 (42.3 – 112.9)
LD_{20} µg a.s./bee (95% confidence intervals)	24 hours: 16.7 (n.d. – 38.9) 48 hours: 7.7 (n.d. – 25.4) 72 hours: 5.3 (0.3 – 12.3) 96 hours: 5.9 (4.0 – 7.7)	24 hours: 44.6 (22.0 – 90.5) 48 hours: 50.4 (24.0 – 105.5) 72 hours: 50.4 (24.0 – 105.5)
LD_{10} µg a.s./bee (95% confidence intervals)	24 hours: 11.0 (n.d. – 24.7) 48 hours: 4.1 (n.d. - 13.1) 72 hours: 2.9 (0.0 – 7.4) 96 hours: 3.7 (2.3 – 5.2)	24 hours: 34.1 (13.2 – 88.1) 48 hours: 42.7 (16.6 – 109.6) 72 hours: 42.7 (16.6 – 109.6)

NOED µg a.s./bee*	24 hours: 7.5 48 hours: 3.4 72 hours: 3.4 96 hours: 3.4	24 hours: 39.8 48 hours: 39.8 72 hours: 39.8

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

The toxicity of BCS-CN88460 EC 50 (50.0 g/L) was tested in both, an acute contact and an acute oral toxicity test on honeybees. The contact LD_{50} values (24, 48, 72 and 96 h) were determined to be 36.8, 25.7, 17.6 and 14.1 µg a.s./bee, respectively. The oral LD_{50} values (24, 48 and 72 h) were determined to be 74.8, 69.1 and 69.1 µg a.s./bee, respectively.

The NOED (96 h) was 3.4 µg a.s./bee in the contact toxicity test and the NOED (72 h) was 39.8 µg a.s./bee in the oral toxicity test.

RMS comments

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

The following was noted by the RMS:

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

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

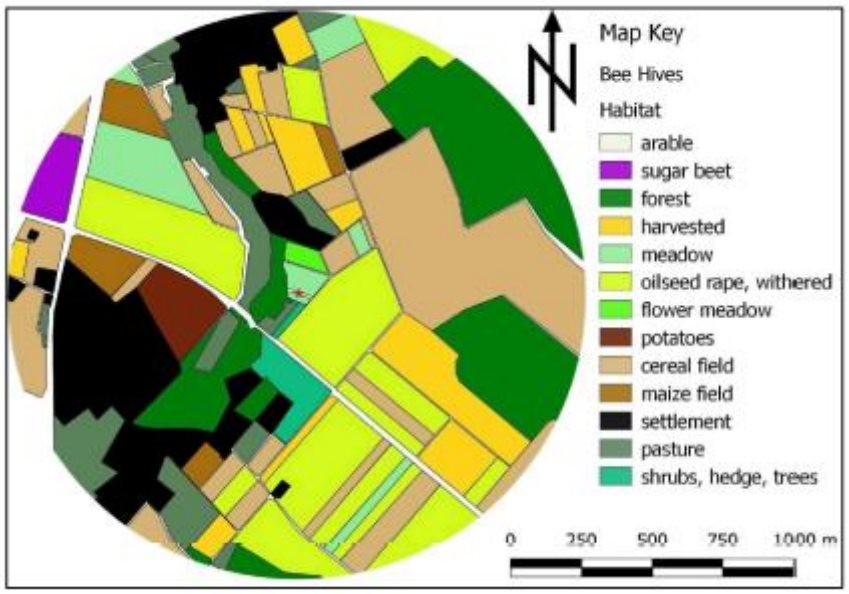
The endpoints are confirmed as:

Exposure	**Contact (96 hours) (solution in Adhasit (0.5 %)/water)**	**Oral (72 hours) (50 % w/v sucrose solution)**
LD_{50} µg a.s./bee (95% confidence intervals)	**14.1(11.0 – 18.2)**	**69.1 (42.3 – 112.9)**
LD_{20} µg a.s./bee (95% confidence intervals)	**5.9 (4.0 – 7.7)**	**50.4 (24.0 – 105.5)**
LD_{10} µg a.s./bee (95% confidence intervals)	**3.7 (2.3 – 5.2)**	**42.7 (16.6 – 109.6)**
NOED µg a.s./bee	**3.4**	**39.8**

Previous evaluation:	New data, submitted for purpose of review

Report:	KCP 10.3.1.5/01; Peters, B.; Rohland, A.; 2016;
Title:	Study on the effect of BCS-CN88460 EC 50 G (50 g/L) on honey bees (*Apis mellifera* L.) under semi-field conditions
Report No.:	P15019
Guideline(s):	OEPP/EPPO (2010): Guideline for the efficacy evaluation of plant protection products - Side effects on honeybees. OEPP/EPPO, PP 1/170(4) update 2010, 313 - 319 OECD No. 75, Guidance Document on the Honey Bee (*Apis mellifera* L.) Brood Test under Semi-Field Conditions, No. 75, ENV/JM/MONO(2007)22
GLP/GEP:	Yes

Material and methods:

Test item:	BCS-CN88460 EC 50 G (50 g/L): BCS-CN88460: 5.12 % w/w (analytical); supplier batch No: 2015-000526; Sample Description: FAR01848-00; Specification No.: 102000029179; density: 0.969 g/mL.
Reference item :	Insegar 25 WG, 250 g/kg fenoxycarb ; batch no. SM02K433
Test organism:	Test species were honeybees (*Apis mellifera L.*; Hymenoptera, Insecta) provided by the Ruhr-University Bochum (Dr. Pia Aumeier, AG Verhaltensbiologie und Didaktik der Biologie, Universitätsstr. 150, Gebäude NCDF 06/495, 44780 Bochum; small honeybee colonies containing 6000 bees each and 3 to 5 brood combs with brood in all stages that did not display clinical symptoms of disease and were in a queenright state. Colonies with sister-queens with the age of one year were chosen.
Study location:	*Exposure phase:* The exposure phase was conducted in Luessem in the district of Zuelpich, North Rhine Westphalia, Germany. One field that served for agricultural production was used for this study (GPS coordinates at the centre of the field: 32 U 336855 [E] 5618666 [N]). *Monitoring site:* After the exposure period hives were removed from tunnels and placed in Sievernich in the district of Vettweiß (approximately 4 km from the exposure field) on an untreated meadow, with no attractive main crops flowering in the surroundings (see figure B9.5.1-1 below) GPS coordinates at the centre of the field: 32 U 335894 [E] 5622377 [N]. Management of the field was performed according to good agricultural practice. The *Phacelia* was sown on 18 April 2015. No pesticides were used on the field prior to sowing or during the growth period of the *Phacelia* before application of the test item.  Figure B9.5.1-1: Post exposure habitat of bee hives (hives marked with a red star)
Test type:	Semi-field tunnel test
Test duration:	34 days (6 days pre-exposure, 7 days exposure, 21 days post-exposure).
Test dates:	11.06.2015 – 15.07.2015
Test concentrations :	*Control:* 400 L water/ha *Treatment:* 75 g a.s. in 400 L water/ha (equivalent to 1.512 mL product/ha) *Reference:* 300 g a.s. in 400 L water/ha (corresponding to nominal 1200 g Insegar/ha)

Application of the test item:	Applications of the test item BCS-CN88460 EC 50 G (50 g/L), control and reference item (Insegar 25 WG, 250 g/kg fenoxycarb) were conducted by spraying the whole area of plants within the tunnel during full bee flight and at full flowering of the crop (BBCH 65) with a calibrated hand-held portable boom sprayer (deviation of max 2.65% of nominal). The test item, control and reference item was applied on 17.06.2015 between 10:50 and 13:23 (control, then test item, then reference item). The crop height was approximately 60 cm and plants were dry. Shortly before application, on average >10 honey bees per m^2/min were actively foraging on representative areas. During exposure mean wind speed was 0.635 and 1.32 m/s inside and outside of the tunnel respectively. Wind speed during application was 0.0 – 1.2 m/s.
Test crop:	*Phacelia tanacetifolia* with an effective crop area of ca. 85 m² (2 × 42.5 m^2) at BBCH stage 60
No. of individuals :	One honeybee colony per replicate containing 6000 bees each.
No. of replicates	4 per treatment group, control and reference item
Test units :	Tunnels 21 m long, 5.5 m wide and 2.5 m high constructed from a tubular steel frame covered with synthetic gauze (2mm) and 2 m from each other. Tunnels were set up between 04 June and 07 June a few days before the start of the test. The crop area inside each tunnel was split down the middle by a path (approximately 50 cm wide), which served as a walkway that was necessary for performing the application. Additionally, at the front and back ends of each tunnel, plants were cleared from an area of 11 m^2 (2 m x 5.5 m) to facilitate the placement of bee hives and also to enable work procedures. The outermost 50 cm of the front and back ends as well as the path were covered with non-woven sheets for collection of dead bees during mortality assessments.
Weather conditions:	Temperature and humidity were recorded with a data logger; however devices wer set up incorrectly and therefore the data recorded were inaccurate. Thus, additional temperature and humidity data was obtained from the nearest weather station in Weilerswist-Lommersum (non- GLP). The distance from the weather station to the exposure location is approximately 7 km and to the post-exposure location approximately 8 km. Precipitation was recorded hourly inside and outside the tunnels using a rain gauge. Wind strength was recorded inside and outside the tunnels using an anemometer. Cloud cover was estimated for mortality and flight activity assessments over the entire study period.
Feeding:	On DAT 7 during Brood area Fixing Day (BFD) 9, each colony was fed with one natural nectar comb that contained 16 dm^2 of nectar (corresponding to 6,400 cells). In each colony either Comb No. 10 or Comb No. 1 was replaced by the food comb. A water supply for the bees was placed at the edge of each tunnel.
Test procedure:	The bee colonies were placed into the tunnels with *Phacelia* (BBCH 60) six days before application. On the evening of 24 June 2015, 7 days following the application (DAT 7), all hives including dead bee traps were removed from the study field and taken to the post-exposure location. At the post-exposure location, hives and corresponding dead-bee traps were placed randomly regardless of whether hives of the same treatment group were adjacent to each other. Tunnels were labelled with consecutive numbers and, after the first condition check on DAT - 1 , tunnels were additionally allocated to treatment groups to assure a similar distribution of weaker and stronger colonies between different treatment groups prior to application. The field phase was subdivided in two consecutive main phases: an exposure phase and a post-exposure phase. The period from the instalment of the colonies until spray application is referred to hereafter as the pre-exposure phase. During the exposure and pre-exposure

	period mortality was assessed using dead-bee traps (from DAT -5 onwards) and non-woven sheets (from DAT -2 onwards). Flight activity during these periods was measured at different times during the day, depending on the weather conditions (from DAT -2 onwards). After removal of the hives from the study field on DAT 7, mortality was investigated at the post-exposure location for another 3 weeks, but only using the dead-bee traps. Brood development and colony condition was observed over the whole experimental period at fixed time intervals. Although bee colonies were placed in the tunnels on DAT -6, regular flight activity and mortality assessments on non-woven sheets could not be performed on DAT -4 and DAT -3, due to a severe thunderstorm on DAT -5 during which 37 mm of precipitation fell within one hour inside the tunnel tents and 43 mm outside the tunnels, resulting in very muddy soil which could not be walked upon.
Observations:	• *Mortality assessment:* Dead worker bees, larvae, pupae and drones were assessed in dead-bee traps and on non-woven sheets on all test days in the morning with the exception of DAT -4 and -5 where they were performed later. • *Flight activity:* Numbers of bees that were both foraging on flowering plants and flying around the crop were recorded for 60 seconds within each tunnel at three different locations which each comprised an area of 1 m^2 on DAT -5, -3, -2, -1, 0, 1, 2, 3, 4 , 5 , 6 and 7. • *Behavioural abnormalities*: recorded DAT -5, -3, -2, -1, 0, 1, 2, 3, 4 , 5 , 6 and 7. • *Colony conditions* :(estimation of bees, brood, pollen and nectar) assessments were conducted on DAT -2, 3, 7, 14, 20 and 26 = BFD0, BFD5, BFD9, BF16 and BFD22 respectively. Strength of colony, presence and vitality of queen, comb area with pollen and nectar storage and comb area containing brood in different stages were all recorded. • *The development of bee brood:* (ontogenesis of eggs) was evaluated for appropriate amount of eggs (> 200) from each colony on DAT -2, 3, 7, 14, and 20 and 26 = BFD0, BFD5, BFD9, BF16 and BFD22 respectively. Brood development was assessed five times using photo-method in parallel to the colony condition check. Photos were taken with a digital camera that was installed in a photo box. For analysis, the different brood stage categories were transcribed into indices (e.g. 0 = empty; 1 = egg; 2 = young larvae; 3 = old larvae; 4 = pupae; 5 = nectar; 6 = pollen) to calculate the brood termination rate (BTR), the brood index and the compensation index for each assessment day and colony. *Brood termination rate:* To obtain the brood termination rate in percent, the number of cells for which a premature termination of the bee brood development was recorded, were summed for each colony, multiplied by 100 and divided by the total number of cells observed. *Brood index:* If a cell did not contain the expected brood stage or food was stored in the cell, the cell was counted with zero on the respective assessment day and also on the following assessment days, even if the cell was later again occupied by brood. The values of all individual cells in each treatment group which were assessed at a given BFDn were summed and divided by the number of observed cells in order to obtain the average brood index for each treatment group at each BFDn. *Compensation index:* Values of all individual cells in each treatment group which were assessed at a given BFDn were summed and divided by the number of observed cells in order to obtain the average compensation index for each treatment group at each BFDn.
Statistical analysis:	GLP-validated linear mixed effect models (lme) were used to interpret and evaluate potential treatment-related effects of the test item on the development of honeybee colonies. ANOVA

	was used to compare fitted models and thus detect the ability of the factors and interactions to explain the encountered variance.

Results

Weather conditions

The below table (Table B9.5.1-2) shows the climatic conditions (temperature, precipitation and humidity) during the pre-exposure, exposure and post-exposure periods inside and outside of the test tunnels. On the day of application, there was no precipitation, however 1, 2, 4, 5 and 6DAT 4.6, 0.2, 1.2, 12.2 and 1.0 mm of rain was recorded inside the tunnel.

Table B9.5.1-2: Climatic conditions during the pre-exposure, exposure and post-exposure periods inside and outside of the tunnel/post-exposure

DAT	Temperature (°C)						Precipitation (mm/day)		Humidity (% RH)					
	Inside tunnel			Outside tunnel/ post exposure			Inside tunnel	Outside tunnel/ post exposure	Inside tunnel			Outside tunnel/ post exposure		
	Min	Max	Mean	Min	Max	Mean			Min	Max	Mean	Min	Max	Mean
Pre-exposure														
-6	14.4	31.7	22.0	13.8	35.8	24.0	0.0	0.0	43.4	54.1	53.6	37.6	51.5	49.8
-5	8.2	36.5	19.8	7.6	36.2	19.2	45.0	51.6	77.0	84.6	81.3	71.2	85.8	78.1
-4	14.1	31.8	20.9	13.9	34.4	21.3	1.4	1.6	79.2	87.0	82.6	71.2	82.2	76.7
-3	9.8	32.9	21.3	9.1	32.4	19.4	0.0	0.0	63.1	76.6	70.9	64.7	77.8	72.1
-2	9.9	25.2	16.8	9.6	27.0	16.4	0.0	0.0	79.8	88.2	83.5	75.6	85.6	81.1
-1	6.4	26.4	15.6	5.5	26.7	13.9	0.0	0.0	67.8	80.4	73.9	69.7	81.3	75.5
Exposure														
0	5.6	31.6	18.1	4.8	35.4	17.6	0.0	0.0	63.6	76.9	70.3	61.4	73.1	66.4
1	9.4	27.5	17.9	9.2	22.9	16.7	4.6	6.4	79.6	87.8	83.9	78.2	85.8	82.6
2	8.4	24.1	13.2	7.8	20.5	12.4	0.2	0.2	84.7	34.9	89.8	82.0	92.8	87.1
3	9.8	24.8	14.4	9.7	18.8	13.1	0.0	0.0	79.5	88.5	84.2	78.8	87.2	83.2
4	11.5	26.8	17.2	11.2	27.0	17.0	1.2	1.8	77.0	89.1	83.8	72.6	85.8	79.6
5	10.0	16.3	12.5	9.2	15.6	12.0	12.2	14.4	95.3	99.2	97.4	95.1	98.9	97.4
6	8.2	24.5	13.3	7.6	19.5	12.4	1.0	1.6	91.8	97.9	92.8	89.1	96.5	92.8
7	10.8	29.0	16.4	10.6	32.6	17.1	0.0	0.0	76.4	87.6	74.7	69.2	81.4	74.7
Post-exposure														
8	-	-	-	10.3	39.7	19.9	-	0.0	-	-	-	-	-	64.2
9	-	-	-	10.4	36.2	21.6	-	0.0	-	-	-	-	-	50.4
10	-	-	-	10.7	38.0	20.8	-	1.4	-	-	-	-	-	48.5
11	-	-	-	6.0	33.5	19.2	-	0.0	-	-	-	-	-	44.5
12	-	-	-	13.7	41.0	23.0	-	0.0	-	-	-	-	-	75.3
13	-	-	-	8.9	43.7	22.7	-	0.0	-	-	-	-	-	38.7
14	-	-	-	9.8	45.1	25.8	-	0.0	-	-	-	-	-	52.4
15	-	-	-	15.3	52.2	30.1	-	0.0	-	-	-	-	-	65.9
16	-	-	-	15.7	45.0	27.9	-	0.0	-	-	-	-	-	67.0
17	-	-	-	18.0	51.3	30.3	-	0.0	-	-	-	-	-	68.7
18	-	-	-	15.3	46.5	23.8	-	9.0	-	-	-	-	-	57.2
19	-	-	-	11.1	42.5	22.8	-	0.2	-	-	-	-	-	37.1
20	-	-	-	10.8	40.4	21.7	-	1.4	-	-	-	-	-	53.1
21	-	-	-	10.1	33.0	17.5	-	0.2	-	-	-	-	-	54.0

DAT	Temperature (°C)						Precipitation (mm/day)		Humidity (% RH)					
	Inside tunnel			Outside tunnel/ post exposure			Inside tunnel	Outside tunnel/ post exposure	Inside tunnel			Outside tunnel/ post exposure		
	Min	Max	Mean	Min	Max	Mean			Min	Max	Mean	Min	Max	Mean
22	-	-	-	7.7	34.0	17.2	-	1.2	-	-	-	-	-	80.3
23	-	-	-	2.9	39.9	17.7	-	0.0	-	-	-	-	-	51.8
24	-	-	-	8.1	40.8	21.8	-	0.0	-	-	-	-	-	63.7
25	-	-	-	9.1	28.0	17.3	-	1.2	-	-	-	-	-	80.6
26	-	-	-	11.4	23.0	17.2	-	1.0	-	-	-	-	-	84.1
27	-	-	-	16.4	32.4	21.6	-	0.0	-	-	-	-	-	82.4
28	-	-	-	15.7	34.2	23.1	-	0.0	-	-	-	-	-	73.8

The below table (Table B9.5.1-3) shows the amount of cloud cover during the mortality and flight activity assessments. During the exposure period 100% cloud cover was recorded most days during the mortality and flight activity assessments.

Table B9.5.1-3: Cloud cover during mortality and flight assessments.

DAT	Cloud cover (%)	
	During mortality assessment	During flight activity assessment
Pre-exposure		
-5	0	0
-4	10	No data collected
-3	0	0
-2	100	100
-1	10	10
Exposure		
0	0 – 5	0 – 55
1	100	40 – 95
2	100	100
3	100	100
4	95	100
5	100	100
6	95	30
7	100	100
Post-exposure		
8	20 (non-woven sheets)	
8	70 (dead-bee trap)	
9	5	
10	90	
11	0	
12	75	
13	0	
14	2	
15	0	
16	0	
17	30	
18	70	
19	0	
20	0	
21	10	
22	40	
23	0	
24	50	

DAT	Cloud cover (%)	
	During mortality assessment	During flight activity assessment
25	0	
26	100	
27	100	
28	80	

Mortality

There was no significant difference ($p > 0.05$) in the mean worker bee and pupal/larval mortality between the test item and the control. Mean worker bee mortality in dead-bee traps was comparable between all treatment groups during the pre-exposure, exposure and post-exposure period.

There was a statistical significant difference between the mortality of immature stages (mainly pupae) in colonies that were exposed to the reference item ($p < 0.001$).

Table B9.5.1-4: Summary table for the average numbers (± SD) of dead workers in dead-bee traps and on non-woven sheets as well as dead pupae and larvae in dead-bee traps per treatment group, throughout all experimental phases including the statistical outcome

	Phase	Control		Test item			Reference item		
		Mean	± SD	Mean	± SD	% difference compared to the control[1)]	Mean	± SD	% difference compared to the control[1)]
Workers in dead –bee traps	pre-exposure (DAT-5 to 0/0)	3.8	1.8	2.8	2.2	-26.3	3.8	1.5	0.0
	exposure (DAT 0/1 to 8	3.3	2.1	4.1	3.3	+24.2	3.0	2.4	-9.1
	post-exposure (DAT 9 to 28)	13.8	16.8	14.4	16.4	-4.3	15.1	6.7	+9.4
Workers on non-woven sheets	pre-exposure (DAT-5 to 0/0)	6.7	5.9	8.5	7.1	+26.9	9.8	10.0	+46.3
	exposure (DAT 0/1 to 8	4.2	2.7	5.9	3.4	+40.5	5.4	3.5	+28.6
Pupae and larvae in dead bee traps	pre-exposure (DAT-5 to 0/0)	0.3	0.4	0.3	0.3	0.0	0.4	0.3	+33.3
	exposure (DAT 0/1 to 8	2.1	2.8	1.0	1.3	-52.4	3.6*	5.3	+71.4*
	post-exposure (DAT 9 to 28)	0.3	0.5	0.2	0.2	-33.3	27.6*	26.8	+9100*
Total of worker bees[1)]	pre-exposure (DAT-5 to 0/0)	10.5		11.3	-	+7.6	13.6	-	+29.5
	exposure (DAT 0/1 to 8	7.5		10	-	+33.3	8.4	-	+12.0
	post-exposure (DAT 9 to 28)	13.8		14.4	-	+4.3	15.1	-	+9.4
Total of all groups[1)]	pre-exposure (DAT-5 to 0/0)	10.8	-	11.6	-	+7.4	14	-	+29.6
	exposure (DAT 0/1 to 8	9.6	-	11.0	-	+14.6	12.0	-	+25.0
	post-exposure (DAT 9 to 28)	14.1	-	14.6	-	+3.5	42.7	-	+203

*statistically signficantly different to the control ; $p<0.001$

\+ = an increase in mortality, compared to the control

\- = a decrease in mortality, compared to the control

1) = calculated by RMS evaluator, no statistical analysis performed

Flight activity

Overall daily mean foraging activity observed before application was similar in all treatments with 18, 17 and 15 bees in control, test item and reference item. During the exposure phase on average 15, 12 and 10 bees were recorded for control, test item and reference item, respectively. No statistically significant differences were observed between control and test item ($p > 0.05$), whilst a reduction ($p < 0.05$) was observed when comparing control and toxic reference item in both the pre-exposure and exposure phase (18.0 and 31.8% reduction in flight intensity respectively). When looking at the individual sampling points, a reduction in flight intensity of up to 82.3% was observed 1 hour after exposure to the test item in comparison to the control, followed by smaller reductions of 49.2, 51.0 and 29.4% 2, 3 and 4 hours after exposure. When comparing the pre-exposure flight activity to the flight activity during exposure in the test item group, reductions in flight activity of up to 80.7% were observed in the four hours following application of the test item. Reductions in flight activity of 98.0% and 99.3% were also observed 1 and 5DAT; however similar reductions were also noted in the control.

Table B9.5.1-5: Summary table for the average numbers (± SD) of foraging bees per treatment group, throughout all experimental phases including the statistical outcome

DAT	Control			Test item				Reference item			
	Mean	± SD	% difference compared to DAT 0/0 [1)]	Mean	± SD	% difference compared to the control[1)]	% difference compared to DAT 0/0 [1)]	Mean	± SD	% difference compared to the control[1)]	% difference compared to DAT 0/0 [1)]
Pre-exposure											
-5	5.6	1.6	-	5.6	1.7	0.0	-	6.3	2.3	+12.5	-
-4	-	-	-	-	-	-	-	-	-	-	-
-3	11.0	2.4	-	9.3	3.6	-15.5	-	8.8	2.9	-27.2	-
-2	22.1	4.7	-	23.8	3.8	+7.7	-	18.3	10.7	-17.2	-
-1	12.9	2.7	-	11.3	3.5	-14.7	-	12.7	6.0	-8.7	-
DAT 0/0	19.9	3.9	-	15.0	2.0	-24.6	-	14.2	1.3	-28.6	-
pre-exposure (DAT-2 to 0/0)	18.3	4.6	-	16.7	6.5	-8.7	-	15.0	2.9	-18.0*	-
Exposure											
0/1	16.4	1.2	-17.6	2.9	0.8	-82.3	-80.7	8.3	1.8	-49.4	-41.5
0/2	19.3	4.0	-3.0	9.8	2.6	-49.2	-34.7	13.9	2.9	-28.0	-2.1
0/3	21.0	2.3	+5.5	10.3	0.5	-51.0	-31.3	12.4	2.5	-41.0	-12.7
0/4	19.7	1.9	-1.0	13.9	1.1	-29.4	-7.3	12.2	0.9	-38.1	-14.1
0/5	19.5	2.3	-2.0	26.8	2.0	+37.4	+78.7	11.7	2.0	-40.0	-17.6
0/6	25.3	1.1	+27.1	15.7	3.7	-37.9	+4.7	10.2	2.5	-59.7	-28.2
1/1	1.3	0.4	-93.5	0.3	0.3	-76.9	-98.0	2.7	1.9	+107.7	-81.0
1/2	9.7	9.1	-51.3	7.1	2.9	-26.8	-52.7	5.3	2.4	-45.4	-62.7
1/3	15.5	1.6	-22.1	11.4	0.9	-26.5	-24.0	11.7	1.6	-24.5	-17.6
2	11.5	4.8	-42.2	14.7	5.7	+27.8	-2.0	12.7	6.9	+10.4	-10.6
3	12.9	5.3	-35.2	11.3	5.1	-12.4	-24.7	9.1	4.9	-29.5	-35.9
4	15.7	3.4	-21.1	14.0	3.7	-10.8	-6.7	12.8	3.5	-18.5	-9.9
5	0.5	1.0	-97.5	0.1	0.2	-80.0	-99.3	0.1	0.2	-80.0	-99.3
6	21.3	3.1	+7.0	20.5	4.7	-3.8	+36.7	17.7	3.5	-16.9	+24.6
7	12.6	3.4	-36.7	13.8	2.5	+9.5	-8.0	11.7	2.1	-7.1	-17.6
exposure (DAT0/1 to 7)	14.8	7.1	-25.6	11.5	7.1	-22.3	-23.3	10.1	4.5	-31.8*	28.9

*statistically signficantly different to the control ; $p<0.05$

DAT 0/0 : Shortly before application ; DAT 0/1 – 0/6 : 1 – 6 hours after application ; DAT 1/1, 1/2 and 1/3 : in the morning, afternoon and evening on DAT 1 respectively.

[1)] = calculated by RMS evaluator

\+ increase in flight activity in comparsion to the control

\- Decrease in flight activity in comparison to the control

Behaviour

No acute symptoms of poisogning (e.g. twitching or cramping) were observed after the applications of the test item during daytime full bee flight. Nevertheless intensive cleaning, coordination problems and apathy were noticed in tunnels of both test item and reference item treatments on the day of application.

Colony condition

One colony in the reference item group (Replicate IV, Colony 05) was found with no eggs present during colony assessments, although the queen was sighted during first condition checks. For this reason, this queenless colony was excluded from data analysis of colony conditions and brood development. Overall the strength of the colonies was similar and within a similar range to the control. The average number of worker bees increased after setup in the tunnels for all treatment groups and there was no statistically significant difference between any of the treatment groups ($p > 0.05$). There was also no statistically significant difference between any of the treatment groups for any brood stage (eggs, open and capped worker brood) or for total brood ($p > 0.05$). The amount of cells containing all brood stages increased in all treatment groups but there was a tendency for a decrease in the amount of capped worker brood for the colonies exposed to the reference item. The storage of nectar and pollen was very similar in the colonies and increased throughout the entire study period. Neither the test item nor the toxic reference item treatment resulted in a statistically significant difference in nectar stores ($p > 0.05$). Linear mixed effect models could not be used for statistical analysis of pollen stores since the baseline in the control was zero. The results for coloby condition are provided in Table B9.5.1-6 below.

Table B9.5.1-6: Summary table for the average numbers (± SD) of workers (colony strength), brood cells, nectar cells and pollen cells in the hive during colony condition checks

	DAT	Control		Test item			Reference item*		
		Mean	±SD	Mean	±SD	**% difference compared to the control[1)]**	Mean	±SD	**% difference compared to the control[1)]**
Colony strength (number of workers)	-2	6046.9	1223.0	6062.5	772.2	+0.3	5416.7	281.8	-10.4
	3	7437.5	1945.2	7765.6	909.1	+4.4	7000.0	1391.9	-5.9
	7	7218.8	2302.9	7500.0	1064.3	+3.9	5979.2	1276.8	-17.2
	14	7312.5	1365.5	9328.1	1274.1	+27.6	6916.7	897.8	-5.4
	20	6984.4	1460.7	9828.1	2209.9	+40.7	4375.0	1532.2	-37.4
	26	10046.9	2077.5	13859.4	1961.5	+37.9	7812.5	2517.9	-22.2
Total worker brood (amount of cells)	-2	13200.0	4023.3	13600.0	1861.9	+3.0	12666.7	923.8	-4.0
	3	15450.0	3279.7	15500.0	1371.1	+0.3	14933.3	461.9	-3.3
	7	14050.0	2048.6	15250.0	1445.7	+8.5	12333.3	461.9	-12.2
	14	25950.0	1330.4	25300.0	3761.2	-2.5	21666.7	3372.4	-16.5
	20	31800.0	2141.7	29500.0	4337.4	-7.2	28533.3	1026.3	-10.3
	26	30800.0	3471.8	31000.0	2597.4	+0.6	24400.0	2778.5	-20.8
Nectar stores (amount of cells)	-2	6200.0	765.9	5800.0	1143.1	-6.5	5933.3	1677.3	-4.3
	3	4800.0	1404.8	5000.0	1657.3	+4.2	4000.0	1587.5	-16.7
	7	2300.0	871.8	2650.0	957.4	+15.2	2600.0	1510.0	+13.0
	14	9650.0	2345.9	10850.0	1087.8	+12.4	10333.3	3008.9	+7.1
	20	12450.0	3247.0	17350.0	3026.0	+39.4	11266.7	2309.4	-9.5
	26	9750.0	2265.0	14150.0	2954.7	+45.1	10266.7	2773.7	+5.3
Pollen stores (amount of cells)[2)]	-2	0.0	0.0	450.0	341.6	+450	1000.0	721.2	+1000
	3	300.0	3446.4	450.0	341.6	+50.0	1200.0	692.8	+300.0
	7	450.0	378.6	550.0	412.3	+22.2	1400.0	592.2	+211.1
	14	4700.0	2042.9	4950.0	1170.5	+5.3	4866.7	1101.5	+3.5
	20	4950.0	2635.0	5150.0	500.0	+4.0	5066.7	1418.9	+2.4
	26	4400.0	2046.1	5550.0	789.5	+26.1	4800.0	1510.0	+9.1

[1)] = calculated by RMS evaluator

[2)] = analysis not conducted as the baseline in the control was zero

*replicate IV excluded from analysis

+ = increase in comparison to the control

- = decrease in comparison to the control

Development of honeybee brood in individual cells (Brood Termination, Brood Index and Compensation Index)

The mean brood termination rates (BTR) on BFD22 were 34.4%, 43.6% and 71.8% for control, test item and reference item colonies, respectively. The statistical analysis showed no significant difference in the brood termination rates of colonies exposed to the control and test item ($p > 0.05$). In contrast, a statistical significant effect on the brood termination rate was detected in the comparison between the control and reference item colonies ($p < 0.05$).

On BFD22, the mean brood indices (BI) for control, test item and reference item colonies were 3.28, 2.82 and 1.41, respectively. An increase in the brood index over time was observed for all treatment groups, indicating normal brood development. The statistical analyses revealed no significant difference in the brood indices of the control and test item colonies ($p > 0.05$) but there was a statistical significant difference in this variable between control and reference colonies ($p < 0.01$).

The mean compensation indices (CI) for control, test item and reference item colonies on BFD22 were 4.10, 3.85 and 3.15, respectively. The compensation index increased during the study in all treatment groups. Similar to the findings for the brood index, there was no statistical significant difference between the control and test item colonies ($p > 0.05$) but there was a statistical significant difference between control and reference item ($p < 0.05$). Results are presented in Table B9.5.1-7 below.

Table B9.5.1-7: Summary table for the average brood termination rate (BTR), brood index (BI) and compensation index (CI) on BFD22 including the standard deviation (± SD) and the statistical outcome (stat.)

	Control		**Test Item**			**Reference Item***		
	Mean	**±SD**	**Mean**	**±SD**	**stat.**	**Mean**	**±SD**	**stat.**
Brood Termination Rate (BTR) [%]	34.38	18.25	43.63	21.77	>0.05	71.83	12.11	<0.05
Brood Index (BI)	3.28	0.91	2.82	1.09	>0.05	1.41	0.61	<0.01
Compensation Index (CI)	4.10	0.11	3.85	0.59	>0.05	3.15	1.05	<0.05

*Replicate IV was excluded from analysis

The RMS evaluator has calculated the brood to food ratio for the observation point prior to application of the test item. This is presented in table Table B9.5.1-8 below :

Table B9.5.1-8: Brood to food ratio prior to treatment

Treatment	Brood (total)	Food (nectar and pollen)	Brood :Food ratio
Control	13200	6200	1:0.47 (4:1.88)
Test item	13600	6250	1:0.46 (4:1.84)
Reference item	12666.7	6933.3	1:0.55 (4:2.19)

Conclusion:

In order to assess potential effects posed by BCS-CN88460 EC 50 G (50 g/L) to honeybees, honeybee colonies were exposed for 7 days under semi-field conditions in tunnels cropped with full flowering of *Phacelia* (BBCH 65) that received treatment with 75 g BCS-CN88460 in 400 L tap water/ha (corresponding to 1.512 mL product/ha). A control group and a reference group were also established for comparison. No statistically significant adverse effects of the test item on brood development (brood termination rate, brood index and compensation index), adult and pupae survival, the condition of the colonies (e.g. on colony strength, the total amount of brood or food stores) or on flight activity were found compared to the control.

RMS comments

This study was conducted according to GLP and following OEPP/EPPO (2010) and OECD 75 guidelines.

The conduct of this study will be discussed further in the risk assessment section (B9.6).

Previous evaluation:	New data, submitted for purpose of review

Report:	KCP 10.3.1.5/02; Hein, R.; 2017
Title:	Assessment of side effects of BCS-CN88460 EC 50 on the honeybee (*Apis mellifera* L.) in the semi-field after one application on *Phacelia tanacetifolia* in Germany 2016
Report No.:	S16-02869
Guideline(s):	Regulation (EC) No 1107/2009; Directive 2003-01 (Canada/PMRA); US EPA OCSPP Not Applicable; OECD Guidance Document No. 75 (2007) and current recommendations of the AG Bienenschutz (Pistorius et al., 2012); OEPP/EPPO Guideline No. 170(4) (2010)
GLP/GEP:	**yes**

Material and methods:

Test item:	BCS-CN88460 EC 50 G (50 g/L): BCS-CN88460: 5.12 % w/w (analytical); supplier batch No: 201-001002; Sample Description: TOX20246-00; Specification No.: 102000031262; density: 0.974 g/mL.
Reference item :	Insegar 25 WG, 250 g/kg fenoxycarb ; batch no. SMO4D0025
Test organism:	Test species were honeybees (*Apis mellifera L.*; Hymenoptera, Insecta) provided by Eurofins Agroscience Services EcoChem GmbH / Agroscience Services Ecotox GmbH, NiefernÖschelbronn, Germany. In order to prepare the colonies before study start, the hives contained 3445 to 7150 bees per colony at the start of the test on 19 Jul 2016. Single box colonies with 10 combs and one queen were used. The colonies were as homogeneous as possible. Sister Queens originated from one breeding line in order to guarantee uniform bee material in all treatment groups. The following criteria for each colony were met: • 4-8 brood combs containing eggs, larvae and capped cells; • 6-10 combs containing honey and pollen;

	• colonies were visibly free of *Nosema* and *Varroa* disease symptoms • and foul brood and other bee diseases; • all brood stages were present at the start of the test.
Study location:	*Semi-field site:* Dußlingen, region Tübingen, Germany, 48°27'11.45" N, 09°01'53.47" E Winter wheat was grown in the field in 2015 and treated with Adexar 2 l/ha, Atlantis 400 g/ha, Primus 100 g/ha in Spring and fertilised with KAS in March and May 2015. *Monitoring site:* After the end of the confined period, the colonies were relocated to a monitoring site without flowering main crops attractive to honeybees within 3 km. The monitoring site was located at a distance of approximately 10.8 km to the field site In Hechingen, Baden-Württemberg, Germany, 48°21'41.25" N, 8°59'12.96" E.
Test type:	Semi-field tunnel test
Test duration:	48 days (5 days pre-exposure, 7 days exposure, 36 days post-exposure).
Test dates:	15.06.2016 – 01.09.2016
Test concentrations :	*Control:* 400 L water/ha *Treatment:* 75 g a.s. in 400 L water/ha (equivalent to 1447.7 g product/ha) *Reference:* 300 g a.s. in 400 L water/ha (corresponding to nominal 1200 g Insegar/ha)
Application of the test item:	Applications of the test item BCS-CN88460 EC 50 G (50 g/L), control and reference item (Insegar 25 WG, 250 g/kg fenoxycarb) were conducted by spraying the whole area of plants within the tunnel during full bee flight and at full flowering of the crop (BBCH 65) with a calibrated multi-nozzle portable boom sprayer with a spray pressure of 2.6 bar, boom width 2.0 m and spray width 2.2 m (deviation of max 7.28% of nominal). The test item, control and reference item was applied on 20.07.2016 between 10:03 and 11:54 (control, then test item, then reference item). Shortly before application, on average >10 honey bees per m^2/min were actively foraging on representative areas. During application mean wind speed was 0.0 – 1.3 m/s and air temperature did not exceed 29.1 – 29.9°C. No rainfall was recorded two hours application. During the application the bee colonies inside the tunnels were covered with plastic sheets and the water supply was moved out of the tunnels until the end of application to avoid direct contamination.
Test crop:	*Phacelia tanacetifolia* planted on 29 Jun 2016 at a seeding rate of 15kg/ha. The area covered by the crop in the tunnels was 87.72 m^2.
No. of individuals :	The initial mean colony sizes per treatment group were in the range of 4501 to 6023 bees.
No. of replicates	4 per treatment group, control and reference item
Test units :	Tunnels 20 m long, 5 m wide and 3.5 m high covered with plastic gauze (1.5mm) and 2.5 m from each other. The area covered by crop was 82.72 m^2 and the area covered by linen sheets was 17.28 m^2.
Weather conditions:	For the period with the bees inside the tunnels (5DBA to 7DAA) the data logger and a rain gauge was placed in one of the tunnels (GLP record). Following climatic data were recorded during this period: • Air temperature (daily minimum/maximum) • relative air humidity (daily minimum/maximum) • daily precipitation

	During the post exposure phase (outside the tunnels: 8DAA to 43DAA), meteorological data (non-GLP) were provided from the EAS weather station in Bodelshausen (distance of approx. 5.2 km). During this period the following climatic data were recorded: • Air temperature (daily minimum/maximum) • relative air humidity (daily minimum/maximum) • daily precipitation During applications the following data were recorded at the field site (GLP record): • Cloud cover (estimated during applications and assessments) • Air temperature, relative air humidity (only during applications) • Wind speed (only during applications)
Feeding:	Extra food was not provided during the study period. A water supply for the bees was placed at the edge of each tunnel.
Test procedure:	The study consisted of three treatment groups: the test item group T (BCS-CN88460 EC 50), a toxic reference item group R (Insegar) and a water-treated control C. The test item (T), the toxic reference item (R) and tap-water (C) were applied on full-flowering *Phacelia tanacetifolia* (BBCH 65) during foraging activity of the honeybees. The test item was applied at a nominal rate of 75.0 g a.s./ha (corresponding to 1447.7 g product/ha). Commercial bee colonies were placed in the tunnels at early flowering of *Phacelia tanacetifolia* (BBCH 62-64, recorded on 5DBA) five days before the application. The mortality, foraging activity, behaviour of the bees, condition of the colonies and the brood development of previously marked eggs was checked regularly after set-up in the tunnels. The effect of BCS-CN88460 EC 50 was evaluated by comparing the data of the assessments of the test item group T to the control C and the reference item group R, and by comparing the pre-application data to the post-application data. The confined phase of the test started on 5DBA (DBA= days before the application) with the set-up of the colonies in the tunnels and ended with the removal of the colonies from the tunnels on 7DAA (DAA = days after the application). The evaluation includes data from 4DBA to 7DAA. The monitoring phase started after removal of the colonies from the tunnels and ended after the colony assessments on 43DAA.
Observations:	• *Mortality assessment:* Mortality of the honeybees was recorded by counting the number of dead honeybees in the dead bee traps in front of the hives, on the bottom drawer inside the hives and on the linen sheets which were spread out in the tunnels. For calculation of the mean values per treatment and day, the number of dead bees on the linen sheets, inside the hives (bottom drawer) and in front of the hives (dead bee trap) were summarized per replicate and counted as one value. Dead bees were differentiated into female worker bees, pupae, larvae, male bees, male bee brood stages and dead bees with malformation. Dead male bees and male bee brood stages are not reported and were not evaluated. Mortality was assessed once daily in the morning during the pre-exposure, exposure and monitoring periods. On the day of application, mortality was checked shortly before application, then 2, 4 and 6 hours after application and in the evening after the daily flight activity of bees. • *Flight activity:* During the presence of the colonies in the tunnels, the flight intensity of honeybees was assessed in three randomly chosen flight observation areas per tunnel (1 m2 per observation area). At each assessment, the number of bees that were both foraging on flowers in the observation areas and flying over the crop was counted for 10-15 seconds in each observation area. Flight activity was assessed once daily during flight activity in the pre-exposure and exposure period; on the day of application flight activity was assessed once shortly before application, 4 times during the first hour after application and 2. 4 and 6 hours after application. One day after application, flight activity was measured 3 times (morning, midday and afternoon).

	• *Behavioural abnormalities*: The behaviour of the honeybees were recorded according to the time schedule of the flight intensity assessments in the tunnels and at the monitoring site according to the mortality assessments until 26DAA • *Colony conditions* : The following parameters were assessed during the colony assessments: Strength of colony, presence and vitality of queen, pollen storage area and area with nectar or honey and area containing cells with eggs, larvae and capped cells. Colony conditions were assessed 1day before application (1DBA = brood fixing day 0; BFD0), 4 DAA (BFD+5), 9DAA (BFD+10), 15DAA (BFD+16), 21DAA (BFD+22), 26DAA (BFD+27), 30DAA (BFD+31), 36DAA (BFD+37) and 43DAA (BFD+44). • *The development of bee brood:* The development of the bee brood was assessed in individually marked brood cells. At the assessment before the application in the treatment groups C, T and R (Brood Area Fixing Day = BFD), one or several brood combs were taken out of each colony to mark areas containing eggs on the comb(s). A minimum of 229 cells per colony containing eggs were marked. The selected combs were uniquely identified. The fixed brood areas were photographed during each brood stage assessment (BFD assessments) and the digital photos were transferred to a computer for analysis. Assessments were made on BFD, BFD+5, BFD+10, BFD+16 and BFD+22. For analysis, the different brood stage categories were transcribed into indices (e.g. 0 = empty; 1 = egg; 2 = young larvae; 3 = old larvae; 4 = pupae; 5 = nectar; 6 = pollen, 7 = dead larvae, 8 = cells which cannot be evaluated for technical reasons) to calculate the brood termination rate (BTR), the brood index and the compensation index for each assessment day and colony. *Brood termination rate:* Gives the number of the marked cells, where a termination of the bee brood development occurred (i.e. no successful development, the bee brood did not reach the expected brood stage at one of the assessment days or food was stored in the cell), expressed at percentage of the sum of all marked cells. *Brood index:* For each cell containing the expected brood stage at the respective day, the assessed value (1-5) was used. For each cell that did not contain the expected brood stage, "0" was used for calculation on this date and on all following assessment dates. The values of all individual cells per assessment day were summed up and divided by the number of observed cells in order to obtain the average brood index. *Compensation index:* For each cell containing the expected brood stage at the respective day, the assessed value (1-5) was used. For each cell that did not contain the expected brood stage, the actually observed cell content was used for calculation. "0" was used for calculation if a cell was empty or filled with nectar or pollen. The values of all individual cells per assessment day were summed up and divided by the number of observed cells in order to obtain the average compensation index.
Statistical analysis:	Before statistical comparison, the respective subsets of the recorded mortality and flight intensity data were tested for normal distribution with the Shapiro-Wilk test ($p > 0.05$) and for homoscedasticity with the folded F-test ($p > 0.05$). Data of the test item group and the reference item group were than compared to the data of the control group C, using either Student's t-test (pooled, $p \leq 0.05$), if the data were found to be normal distributed and showing equally distributed variances or using the t-test Satterthwaite ($p \leq 0.05$) in case of non-homoscedasticity but proven normality or using the Mann-Whitney exact test ($p \leq 0.05$) when the data of the subset was neither normally distributed nor homoscedastic. For statistical analysis of data recorded before application (4DBA to 0DBA) no effects were expected and therefore two sided tests were performed. During the post application phase (0DAA to 26DAA), rightsided tests were used for mortality of T and R compared to C and leftsided tests were used for flight intensity of T and R compared to C.

	Log- transformations were conducted if necessary to achieve better fit to normality and homoscedasticity of data. The data for brood indices, compensation indices and termination rates were compared to the control using the same tests as the data of mortality and flight intensity, with left-sided tests for brood and compensation indices and right-sided tests for the termination rates after the application. All statistical analysis was conducted using SAS release Version 9.3.

Results

Climatic conditions

The below table (Table B9.5.1-9) shows the climatic conditions during the course of the study :

Table B9.5.1-9: Climatic conditions during the study

Timing	Temperature (°C)		Relative humidity (%)		Precipitation (mm)	Clouding (%)
	Min	Max	Min	Max		
Pre-exposure						
5DBA	16.3	23.2	37.5	60.5	-	-
4DBA	12.3	25.8	42.5	82.5	0	5 – 25
3DBA	11.3	26.1	45.5	93.5	0	20 – 40
2DBA	12.6	27.9	47.5	100	0	20
1DBA	14.2	30.4	40.0	100	0	0
Exposure						
0DAA	14.2	33.4	32.4	93.5	0	0 - 50
1DAA	15.7	26.3	53.0	98.0	1	20 - 80
2DAA	13.1	26.6	55.0	100	20	0 - 20
3DAA	15.7	25.0	56.5	100	16	95 - 100
4DAA	15.7	26.1	58.0	100	8	40 - 100
5DAA	15.2	25.3	63.0	100	19	60 - 70
6DAA	15.2	26.1	53.0	100	1	30
7DAA	15.0	25.6	46.0	100	1	100
Post-exposure monitoring period						
8DAA	12.7	24.9	58.1	100	0.7	-
9DAA	14.9	25.5	57.2	100	1.4	-
10DAA	13.4	28.3	45.3	100	0.0	-
11DAA	13.5	21.7	73.3	100	7.6	-
12DAA	10.8	24.0	46.5	100	0.0	-
13DAA	12.4	21.9	60.1	100	4.6	-
14DAA	13.5	26.0	55.3	100	0.0	-
15DAA	10.5	27.5	46.0	100	0.0	-
16DAA	12.5	19.2	76.4	100	14.6	-
17DAA	10.4	21.6	62.3	100	0.0	-
18DAA	9.5	25.2	53.2	100	0.0	-
19DAA	12.6	26.3	53.5	97.6	0.0	-
20DAA	11.7	18.8	65.4	100	1.2	-
21DAA	9.5	17.4	54.1	100	2.2	-
22DAA	5.9	18.7	45.9	100	0.2	-
23DAA	11.4	21.1	66.8	100	0.8	-
24DAA	9.9	27.9	47.3	100	0.0	-
25DAA	12.8	28.2	46.4	100	0.0	-
26DAA	12.3	27.5	50.8	100	0.0	-
27DAA	12.0	27.4	54.3	100	0.0	-
28DAA	10.4	24.5	55.5	100	0.0	-
29DAA	12.8	23.6	67.3	100	0.0	-
30DAA	13.1	24.6	55.0	100	0.0	-
31DAA	12.8	21.9	68.7	100	7.4	-
32DAA	10.7	19.3	58.6	100	0.2	-
33DAA	8.3	23.2	51.0	100	0.0	-
34DAA	9.5	26.4	58.2	100	0.0	-
35DAA	11.3	29.5	42.1	100	0.0	-
36DAA	11.5	30.6	49.7	100	0.0	-
37DAA	16.1	31.6	45.6	100	0.0	-
38DAA	14.8	32.5	42.1	100	0.0	-
39DAA	18.2	32.6	33.4	90.4	1.4	-
40DAA	13.6	23.7	56.9	99.3	0.2	-
41DAA	10.4	24.5	54.7	100	0.0	-
42DAA	11.1	28.2	45.4	100	98.8	-
43DAA	14.2	26.4	39.6	98.8	0.0	-

Mortality

Prior to application, adult worker bee mortality was significantly higher in the treatment group 0DBA in comparison to the control. Adult bee mortality was significantly higher in the treatment group (8.3 dead bees) in comparison to the control (3.3 dead bees) 14DAA but not at any other point during the test following application. There was no statistically significant difference in adult mortality over the whole pre-exposure (4DBA to 0DBA), exposure (0DAA to 7DAA) or post-exposure period (8DAA to 26DAA). There was no statistically significant difference in adult bee mortality between the control and reference item.

During the whole study no dead larvae were recorded at any hive, hereinafter referred to as the "pupal mortality". No statistically significant differences were found between the treatment groups T and C regarding larval/pupal mortality (dead larvae/pupae/colony/day), neither on any individual day of the pre- or post-application period, nor if calculated over the periods in the tunnels before exposure (4DBA to 0DBA), in the tunnels after application until relocation to the monitoring site (0DAA to 7DAA), at the monitoring site (8DAA to 26DAA) and the whole exposure period from application to end of the study at the monitoring site (0DAA to 26DAA).

In the treatment group R a strong effect of the toxic reference item was detected by statistically significant higher pupal mortality rates in R on 11, 12, 13, 14, 15, 16, 17 and 24DAA. The pupal mortality rates in R were also statistically significant higher if calculated over the periods at the monitoring site (8DAA to 26DAA, C: 1.3 and R: 16.4 dead pupae/hive/ day) and over the whole period after application (0DAA to 26DAA, C: 1.3 and R: 11.7 dead pupae/hive/day).

In replicate Tb high pupal mortality rates were recorded prior to application. The mean daily pupal mortality rate from 4DBA to 0DBA in Tb was 60.4, while at the other hives the mean daily pupal mortality rates ranged from 4.6 (Cb) to 0 (Ca, Cd and Tc) dead pupae/day. Therefore no equal conditions before application could be assumed for replicate Tb and the Grubbs test was performed on the pupal mortality data in order to test the mean pupal mortality rate in Tb for outlier. Over the period 4DBA to 0DBA mean pupal mortality data of replicate Tb was tested against the mean pupal mortality data of hives Ca, Cb, Cc, Cd, Ta, Tc, Td, Ra, Rb, Rc, Rd. The mortality rate recorded at replicate Tb was identified (highly significant with $p < 0.01$) as an outlier. Therefore, no equal pre-conditions were verified in this regard and pupal mortality of replicate Tb was excluded from statistical analysis and evaluation

Pupae with malformations (white eyes) on 8, 9, 11, 13, 14, 15, 17, 18, 22 and 25DAA was also observed in the reference item group.

Results for the mortality assessments are shown in Table B9.5.1-10 below.

Table B9.5.1-10: Summary table for the average numbers (± SD) of dead workers in dead-bee traps and on non-woven sheets as well as dead pupae and larvae in dead-bee traps per treatment group, throughout all experimental phases including the statistical outcome

Treatment group		Control (C) ± SD	Test item(T) ± SD	% difference compared to the control[1]	Reference Item (R) ± SD	% difference compared to the control[1]
Daily mean mortality (dead worker bees/colony) ± STD	4DBA to 0DBA	42.5 ± 14.4	48.1 ± 15.9	+13.2	59.5 ± 34.3	+40.0
	0DBA	54.5 ± 2.4	63.5* ± 5.7	+16.5	87.8 ± 22.9	+61.1
	0DAA	86.0 ± 20.8	80.5 ± 36.5	-6.4	109.3 ± 22.9	+27.2
	0DAA to 7DAA	60.3 ± 16.7	55.5 ± 16.8	-8.0	72.6 ± 29.8	+20.4
	14DAA	3.3 ± 3.3	8.3* ± 3.1	+151.5	6.3 ± 3.9	+90.9
	8DAA to 26DAA	17.1 ± 5.4	13.7 ± 3.2	-19.9	20.7 ± 11.4	+21.1
	0DAA to 26DAA	29.9 ± 8.1	26.1 ± 6.5	-12.7	36.1 ± 7.3	+20.7
Daily mean mortality (dead larvae + pupae/ colony) ± STD	4DBA to 0DBA	1.2 ± 2.3	0.6 ± 0.7	-50.0	0.5 ± 0.4	-58.3
	0DAA	0.0 ± 0.0	0.7 ± 1.2	+0.7	0.3 ± 0.5	+0.3
	0DAA to 7DAA	1.5 ± 1.9	0.7 ± 0.8	-53.3	0.4 ± 0.4	-73.3
	8 DAA to 26DAA	1.3 ± 1.7	0.4 ± 0.6	-69.2	16.4 * ± 21.7	+1161.5
	0DAA to 26DAA	1.3 ± 1.4	0.4 ± 0.7	-69.2	11.7 * ± 15.2	+800.0

* = statistically significant higher than control group
+ = increase compared to the control
- = decrease compared to control
[1]Calculated by the RMS evaluator

Flight activity

The mean flight activity over the period before application was similar in all treatment groups, with no statistically significant differences.

After application on the application day (0DAA), the flight activity in the treatment group T was statistically significantly lower than the mean flight activity in the control C (T: 14.7, C: 21.7 forager bees/m²; Student's t-test, method pooled, left-sided, $\alpha = 0.05$). On the following day (1DAA) there was no statistically significant difference between the flight activity in T and C (T: 20.4, C: 20.5 forager bees/m²). A statistically significant difference was found again on 2DAA (T: 21.6, C: 26.5 forager bees/m²) but on all following days until 7DAA no statistically significant differences were found between the test item treatment group T and the control C.

Over the exposure period in the tunnels (0DAA to 7DAA) the difference between the mean flight activity T was statistically lower than that in C (T: 18.2, C: 20.7 forager bees/m²).

When comparing the foraging activity of the test item group on 0DBA to that in the exposure period, a reduction in flight activity of 42.3, 19.7 and 8.5% was observed 15, 30 and 45 minutes after application of the test item. After this, foraging activity increased.

A summary of the results is presented in Table B9.5.1-11 below:

Table B9.5.1-11: Summary table for the average numbers (± SD) of foraging bees per treatment group, throughout all experimental phases including the statistical outcome

Treatment group		Control (C)	% difference compared to 0DBA[1]	Test item (T)	% difference compared to the control[1]	% difference compared to 0DBA[1]	Reference Item (R)	% difference compared to the control[1]	% difference compared to 0DBA[1]
Daily mean flight intensity (bees/m²) ± STD	Pre-exposure								
	4DBA	6.6 ± 3.9	-	10.1 ± 4.9	+53.0	-	10.4 ± 4.8	+57.6	-
	3DBA	15.7 ± 3.8	-	16.7 ± 1.8	+6.4	-	15.6 ± 3.8	-0.6	-
	2DBA	16.4 ± 0.5	-	17.4 ± 4.9	+6.1	-	13.6 ± 2.5	-17.1	-
	1DBA	19.7 ± 7.4	-	16.9 ± 2.9	-14.2	-	18.8 ± 5.4	-4.6	-
	0DBA	12.4 ± 1.6	-	14.2 ± 3.0	+14.5	-	14.8 ± 3.3	+19.4	-
	4DBA to 0DBA	14.1 ± 2.8	-	15.1 ± 2.3	+7.1	-	14.6 ± 2.0	+3.5	-
	Exposure								
	15MAA	15.4 ± 4.4	+24.2	8.2 ± 1.8	-46.8	-42.3	16.5 ± 2.7	+7.1	+11.5
	30MAA	24.6 ± 10.6	+98.4	11.4 ± 0.8	-53.7	-19.7	19.3 ± 2.0	-21.5	+30.4
	45MAA	22.2 ± 6.5	+79.0	13.0 ± 1.7	-41.4	-8.5	19.6 ± 3.4	-11.7	+32.4
	1HAA	25.3 ± 6.8	+104.0	14.5 ± 0.6	-42.7	+2.1	18.5 ± 1.7	-26.9	+25.0
	2HAA	24.2 ± 8.9	+95.2	18.3 ± 2.0	-24.4	+28.9	17.1 ± 2.2	-29.3	+15.5
	4HAA	19.8 ± 4.4	+59.7	17.8 ± 3.1	-10.1	+25.4	17.7 ± 1.2	-10.6	+19.6
	6HAA	20.7 ± 3.3	+66.9	19.3 ± 3.4	-6.8	+35.9	19.3 ± 2.6	-6.8	+30.4
	Mean 0DAA	21.7 ± 6.0	+75.0	14.7 * ± 1.7	-32.3	+3.5	18.3 ± 1.6	-15.7	+23.6
	1DAA	20.5 ± 4.2	+65.3	20.4 ± 3.0	-0.49	+43.7	16.3 ± 1.1	-20.5	+10.1
	2DAA	26.5 ± 3.3	+113.7	21.6 * ± 2.3	-18.5	+52.1	24.9 ± 3.8	-6.0	+68.2
	3DAA	14.2 ± 1.0	+14.5	13.7 ± 15	-3.5	+3.5	13.7 ± 1.3	-3.5	-7.4
	4DAA	17.4 ± 6.6	+40.3	18.9 ±3.9	+8.0	+33.1	22.7 ± 3.7	+30.5	+53.4
	5DAA	24.6 ± 8.3	+98.4	18.3 ± 1.6	-25.6	+28.9	24.6 ± 5.2	0.0	+66.2
	6DAA	22.6 ± 3.0	+82.3	20.3 ± 2.1	-10.2	+43.0	22.3 ± 3.9	-1.3	+50.7
	7DAA	18.4 ± 1.5	+48.4	18.2 ± 2.2	-1.1	+28.2	20.2 ± 2.6	+9.8	+36.5
	0DAA to 7DAA	20.7 ± 2.3	+66.9	18.2 * ± 0.7	-12.1	+28.2	20.4 ± 0.8	-1.4	+37.8

DAA = days after application; DBA = days before application; MAA = minutes after application; HAA = hours after application; STD = standard deviation
* = statistically significant lower than control group
+ = increase compared to the control
- = decrease compared to control
[1]Calculated by the RMS evaluator

Behaviour of the bees

In the test item treatment group 150 bees were recorded, clustering at the trap of replicate Td, 6hours after application 0DAA. On the application day temperature records were the highest during the study.

No adverse effect of the test item treatment on honeybee behaviour could be discerned.

Strength of the colonies

No statistically significant test-item related adverse effects on colony strength were observed.The results of colony strength, total worker brood and total food are presented in Table B9.5.1-12below.

Table B9.5.1-12: Summary table for the average numbers (± SD) of workers (colony strength), brood cells and food cells in the hive during colony condition checks

Measurement	Treatment day	Control		Test item			Reference item		
		Mean	±SD	Mean	±SD	% difference compared to the control[1)]	Mean	±SD	% difference compared to the control[1)]
Colony strength (number of workers)	1DBA	4637	1501	4501	1101	-2.9	6023	1291	+29.9
	4DAA	6847	993	7101	907	+3.7	7930	1633	+15.8
	9DAA	8862	1790	7686	267	-13.3	7648	1057	-13.7
	15DAA	9360	1578	7768	962	-17.0	6348	2546	-32.2
	21DAA	7648	2271	7638	985	-0.1	8407	2136	+9.9
	26DAA	6110	1214	6630	990	+8.5	6002	2376	-1.8
	30DAA	6890	1705	6191	709	-10.1	5308	1491	-23.0
	36DAA	7063	1560	6549	878	-7.3	5720	879	-19.0
	43DAA	7150	2364	6029	577	-15.7	5200	469	-27.3
Total worker brood (amount of cells)	1DBA	15933	2157	14000	712	-12.1	18733	4997	+17.6
	4DAA	18733	3802	15100	1997	-19.4	17267	2194	-7.8
	9DAA	19333	3202	16050	1754	-17.0	14267	3495	+26.2
	15DAA	19067	3818	15650	2548	-17.9	12533	416	-34.3
	21DAA	19200	3934	16300	931	-15.1	13333	577	-30.6
	26DAA	18933	5065	16450	1147	-13.1	15000	1637	-20.8
	30DAA	20000	4133	16900	931	-15.5	14800	2117	-26.0
	36DAA	17533	6201	14750	2484	-15.9	14800	800	-15.6
	43DAA	15333	3233	14600	1461	-4.8	15600	1039	+1.7
Total food (amount of cells)	1DBA	23933	8093	18200	5937	-24.0	13467	2230	-43.7
	4DAA	24267	10130	19550	6098	-19.4	14133	2501	-41.8
	9DAA	24267	9617	20100	5212	-17.2	12933	1701	-46.7
	15DAA	20267	8764	16350	6157	-19.3	10267	2517	-49.3
	21DAA	18467	7575	15550	5370	-15.8	8800	3464	-52.3
	26DAA	22600	8488	19450	5422	-13.9	13667	2887	-39.5
	30DAA	21667	9065	17200	4324	-20.6	12467	2701	-42.5
	36DAA	18333	6586	15700	3357	-14.4	9333	1405	-49.1
	43DAA	20000	6823	20200	3433	+1.0	12733	1137	-36.3

[1)] = calculated by RMS evaluator
+ = increase compared to the control
- = decrease compared to control

Development of the brood area
The mean amount of brood in the colonies (sum of cells containing eggs, larvae and pupae) was assessed. There was no statistically significant decline in brood area in the treatment groups when compared to the control.

Development of the food storage area
The mean amount of food stores in the colonies (sum of cells containing nectar and pollen) was assessed. There was no statistically significant decline in pollen or nectar stores in the test item treated groups in comparison to the control groups. In there reference item group, there was a decline in mean colony strength and food storage area at the end of the first brood cycle (21DAA). No test-item related adverse effects on the development of the food storage area were observed.

Development of honeybee brood in individual cells (Brood Termination, Brood Index and Compensation Index)
In the control group C, successful development was observed in the majority of the marked brood cells, indicating a healthy development of brood. The mean termination rate at the end of the observation period (BFD+22) was 12.70 %.
In the reference item treatment group R, the mean values of the brood and compensation indices were not statistically significantly lower than those observed in the control. The brood termination rate in R was 38.56 %. However, adverse reference item effects were documented by high pupal mortality rates and malformations of the pupae in the typical period from 10 days after application.
In the test item treatment group T the brood and compensation indices were comparable to those in the control on all assessment dates after BFD 0. The mean termination rate of 9.30 % was not statistically significantly different to the control.

Table B9.5.1-13: Summary table for the average brood termination rate (BTR), brood index (BI) and compensation index (CI) on BFD22 including the standard deviation (± SD) and the statistical outcome (stat.)

Replicate	**Brood index / Compensation index at x days after brood area fixing day (BFD)**					**Termination rate (BFD +22)**
	0	**+5**	**+10**	**+16**	**+22**	
Control	**1.00 / 1.00**	**2.62 / 2.63**	**3.52 / 3.53**	**3.50 / 3.52**	**4.37 / 4.44**	**12.70**
STD	0.00 / 0.00	0.48 / 0.48	0.60 / 0.61	0.59 / 0.61	0.74 / 0.79	14.80
Test item T	**1.00 / 1.00**	**2.79 / 2.80**	**3.72 / 3.74**	**3.65 / 3.74**	**4.53 / 4.71**	**9.30**
STD	0.00 / 0.00	0.04 / 0.04	0.03 / 0.04	0.05 / 0.07	0.04 / 0.06	0.82
Reference item R	**1.00 / 1.00**	**1.93 / 1.96**	**2.57 / 2.73**	**2.48 / 2.71**	**3.07 / 3.43**	**38.56**
STD	0.00 / 0.00	1.22 / 1.19	1.65 / 1.55	1.60 / 1.43	1.99 / 1.78	39.82

BFD = Brood area fixing day; STD = Standard deviation

The RMS evaluator has calculated the brood to food ratio for the observation point prior to application of the test item. This is presented in table Table B9.5.1-14 below :

Table B9.5.1-14: Brood to food ratio prior to treatment

Treatment	Brood (total)	Food (nectar and pollen)	Brood :Food ratio
Control	15993	23933	1:1.50 (4:5.99)
Test item	14000	18200	1:1.30 (4:5.20)
Reference item	18733	13467	1:0.72 (4:2.88)

Conclusion:

BCS-CN88460 EC 50 was applied at a target rate corresponding to 75 g a.s./ha at full-flowering *Phacelia tanacetifolia* during honeybee foraging activity. The effects on honeybee colonies under confined conditions considering mortality, flight intensity, behaviour, colony strength, amount of brood and brood cell development were evaluated.
No test-item related adverse effects on larval, pupal and adult worker bee mortality were observed over the entire test period. Adverse effects on flight intensity and behavior potentially attributable to the test item were observed but these were transient and not clearly test-item related.
The effect of the toxicity of the reference item was detected by increased pupal mortality rates and the occurrence of pupae with malformations, reported in the treatment group R.

The quantitative assessments of brood development in individually marked cells containing eggs did not result in statistically significant differences on honeybee brood development.
No test-item related adverse effects on colony strength (mean number of bees per colony), amount of brood (mean number of cells covered with the different types of brood) or on the development of the food storage area were observed.

RMS comments

This study was conducted according to GLP and following OEPP/EPPO (2010) and OECD 75 guidelines.

The conduct of this study will be discussed in the risk assessment in section B9.6.

Previous evaluation:	New data, submitted for purpose of review

Report:	KCP 10.3.1.5/03; Schmitzer, S.; 2017
Title:	Isoflucypram EC 50 G: Toxicity testing on honey bees (*Apis mellifera* L.) under semi-field conditions in Germany - Tunnel test
Report No.:	122701037
Guideline(s):	OEPP/EPPO No. 170 (4)(2010) Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP Not Applicable
GLP/GEP:	**Yes**

Material and methods:

Test item:	Isoflucypram EC 50 G (50 g/L): BCS-CN88460: 5.28% w/w (analytical); batch ID: 2016-001002; Sample Description: TOX20246-01; Specification No.: 102000031262; density: 0.975 g/mL.
Reference item :	BAS 152 11 I (400g dimethoate/L nominal ; 405.2 g dimethoate/L analysed) ; batch no. : FRE-001302
Test organism:	Honey bees (*Apis mellifera carnica* L.); small bee colonies, maintained according to normal beekeeping practice, containing 11 combs with honey, pollen and brood. The preliminary brood check indicated healthy colonies, with all brood stages present and a sufficient amount of pollen and honey to guarantee colony viability. The mean strength of the colonies per treatment group, three days before the application, was similar and ranged between 4770 and 6233 adult bees per colony. No medical treatments were given 4 weeks prior to the test. Bees were placed in the tunnel 6 days prior to application.
Study location:	*Semi-field site:* Darmstadt-Dieburg, Germany, 64354 Reinheim (Zeilhard), Flur 2, Flurstück 231 – 238; 49° 83' 9.50" north, 008° 79' 21.34" east. Mustard was grown in the field in 2016/2017. Fields were previously treated with the following: March 2016: 1 L/ha Atlantis (5.6 g/kg iodosulfuron + 29.2 g/kg mesosulfuron, Herbicide) 80 g/ha Husar (93.2 g/L iodosulfuron), Herbicide, 0.2 L/ha Moddus Start (222 g/L trinexapac, Plant Growth Regulator) April 2016: 165 kg N-Fertilizer April 2016: 0.3 L/ha CCC (558.3 g/L chlormequat, Plant Growth Regulator) 0.25 L/ha Moddus (222 g/L trinexapac, Plant Growth Regulator) 1 L/ha Matador (75 g/L triadimenol + 225 g/L tebuconazole, Fungicide)

	May 2016: 2 L/ha Ceriax (41.6 g/L epoxiconazole, 66.6 g/L pyraclostrobin, 41.6 g/L fluxapyroxad, Fungicide, Plant Growth Regulator) June 2016: 38 kg N-Fertilizer June 2016: 2.5 L/ha Osiris (37.4 g/L epoxiconazole, 27.5 g/L metconazole, Fungicide) *Monitoring site:* After 7 complete days of confined exposure all bee colonies were removed from their respective tunnels and placed in an area with no bee attractive main crops in bloom in the evening on day 7. The bees were relocated to a field ("Kaiser und Seibert-Koppel/D-64380 Rossdorf: longitude: 49° 51' 3.50" north, latitude: 8° 44' 49.24" east) at a distance of ca. 4 km to the original experimental plot. There were no main flowering crops in the surrounding area.
Test type:	Semi-field tunnel test
Test duration:	45 days (3 days pre-exposure, 7 days exposure, 22 days post-exposure).
Test dates:	14.07.17 – 11.08.17
Test concentrations :	*Control:* 400 L water/ha *Treatment:* 75 g a.s. in 400 L water/ha (equivalent to 1.42 kg product/ha) *Reference:* 1.2 L BAS 152 11 I in 400 L water/ha (corresponding 3.0mL/L or 3.22 g/L)
Application of the test item:	Applications were conducted during daytime and during full flowering of the Phacelia-crop (BBCH 65), with confined honey bees actively foraging on the crop during application on 14 July 2017 between 10:35 – 12:50. The whole plot of plants was sprayed evenly and in two subsequent operations with a hand-held portable boom sprayer (manufacturer: Schachtner Gerätetechnik, Voithstr. 8/1, 71640 Ludwigsburg, Germany) equipped with an extension tube including 5 spraying nozzles (Lechler IDK 80-02 (2) and 120-02 (3), manufacturer: Lechler GmbH, D-72555 Metzingen) by compressed air; distance between nozzles: 50 cm. The temperature during application was 19 – 24 °C, relative humidity was 61 – 77 % and wind velocity was 0.0 – 2.1 m/s. There was partial cloud cover. The bees were very actively foraging at the time of the spraying during the application (mean of 12.2 to 13.7 bees/m^2). The sprayer was calibrated before application (once before start of the bee season) in order to ensure the accuracy of the spray application within the validity criterion. This procedure was repeated 5 times to ensure that the application rate was within 400 L/ha ± 10 %, without changing the sprayer settings.
Test crop:	*Phacelia tanacetifolia* planted 8 weeks prior to study initiation at a seeding rate of 9 kg/ha, covering ~ 80m^2.
No. of individuals :	The initial mean colony sizes per treatment group were in the range of 4770 and 6233 adult bees.
No. of replicates	4 per treatment group, control and reference item with a further 3 tunnels with 1 colony for sampling for residual analysis (test item only).
Test units :	20 m length x 5.5 m width x 2.5 m height, tunnels were semi-circular in cross-section and constructed out of a tubular steel frame, covered with synthetic gauze (mesh size ca. 2 mm). The tunnels were placed over the flowering plants a few days before experimental starting date with a distance of ≥ 2 meters in between. Each plot was subdivided in the middle by a cleared alleyway (ca. 50 cm), which served as a trail for carrying out the application. To facilitate the collection of the dead bees, in the middle of the tunnels, the plants were removed in order to prepare plane paths at ground level and covered by 50 cm wide gauze. Additionally at the front and head side of each tunnel, gauze was laid on the ground in order to collect the dead bees. A water supply for the bees was placed into each tunnel.

Weather conditions:	Temperature, relative humidity and precipitation were recorded during the whole study duration. Wind speed was recorded during application. Cloud cover was recorded during the exposure phase in the tunnels. All measurements were recorded with data loggers.
Feeding:	At the 3rd colony assessment (28 days after application) some colonies had low food stores due to limited natural nectar resources. In order to avoid artefacts from insufficient food supply, 5 L commercial ready-to-use syrup (Apiinvert; 30 % glucose, 31 % sucrose, 39 % fructose) was offered to each colony. Food uptake was finished after 5 days (food uptake from July 28 - August 02, 2017). A water supply for the bees was placed at the edge of each tunnel.
Test procedure:	The study design comprised one of three treatment groups in total, one being treated with the test item, one treated with the reference item dimethoate and one treated with tap water, each with four replicates (tunnels). Applications were made at full-flowering (BBCH 65) while honeybees were actively foraging on the crop. The test item Isoflucpyram EC 50 was applied at a target rate of 75 g a.s./ha in 400 L water/ha in the 4 tunnels (replicates) for biological assessments. Three additional tunnels (replicates) treated with the test item were set up to measure the concentration of isoflucypram in pollen and nectar after the application. Dimethoate used as reference item was applied in the 4 replicates at a target rate of 480 g a.s./ha in 400 L water/ha. Small bee colonies were introduced to the tunnels 6 days before the application of the test item, the control and the reference item respectively. The confined exposure phase of the honeybees to the control, test item and reference item was 7 days following the application. In the evening of the 7th day after application, all bee colonies were relocated from their respective tunnels and placed in an area with no main flowering, bee attractive crops.
Observations:	• *Mortality assessment:* To assess honey bee mortality, dead bees were collected from the gauze strips in the tunnels and from dead-bee traps placed in front of each hive. Observations were made once daily before application, once shortly before application then 3 times on the day of application until the evening. Following application. observations were made once daily during the 7 day exposure phase and post-exposure period (outside the tunnels up to day 21) and 3 times per week from day 22 to day 42. • *Flight activity:* Foraging activity on the plants was recorded within each tunnel at three different places on an area of 1 m2, each. Foraging bees were counted there in order to assess the foraging activity per m² (number of bees that are foraging on flowering plants were counted for a short time period (e.g. 10-15 seconds) per marked area). Observations were made once daily before application, once shortly before application then 3 times on the day of application until the evening. Following application. observations were made once daily during the 7 day exposure phase. • *Behavioural abnormalities*: Sub-lethal effects such as symptoms of poisoning or any abnormal behaviour at the entrance hole or on the plants in comparison to the control were recorded during the assessments of mortality and foraging activity. • *Colony conditions* : The following parameters were assessed during the colony assessments: Strength of colony, presence and vitality of queen, pollen storage area and area with nectar or honey and area containing cells with eggs, larvae and capped cells. Colony conditions were assessed once before application (day -6) and 6 × after the application (days 7, 14, 21, 28, 34 and day 42).
Residue analysis:	The sampling of foraging bees for pollen and nectar was performed with suitable instruments (exhauster, forceps) and collected from the hive entrance when the bees were returning back to the hive. The foraging bees were collected in a container with dry ice. Sampling was conducted 2 hours after application and 1 day after application. Afterwards the total number of collected foraging bees for pollen and nectar sampling were counted (150 bees/sampling day for pollen and 200 bees/sampling day for nectar per

	sampling day and residue monitoring tunnel were collected) and transferred into a suitable sampling container (sampling vial) and accurately sealed. The total numbers of collected foraging bees per residue monitoring tunnel, for pollen and/or nectar are documented in the raw data. Afterwards all samples were transported deep-frozen (≤ -20 °C) to the ibacon laboratory, Germany for further processing. The residues were quantified and detected by reversed phase HPLC-MS/MS with electrospray ionization in the positive ionization mode (ESI+) using internal stable labelled standards. In this study only BCS-CN88460 was analysed.
Statistical analysis:	Statistical evaluation was done for mortality, foraging activity and colony strength using Shapiro-Wilk`s test (check for normal distribution), Levene`s test (check for homogeneity of variance), Student or Welch t-test and Mann-Whitney U-test (pairwise comparison), (software: TOX Rat Professional, Version 3.2.1, ® ToxRat Solutions GmbH).

Results

Climatic conditions

The below table (Table B9.5.1-15) shows the climatic conditions during the course of the study :

Table B9.5.1-15: Climatic conditions during the study

Timing	Temperature (°C)		Mean relative humidity (%)	Precipitation (mm)
	Min	Max		
3DBA	15.9	26.3	77.6	23.0
2DBA	12.6	20.8	92.6	5.0
1DBA	8.5	24.7	75.9	0.0
0DAA	10.1	24.3	72.2	0.0
1DAA	10.3	23.5	72.3	0.0
2DAA	11.8	26.1	67.8	0.0
3DAA	15.1	31.0	68.7	0.0
4DAA	15.3	32.7	67.2	0.0
5DAA	17.1	37.0	59.1	0.0
6DAA	17.0	26.9	76.8	0.0
7DAA	13.9	32.0	65.2	0.0
8DAA	16.1	40.6	88.5	25.0
9DAA	14.4	26.5	74.9	7.0
10DAA	11.9	26.6	93.5	13.0
11DAA	12.1	15.0	99.7	14.0
12DAA	13.4	38.6	90.0	14.0
13DAA	12.9	36.7	90.9	2.0
14DAA	12.5	31.5	84.2	5.0
15DAA	14.2	40.5	74.1	0.0
16DAA	16.4	32.6	80.1	0.0
17DAA	15.8	31.2	89.1	1.0
18DAA	16.6	31.6	90.6	20.0
19DAA	16.5	36.0	80.5	2.0
20DAA	17.2	36.1	87.3	13.0
21DAA	16.8	39.5	73.8	1.0
22DAA	14.3	38.3	78.3	*
23DAA	10.7	44.9	76.0	*
24DAA	8.8	45.9	69.3	0.0
25DAA	11.9	28.0	81.7	*
26DAA	11.6	42.5	70.4	0.0
27DAA	13.6	15.5	99.8	*
28DAA	12.8	15.7	100.0	31.0
29DAA	11.9	19.2	97.7	*
30DAA	14.6	40.5	85.3	*
31DAA	12.4	39.0	83.8	32.0
32DAA	13.8	35.5	91.5	*
33DAA	15.1	38.8	86.3	9.0
34DAA	14.7	40.6	84.7	*
35DAA	15.0	33.5	89.7	2.0
36DAA	12.4	40.5	79.6	*
37DAA	9.9	35.5	78.9	*
38DAA	9.7	37.9	79.1	11.0
39DAA	8.8	43.4	76.4	*
40DAA	10.6	47.0	76.4	0.0
41DAA	13.6	39.4	77.2	*
42DAA	13.2	39.0	73.4	0.0

DBA = Days before application, DAA = Days after application

*from day 21 onwards rain was only assessed every 2 - 3 days. Values are precipitations for a period of 2 - 3 days

Mortality

Mortality of the pre-application phase (day - 3 to day -1) in the control, test item and reference item group was 43.2, 31.2 and 42.6 dead bees/colony/day, respectively. This was not statistically significantly different compared to the water control.

The comparison of the daily and the overall mortality values (day 0 to day 7) between the test item treatment and the control group showed no statistical significant difference to the control. Average control mortality of adult bees during the exposure phase (day 0 to day 7 following the application) were 42.6 dead bees/colony/day and 36.8 dead bees/colony/day in the test item group. The average mortality in the reference item group was 246.1 dead bees/colony/day. From day 0 to day 3 following the application the number of dead bees found in the reference item treatment were statistically significantly increased compared to the control values.

During the period from day 8 to day 21 after treatment the number of dead bees in the test item treatment was on average 10.9 dead bees per day and colony, which was not statistically significantly different to the control (14.6 dead bees/day/colony). On day 18, a mean of 27.8 dead bees was found in the test item group, (*vs* 12.8 dead bees in the control), which was statistically significantly higher in comparison to the control.

The overall comparison from day 22 to day 42 showed that the number of dead bees found in the test item treatment (6.6 dead bees/day/colony) was not statistically significantly higher compared to the number of dead bees found in the control group (5.8 dead bees/day/colony). The pairwise comparison on days 33 - 35 and 40 - 42 displayed a statistical significant difference of the test item group to the control. However, these mean values (26.3 and 9.8 mean dead bees/colony) were lower compared to the mean values in the control group for *e.g*. days 26 - 28 (37.8 mean dead bees) or day 28 - 31 (33.3 mean dead bees). The summarised results are presented in Table B9.5.1-16 below.

Table B9.5.1-16: Mortality of worker bees exposed to the test item and reference item in comparison to the control

Treatment group		**Control**	**Test item**	**% difference in comparison to the control[1]**	**Reference Item**	**% difference in comparison to the control[1]**
Daily mean mortality (dead worker bees/colony) ± STD	3DBA to 1DBA	43.2 ± 14.7	31.2 ± 9.7	-27.8	42.6 ± 15.4	-1.4
	0DBA	35.3 ± 12.2	32.0 ± 6.9	-9.3	22.0 ± 10.8	-37.7
	0DAA	17.8 ± 6.4	23.3 ± 3.3	+30.9	1287.5 ± 234.6	+7133.1*
	0DAA to 7DAA	42.6 ± 23.9	36.8 ± 17.5	-13.6	246.1 ± 430.5	+477.7
	8DAA to 21DAA	14.6 ± 9.7	10.9 ± 7.9	-25.3	5.5 ± 5.0	-62.3
	22DAA to 42DAA	5.8 ± 2.6	6.6 ± 1.6	+13.8	4.6 ± 1.5	-20.7
	0DAA to 42DAA	15.3 ± 6.6	13.5 ± 2.1	-11.8	48.8 ± 4.9	+219.0

DBA = Days before application, DAA = Days after application
+ = increase in mortality in comparison to the control
- = decrease in mortality in comparison to the control
*statistically significant compared to the control
[1]Calculated by the RMS evaluator

Foraging activity

The mean foraging activity over the period before application (day - 3 to day -1) was comparable in all treatment groups, with no statistically significant differences.

Overall, from day 0 to day 7, mean foraging activities in the test item group were comparable to the control values (16.3 bees/m²/day and 16.8 bees/m²/day, respectively), and thus not statistically significantly different. The overall daily mean foraging activity from day 0 to day 7 in the reference item group was 0.1 bees/m²/day, which was statistically significantly reduced compared to the control group.

When comparing the pre-treatment forgaing activity to the foraging activity during exposure in the treatment group, no reduction in foraging activity was observed apart from 6DAA, however a reduction in foraging activity was also observed in the control at this time point.

This is summarised in Table B9.5.1-17 below.

Table B9.5.1-17: Flight activity of worker bees exposed to the test item and reference item in comparison to the control

Treatment group		**Control (C)**	**% difference to 0DBA[1]**	**Test item (T)**	**% difference to control[1]**	**% difference to 0DBA[1]**	**Reference Item**	**% difference to control[1]**	**% difference to 0DBA[1]**
Daily mean foraging activity (bees/m²) ± STD	Pre-exposure								
	1DBA	7.0 ± 2.7	-	5.8 ± 1.3	-17.1	-	6.1 ± 1.2	-12.9	-
	2DBA	0.0 ± 0.0	-	0.0 ± 0.0	0.0	-	0.0 ± 0.0	0.0	-
	3DBA	18.9 ± 0.9	-	20.7 ± 2.2	+9.5	-	21.6 ± 2.3	+14.5	-
	3DBA to 1DBA	13.0 ± 8.4	-	13.3 ± 10.5	+2.3	-	13.8 ± 11.0	+6.2	-
	0DBA	12.8 ± 1.9	-	12.2 ± 1.1	-4.7	-	13.7 ± 0.9	+7.0	-
	Exposure								
	0DAA/1	16.2 ± 3.7	+26.6	14.1 ± 0.7	-13.0	+15.6	0.3 ± 0.3	-98.1	-97.8
	0DAA/2	17.8 ± 2.6	+39.1	21.6 ± 2.2	+21.3	+77.0	0.0 ± 0.0	-100.0	-100.0
	0DAA/3	21.2 ± 3.9	+65.6	15.3 ± 1.8	-27.8	+25.4	0.2 ± 0.3	-99.1	-98.5
	0DAA	18.4 ± 3.2	+43.8	17.0 ± 1.4	-7.6	+39.3	0.1 ± 0.1	-99.5	-99.3
	1DAA	14.2 ± 1.0	+10.9	12.8 ± 1.4	-9.9	+4.9	0.0 ± 0.1	-100.0	-100.0

	2DAA	17.4 ± 7.5	+35.9	19.4 ± 3.4	+11.5	+59.0	0.1 ± 0.2	-99.4	-99.3
	3DAA	12.6 ± 1.2	+1.6	13.3 ± 1.3	+5.6	+9.0	0.0 ± 0.1	-100.0	-100.0
	4DAA	26.5 ± 6.3	+107.0	29.1 ± 4.3	+9.8	+138.5	0.1 ± 0.2	-99.6	-99.3
	5DAA	26.2 ± 2.5	+104.7	19.8 ± 4.1	-24.4	+62.3	0.3 ± 0.3	-98.9	-97.8
	6DAA	2.1 ± 1.1	-83.6	1.8 ± 1.2	-14.3	-85.2	0.0 ± 0.0	-100.0	-100.0
	7DAA	17.3 ± 1.5	+35.2	17.3 ± 2.1	0.0	+41.8	0.1 ± 0.2	-99.4	-99.3
	0DAA to 7DAA	16.8 ± 7.8	+31.3	16.3 ± 7.7	-3.0	+33.6	0.1 ± 0.1*	-99.4	-99.3

DAA = days after application; DBA = days before application ; 0DAA/1, 0DAA/2 and 0DAA/3 = morning, afternoon and evening after application respectively.
* = Statistically significant lower than control group
+ = increase in mortality in comparison to the control
- = decrease in mortality in comparison to the control
[1]Calculated by the RMS evalauator

Behavioural abnormalities

No behavioural abnormalities occurred in the test item treated group at any assessment day.

The reference item caused behavioural abnormalities (moribund and affected bees) for three days following day 0

Strength of the colonies

The mean number of honey bees per colony in all treatment groups did not differ statistically significantly three days before application (mean of 4770 to 6233 per colony). The subsequent development of the colony strength among the colonies in the control and test item treatment groups followed the same pattern. Overall, no adverse effects of the test item on colony strength and population development were observed throughout the study. A summary of the results is presented in Table B9.5.1-18 below:

Table B9.5.1-18: Summary of the strength of the colonies

Day	Water control No. of bees			Test item No. of bees				Reference item No. of bees			
	Mean	SD	(%)[1]	Mean	SD	(%)[1]	Difference compared to control[2]	Mean	SD	(%)[1]	Diff. compared to control[2]
3DBA	4770	1208	100	5378	2405	100	+12.7	6233	423	100	+30.7
7DAA	5490	1353	115	5310	1333	99	-3.3	3746	958	60	-31.8*
14DAA	8033	1297	168	7695	2320	143	-4.2	6019	2123	97	-25.1
21DAA	6885	992	144	8865	608	165	+28.8	7425	1213	119	+7.8
24DAA	7751	680	163	7841	281	146	+1.2	6165	1417	99	-20.5*
34DAA	9068	919	190	9428	552	175	+4.0	8550	798	137	-5.7
42DAA	9428	687	198	10406	315	194	+10.4	8719	790	140	-7.5

[1]In relation to first assessment on day-3
[2]Statistics: Before Application: ANOVA, after application: Student t-test, pair-wise, one-sided smaller, $\alpha = 0.0$
* = statistically significant compared to the control

Development of the brood area

At the beginning of the trial all colonies to be used for the test were within the range of the control. All queens (or eggs) and brood stages (eggs, larvae and closed brood) were found in all colonies as an indication of healthy colonies. Compared to the control, a similar amount of brood could be found during the assessments with no indication of a test item related effect. All colonies exposed to the test item remained vital with increasing bee numbers and healthy brood. The amount of individual brood stages (eggs, larvae and pupae) present in the colonies of the different treatment groups fluctuated and was alternating higher in the different treatment groups on the different assessment days. There was no indication of any effect of the test item on the condition of the bee colonies. A summary of the results is presented in the following table (Table B9.5.1-19):

Table B9.5.1-19: Development of brood during the experimental period.

Day	Mean number of total brood per colony per comb			Mean number of eggs per colony per comb			Mean number of larvae per colony per comb			Mean number of closed brood per colony per comb		
	C (%)	T (%)	R (%)	C (%)	T (%)	R (%)	C (%)	T (%)	R (%)	C (%)	T (%)	R (%)
3DBA	26	24	28	7	7	7	8	8	10	11	9	11
7DAA	32	30	25	5	6	4	7	8	5	21	16	16
14DAA	37	33	25	6	8	5	13	9	7	18	17	13
21DAA	38	39	29	7	7	4	10	13	10	21	19	15
24DAA	44	46	36	4	6	5	12	11	9	28	29	22
34DAA	46	48	40	7	7	6	13	13	11	26	29	22
42DAA	41	46	37	8	7	6	10	11	10	23	28	21

C= Control
T = Test item
R = Reference item

Analytical findings

The exposure of the honeybees to the test item was confirmed by analytical measurement of the active substance isoflucypram in the spray solution samples taken from the biological assessment tunnels TS and the extra residue tunnels TR. The concentration of isoflucypram in both groups of tunnels was in a comparable range so that it is assumed that the exposure conditions were comparable in all tunnels treated with the test item. Separate tunnels were used for the residue determination; honeybees were used as sampling device. The concentration of isoflucypram measured in the collected pollen and nectar samples of the day of application and the day after allows for confirmation of the exposure of the bees inside the tunnels.

The following table (Table B9.5.1-20) gives an overview of the concentration of isoflucypram in the analysed sample materials after application of Isoflucypram EC 50 G with 75 g a.s./ha in 400 L water/ha.

Table B9.5.1-20: Analytical results

Sample Material	**Test Item**	**Sampling Day**	**BCS-CN88460**	
			Concentration [mg/kg]	**Mean Concentration [mg/kg]**
Nectar	BCS-CN88460	DAA0	0.0156 – 0.0237	0.0206
		DAA1	<LOQ – 0.0107	0.00859
Pollen		DAA0	11.2 – 15.1	13.1
		DAA1	0.527 – 1.83	1.08
Spray Solution		DAA0	TS: 130 - 168	TS: 154
			TR: 138 - 184	TR: 162

LOQ = Limit of Quantification = 0.01 mg/kg (= 10 µg/kg = 10 ppb) for BCS-CN88460
LOD = Limit of Detection = 0.003 mg/kg (= 3 µg/kg = 3 ppb) for BCS-CN88460
DAA = Days after application
TS = biological assessment tunnel, TR = residue analysis tunnel

Conclusion:

Isoflucypram EC 50 G was applied at 75 g a.s. in 400 L/ha (1.46 L product/ha) during full flowering of the surrogate crop *Phacelia tanacetifolia* and with honey bees present.
No clear effects on mortality of adult and immature honey bees were observed. Foraging activity, behaviour, nectar- and pollen storage as well as queen survival was not affected. There was no effect on overall colony development, development of brood and colony strength observed.
Based on the results of this study, it can be concluded that Isoflucypram EC 50 G does not adversely affect honey bee behaviour, brood development, colony strength and queen survival when applied at a rate of 75 g a.s. in 400 L/ha (1.46 L product/ha) under the above described conditions.

RMS comments

This study was conducted according to GLP and following OEPP/EPPO (2010) guidelines.

The analytical methods from this study have been acceptably validated (see section B5.1.2.6.5 of the CA document for further details).

The conduct of this study will be considered further in the risk assessment in section B9.6.

Previous evaluation:	New data, submitted for purpose of review

Report:	KCP 10.3.1.5/04; Vallon, A.; 2017
Title:	Assessment of side-effects of isoflucypram EC 50 G on the honeybee (*Apis mellifera* L.) in a semi-field study after application in flowering *Phacelia tanacetifolia* in Spain 2017
Report No.:	EBLN0008
Guideline(s):	OEPP/EPPO Guideline No. 170(4), 2010; EU Guideline 7029/VI/95 rev. 5 Regulation (EC) No 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP Not Applicable
GLP/GEP:	**yes**

Materials and methods:

Test item:	Isoflucypram EC 50 G (50 g/L): BCS-CN88460: 5.28% w/w (analytical); batch ID: 2016-001002; Sample Description: TOX20246-01; Specification No.: 10200003162; density: 0.975 g/mL.
Reference item :	Perfekthion 405.2 g/L dimethoate; batch no. FRE-001302
Test organism:	Honeybees (*Apis mellifera* L.; Hymenoptera, Insecta). Colonies with 6 combs (2 – 5 brood combs) and one queen were used. At the start of the test colonies sizes were in the range of 4436 to 9214 bees. The colonies were produced with sister queens in order to guarantee uniform bee material in all treatment groups. All test colonies were provided by Antonio Escriva Moreno, C/Divina Aurora 29, Ayora, Valencia, Spain. The following criteria for each colony were met: • 2 – 5 brood combs containing eggs, larvae and capped cells; • 3 - 6 combs containing honey and pollen; • Colonies were visibly free of *Nosema* and *Varroa* disease symptomsand other bee diseases; • all brood stages were present at the start of the test.
Study location:	*Semi-field site:* Ayora, Valencia Spain; 39.073653 N; -1.044299 W *Phacelia tanacetifolia* was grown in the field in 2017 and 2017 and fertilised with rabbit manure (2000 kg/ha) and ammonium nitrate (125 kg/ha) in 2016 and 2017 respectively. *Monitoring site:* After the end of the confined period, the colonies were relocated to a monitoring site without flowering main crops attractive to honeybees within a radius of at least 3 km. This monitoring site (after exposure) was located at a distance of 10.1 km to the field site. The details of the monitoring sites (before and after exposure) are given in Table B9.5.1-21:

Table B9.5.1-22: Description of the monitoring site

	Monitoring site (before exposure)	**Monitoring site (after exposure)**
Location	Montroi	Teresa de Cofrentes
Zip code	46193	46622
Federal state	Valencia	Valencia
Country	Spain	Spain
Meters above sea level	241 m	970 m
Latitude	39.329711 N	34.120068 N
Longitude	-0.67151 W	-0.951253 W

	Distance to field site	41.7 km	10.1 km
	Surroundings	Forest (Pine trees, scrubs), *Teucrium polium*, *Thymus vulgaris*, *Satureja montana*, *Papaver rhoeas*,*Diplotaxis erucoides*, *Sorbus* spec., *Citrus* spec., wild weeds	Forest (Pine trees, scrubs), *Citrus* spec., *Lavendula angustifolia*,
Test type:	Semi-field tunnel test		
Test duration:	47 days (4 days pre-exposure, 7 days exposure, 36 days post-exposure).		
Test dates:	18.05.2017 – 04/07/2017		
Test concentrations :	*Control:* 400 L water/ha *Treatment:* 75 g a.s. in 400 L water/ha (equivalent to 1447.7 g product/ha) *Reference:* 400 g dimethoate in 400 L water/ha (corresponding to nominal 1000 mL Perfekthion/ha)		
Application of the test item:	The test item solution was prepared shortly before the application. The application was carried out with a calibrated portable boom sprayer (spray pressure 3.0 bar; boom width 2.5 m) simulating a commercial application. Deviations to the target spray volume of ±10% were met for all replicates with the exception of Tc where a deviation of +12.78% was recorded. The following conditions during the applications were met: • Crop at full flowering during application in C, T and R (BBCH 65); • Bees were actively foraging shortly before application in C, T and R (≥ 10 bees/m² per treatment group); • Wind speed did not exceed 0.2 m/s during all applications inside the tunnels (actual 0 – 0.1 m/s); • Air temperature did not exceed 35 °C during all applications (actual max. 23.9 °C); • There was no rainfall on the day of application. • The amount of test item, control or reference item solution actually applied was determined by weighing the prepared and the remaining spray solution per plot. • The control plot Ce remained untreated. During the application the bee colonies inside the tunnels were covered with plastic sheets and the water supply was moved out of the tunnels until the end of application to avoid direct contamination. Samples of the test item solution were taken directly after preparation and mixing of the spray solution before filling up the boom sprayer shortly before the application. Two sub-samples (A and R, each sub-sample ≥ 10 g), one for analysis (A) and one as a retained sample (R) were taken in Ta, Tb, Tc, Td, Te, Tf and Tg,respectively.		
Test crop:	*Phacelia tanacetifolia* planted on 03 March 2017 at a seeding rate of 12 kg/ha. The area covered by the crop in the tunnels was 82.72 m^2.		
No. of individuals :	At the start of the test colonies sizes were in the range of 4436 to 9214 bees.		
No. of replicates	4 per treatment group (Ta – Td), control (Ca – Cd) and reference item (Ra – Rd); 3 for residue analysis (Te – Tg).		

Test units :	Tunnels 20 m long, 5 m wide and 3.5 m high covered with plastic gauze (1.5mm) and ≥ 2 m from each other. The area covered by crop was 82.72 m^2 and the area covered by linen sheets was 17.28 m^2.
Weather conditions:	For the period with the bees inside the tunnels (3DBA to 7DAA) the data logger and a rain gauge was placed in one of the tunnels (GLP record). Following climatic data were recorded during this period: • Air temperature (daily minimum/maximum) • relative air humidity (daily minimum/maximum) • daily precipitation During the post exposure phase (outside the tunnels: 8DAA to 43DAA), meteorological data (non-GLP) were provided from the weather station in Almansa (distance of approx. 29km) During this period the following climatic data were recorded: • Air temperature (daily minimum/maximum) • relative air humidity (daily minimum/maximum) • daily precipitation During applications the following data were recorded at the field site: • Cloud cover (estimated during applications and assessments) • Air temperature, relative air humidity (only during applications) • Wind speed (only during applications)
Feeding:	Bee colonies were fed 35DAA nd 37DAA with 400 g 70%-sucrose solution + 250g Neopoll. A container filled with a sufficient amount of water was placed in each tunnel as water supply for the bees.
Test procedure:	The aim of the study was to evaluate potential side effects of Isoflucypram EC 50 G on the honeybee (*Apis mellifera* L.) under semi-field conditions after one application during daily bee flight on flowering *Phacelia tanacetifolia*. The study was conducted in Ayora, Valencia, Spain. The study consisted of three treatment groups: a water-treated control C, the test item group T (Isoflucypram EC 50 G) and a toxic reference item group R (BAS 152 11 I). All treatment groups contained 4 replicates for biological assessments. For the test item treatment group T, three additional replicates were established for residue sampling, i.e. to obtain pollen and nectar samples collected by the foraging bees. No biological assessments were performed in the three test item "residue tunnels". In order to verify application and exposure of the bees, samples of the test item spray solution were taken from each (in total 7) of the test item treated replicates. The tap water (C), test item (T) and reference item (R), were applied on *Phacelia tanacetifolia* during full-flowering and daily bee flight (BBCH 65). The application rate in the test item treatment was 75.0 g a.s./ha (actual mean rate applied was 78.9 g a.s./ha) and the application rate in the toxic reference item (R) was 1000 mL product/ha, corresponding to 400 g a.s./ha (actual mean rate applied was 1036 mL product/ha, corresponding to 415 g a.s./ha). Small bee colonies were introduced to the tunnels 3 days before the application of the test item, the control and the reference item respectively. The confined exposure phase of the honeybees to the control, test item and reference item was 7 days following the application. In the evening of the 7^{th} day after application, all bee colonies were relocated from their respective tunnels and placed in an area with no main flowering, bee attractive crops. The following endpoints were assessed: - Mortality: 3 days before to 43 days after application - Flight intensity: 3 days before to 7 days after application - Behavioural abnormalities: 3 days before to 7 days after application

	- Condition of the colonies: Number of bees (colony strength) and development of the bee brood and food storage area: 4 days before to 43 days after application - Results of residue analysis
Observations:	• *Mortality assessment:* Mortality of the honeybees was recorded by counting the number of dead honeybees in the dead bee traps in front of the hives, on the bottom drawer inside the hives and on the linen sheets which were spread out in the tunnels. For calculation of the mean values per treatment and day, the number of dead bees on the linen sheets, inside the hives and in front of the hives (dead bee trap) were summarized per replicate and counted as one value. Dead bees were differentiated into female worker bees, pupae, larvae, male bees, male bee brood stages and dead bees with malformation. Dead male bees and male bee brood stages are not reported and were not evaluated. Mortality was assessed once daily in the morning during the pre-exposure, exposure and monitoring periods. On the day of application, mortality was checked shortly before application, then 2, 4 and 6 hours after application and in the evening after the daily flight activity of bees. • *Flight activity:* During the presence of the colonies in the tunnels, the flight intensity of honeybees was assessed in three randomly chosen flight observation areas per tunnel ($1m^2$ per observation area). At each assessment, the number of bees that were both foraging on flowers in the observation areas and flying over the crop was counted for 30 seconds in each observation area. Flight activity was assessed once daily during flight activity in the pre-exposure and exposure period; on the day of application flight activity was assessed once shortly before application, and 1, 2 4 and 6 hours after application. One day after application, flight activity was measured 3 times (morning, midday and afternoon). • *Behavioural abnormalities*: The behaviour of the honeybees were recorded according to the time schedule of the flight intensity assessments and mortality assessments. • *Colony conditions* : The following parameters were assessed during the colony assessments: Strength of colony, presence of a healthy queen, pollen storage area and area with nectar or honey and area containing cells with eggs, larvae and capped cells. Colony conditions were assessed 4 days before application (DBA), 5 days after application (DAA), 14DAA, 21DAA, 28DAA, 35DAA and 43DAA.
Residue analysis:	*Forager bees:* Forager bees were collected in replicates Te, Tf and Tg on two sampling occasions for the preparation of nectar and pollen loads for subsequent residue analysis. At each sampling, the hive entrances were sealed before the sampling and the forager bees were subsequently collected as they returned to the hive by collecting them into a box containing dry ice using modified hoovers ("bee vac"). After sampling the hives were re-opened. Each sample was divided into two subsamples in order to obtain one A sample (for residue analysis) and one R sample (retain sample). The samples contained at least 200 forager bees (per A sample) or 150 forager bees (per R-sample). The preparation of the honey stomachs and pollen loads from forager bees was done at the test facility. Preparation was done as follows: • The total no. of bees per A-sample was counted; • At least 50 % of the bees of the A-sample was prepared; • If the minimum amount could not be obtained from the sub-sample A, then sub-sample R was prepared and added to sub-sample A, until the requested amount was achieved; • The duration of any samples remaining outside of the freezer did not exceed 2 hours; • The total number of prepared bees and the sub-samples used were recorded;

	• The pollen loads were detached from the legs of the forager bees and transferred into a vial; • Bees were fixed at the thorax and the abdomen was separated with a pair of tweezers. The honey stomach content was transferred directly into a vial and stored deep frozen immediately; After preparation, the contents of the honey stomachs and the pollen were stored separately for each sample at <-18°C at the test facility until shipment of thesamples to the analytical laboratory for residue analysis. The residues were quantified and detected by reversed phase HPLC-MS/MS with electrospray ionization in the positive ionization mode (ESI+) using internal stable labelled standards.
Statistical analysis:	For statistical data analysis of mortality and flight activity, the test item group and the reference item group were compared separately to the control. The data of the post-application period were tested for normality and homoscedasticity using the Shapiro-Wilk Test ($p > 0.05$) and folded F-Test ($p > 0.05$), respectively. Data were statistically compared using Student's t-Test (method pooled, one-sided, $p \leq 0.05$) in case of normality and homoscedasticity. In case of non-homoscedasticity but proven normality, t-Test was conducted with method Satterthwaite (one-sided, $p \leq 0.05$). In case of non-normality and non-homoscedasticity, Mann-Whitney exact test (one-sided, $p \leq 0.05$) was used. Log-transformations were conducted if necessary to achieve better fit to normality and homoscedasticity of data. During the post-application period, right-sided tests were used for mortality and left-sided tests were used for flight intensity. For the pre-application period, the means of mortality and flight intensity were compared through comparisons with the Tukey's test (two-sided, $p \leq 0.05$). All statistical analysis was conducted using SAS version 9.3.

Results

Weather conditions

Table B9.5.1-23: Climatic conditions during the study

Timing	Temperature (°C)		Relative humidity (%)		Precipitation (mm)	Cloud cover (%)
	Min	Max	Min	Max		
Pre-exposure						
3DBA	12.01	26.15	30.2	100.0	0.0	0
2DBA	13.46	30.62	37.7	97.4	0.0	0
1DBA	6.56	29.75	30.9	100.0	0.0	40 - 70
Exposure phase						
0DAA	11.09	35.21	24.2	98.0	0.0	0 - 20
1DAA	6.15	30.20	27.5	100.0	0.0	0
2DAA	7.64	33.81	28.8	100.0	0.0	0
3DAA	6.46	36.04	24.7	100.0	0.0	0
4DAA	9.84	33.90	31.4	100.0	0.0	0
5DAA	9.73	32.17	45.8	100.0	0.0	10 – 25
6DAA	8.79	31.56	39.9	100.0	0.0	25 – 40
7DAA	9.76	33.31	29.8	100.0	0.0	0
Monitoring phase						
8DAA	12.1	22.4	59.8	100.0	15.9	-
9DAA	9.6	25.9	45.6	100.0	1.4	-
10DAA	11.0	29.0	30.5	100.0	0.0	-
11DAA	11.2	30.6	25.4	100.0	0.0	-
12DAA	14.1	29.0	35.2	100.0	0.0	-
13DAA	11.9	22.2	69.0	100.0	21.9	-
14DAA	9.8	24.3	34.1	100.0	0.2	-
15DAA	12.2	28.9	28.5	100.0	0.0	-
16DAA	10.4	27.3	47.8	100.0	2.6	-
17DAA	14.4	29.9	31.3	100.0	0.0	-
18DAA	15.1	30.6	36.9	100.0	0.0	-
19DAA	15.1	30.8	34.7	100.0	0.0	-
20DAA	13.5	33.1	18.7	100.0	0.0	-
21DAA	12.3	31.4	26.2	72.9	0.0	-
22DAA	12.1	32.0	27.5	82.6	1.2	-
23DAA	12.6	36.6	18.8	79.1	0.0	-
24DAA	15.6	37.9	16.3	67.1	0.0	-
25DAA	16.9	33.9	27.2	97.0	0.0	-
26DAA	16.2	36.3	23.8	100.0	0.0	-
27DAA	17.2	33.1	26.8	100.0	0.0	-
28DAA	14.8	31.7	32.8	100.0	0.0	-
29DAA	15.4	33.4	21.9	91.4	0.0	-
30DAA	15.2	34.8	20.5	77.4	0.0	-
31DAA	16.8	35.3	21.1	64.2	0.0	-
32DAA	17.1	37.7	15.1	68.7	0.0	-
33DAA	16.6	37.1	13.8	72.8	0.0	-
34DAA	16.7	32.1	33.7	87.6	0.0	-
35DAA	15.9	34.6	12.5	95.4	1.6	-
36DAA	13.9	33.2	8.9	70.7	0.0	-
37DAA	18.3	28.0	26.6	53.7	0.0	-
38DAA	13.9	26.2	18.0	55.8	0.0	-
39DAA	13.2	25.3	16.7	73.2	0.0	-
40DAA	10.6	23.4	40.5	84.0	0.0	-
41DAA	8.4	28.6	27.3	90.4	0.0	-
42DAA	9.4	29.7	24.0	88.2	0.0	-
43DAA	10.9	31.2	24.0	80.4	0.0	-

Mortality

During the pre-application period (3DBA to 0DBA), the mean daily honey bee mortality was 17.0, 38.5 and 33.9 dead honey bees/colony/day in the treatment groups C, T and R, respectively. No statistically significantly different values of mortality compared to the control were observed in this period.

During the exposure periods inside the tunnels (0DAA to 7DAA), the mean daily mortality values were 14.8 and 24.1 honey bees/colony/day in the treatment groups C and T, respectively and no statistically significant higher values compared to the control group were found except for 0DAA. On the day of application mortality in T was statistically significantly higher compared to the control. During the entire observation period after application (0DAA to 43DAA), the mean daily mortality was 3.7 and 5.2 dead honey bees/colony/day in the treatment groups C and T, respectively. During the monitoring period after removal of the colonies from the tunnels (8DAA to 43DAA), the mean daily mortality was 1.2 and 1.1 dead honey bees/colony/day in the treatment groups C and T, respectively.

During the post-application period (0DAA to 7DAA), mortality was in the range 51.0 - 890.5 dead honey bees/colony/day (mean: 307.2 dead honey bees/day) in the reference item group compared to 8.3 to 21.3 dead honey bees/colony/day (mean: 14.8 dead honey bees/colony/day) in the control. These differences were statistically significant from 0DAA to 6DAA, for the mean post-application mortality (0DAA to 7DAA) and for the entire observation period after application (0DAA to 43DAA). Elevated, but not statistically significantly higher values were observed on 7DAA. During the monitoring period after removal of the colonies from the tunnels (8DAA to 43DAA), the mean daily mortality was 1.2 and 7.6 dead honey bees/colony/day in the treatment groups C and R, respectively, and no statistically significant higher values compared to the control group were found.

Overall, no adverse effects on mortality of honey bees were found for test item treatment group T, except for the day of application (0DAA) with a statistically significantly higher value compared to the control. In the reference item treatment group R a clear impact on honey bee mortality was observed after the application.

A comparison was also made of the mortality 0DBA and that following exposure. There was a decrease in mortality in the treatment group following the hours after exposure; after this mortality increased in comparison to that 0DBA. However, it is noted that mortality in the control was also increasing at this point.

Results are summarised in Table B9.5.1-24 below :

Table B9.5.1-24: Mortality of honey bees in control, test item and reference groups

Treatment group		**Control**		**Test item**			**Reference Item**		
		Mean ± SD	**% difference to 0DBA[1]**	**Mean** ± SD	**% difference to control[1]**	**% difference to 0DBA[1]**	**Mean ± SD**	**% difference to control[1]**	**% difference to 0DBA[1]**
Daily mean mortality (dead worker bees, larvae and pupae/colony) ± STD		Pre-exposure							
	3DBA	6.8 ± 5.7	-	5.5 ± 3.7	-19.1	-	3.8 ± 2.2	-44.1	-
	2DBA	39.3 ± 18	-	88.3 ± 67.5	+124.7	-	81.5 ± 37.8	+107.4	-
	1DBA	16.7 ± 12.7	-	41.5 ± 39.0	+148.5	-	30.8 ± 13.9	+84.4	-
	0DBA	5.0 ± 2.7	-	18.8 ± 10.0	+276.0	-	19.5 ± 14.4	+290.0	-
	Mean 3DBA to 0DBA	17.0 ± 7.0	-	38.5 ± 29.2	+126.5	-	33.9 ± 7.7	+99.4	-
		Exposure							
	2HAA	3.5 ± 1.9	-30.0	10.3 ± 2.8	+194.3	-45.2	217.3 ± 59.0	+6108.6	+1014.4
	4HAA	2.5 ± 1.3	-50.0	4.3 ± 2.2	+72.0	-77.1	390.0 ± 66.6	+15500.0	+1900.0

	6HAA	5.5 ± 2.6	+10.0	3.0 ± 1.4	-45.5	-84.0	283.3 ± 89.6	+5050.9	+1352.8
	Sum 0DAA	11.5 ± 3.4	+130.0	17.5* ± 4.7	+52.2	-6.9	890.5* ± 79.2	+7643.5*	+4466.7
	1DAA	8.3 ± 3.1	+66.0	37.5 ± 52.7	+351.8	+99.5	623.5 ± 110.2	+7412.0	+3097.4
	2DAA	13.8 ± 6.2	+176.0	42.8 ± 59.8	+210.1	+127.7	352.5 ± 28.1	+2454.3	+1707.7
	3DAA	15.8 ± 6.6	+216.0	22 ± 20	+39.2	+17.0	187.0 ± 60.0	+1083.5	+859.0
	4DAA	13.8 ± 8.2	+176.0	19.5 ± 17.1	+41.3	+3.7	180.8 ± 96.4	+1210.1	+827.2
	5DAA	15.0 ± 11.6	+200.0	13.8 ± 6.3	-8.0	-26.6	92.5 ± 56.5	+516.7	+374.4
	6DAA	21.3 ± 14.2	+326.0	20.3 ± 7.4	-4.7	+8.0	79.5 ± 48.9	+273.2	+307.7
	7DAA	19.3 ± 17.8	+286.0	19.3 ± 10.5	0.0	+2.7	51.0 ± 47.8	+164.2	+161.5
	0DAA to 7DAA	14.8 ± 7.4	+196.0	24.1 ± 19.8	+62.8	+28.2	307.2* ± 36.5	+1975.7	+1475.4
	0DAA to 43 DAA	3.7 ± 1.8	-26.0	5.2 ± 3.6	+40.5	-72.3	62.0* ± 14.2	+1575.7	+217.9
	8DAA to 43 DAA	1.2 ± 0.7	-76.0	1.1 ± 0.4	-8.3	-94.1	7.6 ± 13.0	+533.3	-61.0

DBA = Days before application, DAA = Days after application ; HAA = Hours after application
* Statistically significantly higher compared to the control
+ Increase in mortality in mortality in comparison to the control
- Decrease in mortality in comparison to the control
[1]Calculated by RMS

Flight intensity

Flight intensity was on a comparable level in all treatment groups during the pre-application period (3DBA to 0DBA), indicating similarly intense foraging on the crop. The mean flight intensity was 21.5, 18.1 and 16.3 forager bees/m² in the treatment groups C, T and R, respectively. The day before application flight activity was statistically significantly lower in the test item group in comparison to the control (16.0 in compraison to 30.7 forager bees/m^2).

During the exposure periods inside the tunnels (0DAA to 7DAA), the mean flight intensity was 28.4 and 27.5 forager bees/m² in the treatment groups C and T, respectively. The values for flight intensity were therefore on a similar level in both treatment groups C and T. No significantly lower daily flight intensity values compared to the control group were detected in T during this period, however a reduction in flight intensity of 38.1% in comparison to the control was observed one hour after application.

When comparing the pre- exposure flight activity levels to the flight activity levels during exposure in the treatment group, an increase in flight activity was observed at all time points.

A statistically significantly different value in R compared to the control group was observed on 0DBA and for the mean pre-application period (3DBA to 0DBA). Flight activity after the application in R was significantly reduced from 0DAA to 7DAA as well as for the mean post-application period (0DAA to 7DAA).

Overall, no adverse effects on flight activity were observed in the test item treatment group (T), however a significant decrease in flight intensity was observed in the reference item treatment R after the application.

Results are summarised in Table B9.5.1-25 below :

Table B9.5.1-25: Flight intensity of honey bees in control, test item and reference groups

Treatment group		Control (C)		Test item (T)			Reference Item (R)		
		Mean ± SD	Difference to 0DBA (%)	Mean ± SD	Difference to control (%)	Difference to 0DBA (%)	Mean ± SD	Difference to control (%)	Difference to 0DBA (%)
Daily mean flight intensity (bees/m²) ± STD	Pre-exposure								
	3DBA	9.1 ± 1.0	-	11.4 ± 2.5	+25.3	-	7.9 ± 3.7	-13.2	-
	2DBA	24.5 ± 0.8	-	22.9 ± 4.3	-6.5	-	21.4 ± 4.7	-12.7	-
	1DBA	21.5 ± 2.7		22.1 ± 2.4	+2.8	-	21.5 ± 4.8	0.0	-
	0DBA	30.7 ± 3.5	-	16.0** ± 4.6	-47.9	-	14.4** ± 3.2	-53.1	-
	Mean 3DBA to 0DBA	21.5 ± 1.2	-	18.1 ± 2.3	-15.8	-	16.3** ± 3.4	-24.2	-
	Exposure								
	0DAA/1h	32.3 ± 6.2	+5.2	20.0 ± 2.3	-38.1	+25.0	7.3 ± 4.7	-77.4	-49.3
	0DAA/2h	25.4 ± 3.8	-17.3	24.8 ± 3.1	-2.4	+55.0	4.3 ± 1.5	-83.1	-70.1
	0DAA/4h	26.8 ± 3.5	-12.7	28.2 ± 1.7	+5.2	+76.3	1.2 ± 1.3	-95.5	-91.7
	0DAA/6h	29.3 ± 7.4	-4.6	31.3 ± 3.6	+6.8	+95.6	0.5 ± 0.6	-98.3	-96.5
	Mean 0DAA	28.5 ± 4.5	-7.2	26.1 ± 2.3	-8.4	+63.1	3.3** ± 1.6	-88.4	-77.1
	1DAA/1	22.0 ± 1.1	-28.3	19.2 ± 6.8	-12.7	+20.0	0.0 ± 0.0	-100.0	-100.0
	1DAA/2	24.4 ± 1.4	-20.5	26.0 ± 5.6	+6.6	+62.5	0.0 ± 0.0	-100.0	-100.0
	1DAA/3	23.3 ± 3.3	-24.1	28.4 ± 10.5	+21.9	+77.5	0.0 ± 0.0	-100.0	-100.0
	Mean 1DAA	23.3 ± 1.4	-24.1	24.6 ± 7.2	+5.6	+53.8	0.0** ± 0.0	-100.0	-100.0
	2DAA	36.0 ± 5.0	+17.3	30.2 ± 9.7	-16.1	+88.8	0.0** ± 0.0	-100.0	-100.0
	3DAA	35.0 ± 4.3	+14.0	30.8 ± 6.7	-12.0	+92.5	0.0** ± 0.0	-100.0	-100.0
	4DAA	28.9 ± 5.3	-5.9	32.8 ± 6.5	+13.5	+105.0	0.0** ± 0.0	-100.0	-100.0
	5DAA	19.6 ± 4.1	-36.2	18.2 ± 1.9	-7.1	+13.8	0.0** ± 0.0	-100.0	-100.0
	6DAA	25.3 ± 2.6	-17.6	28.8 ± 3.8	+13.8	+80.0	0.3** ± 0.3	-98.8	-97.9
	7DAA	30.7 ± 2.7	0.0	28.2 ± 3.7	-16.1	+76.3	0.3** ± 0.5	-99.0	-97.9

	0DAA to 7DAA	28.4 ± 1.9	-7.5	27.5 ± 4.6	-3.2	+71.9	0.5** ± 0.2	-98.2	-96.5

DBA = Days before application; DAA = Days after application ; 0DAA/1h – 6 h = 1 - 6 hours after application ; 1DAA/1, 2 and 3 = morning, afternnon and evening 1 day after application
** = Statistically significantly lower compared to the control
+ Increase in flight intensity in comparison to the control
- Decrease in flight intensity in comparison to the control

Behavioural abnormalities

In the test item group T, normal behavior was observed throughout the assessment period.

A total of 62 bees with locomotion problems and 1250 clustering bees were observed across all four replicates in reference item group R on the day of application. On 1DAA2, 60 bees with locomotion problems were documented. On 2DAA, 8 bees with locomotion problems were recorded and on 6DAA, 5 bees with locomotion problems and 1 cramping bee were observed.

Strength of the colonies

The mean number of bees per colony assessed during the first colony assessment on 4DBA was 7217 bees/colony in C (range: 4436 to 8804), 7559 bees/colony in treatment group T (range: 4436 to 8804) and 7388 bees/colony in R (range: 4505 to 9214).

At the second colony assessment on 5DAA (during confinement), the mean colony strength slightly increased in treatment groups C and T (C: 7508 bees/colony; range: 6074 to 8873; T: 7576 bees/colony, range: 6689 to 8395), but decreased in R (3498 bees/colony; range 2116 – 4573). On the following assessment (14DAA, after the end of the confinement period) an increase in colony size was seen in all treatment groups (C: 7661 bees/colony; range: 6143 to 10579; T: 8054 bees/colony, range: 6074 to 10306; R: 4658 bees/colony, range: 2730 to 6211). Thereafter, the colony strength decreased from 21DAA (C: 7320 bees/colony; range: 5187 to 9214; T: 6416 bees/colony, range: 4846 to 7985) to 35DAA (C: 6279 bees/colony; range: 5733 to 7098; T: 5341 bees/colony, range: 3754 to 7030) in the control group C and the test item treatment group T.

On the last colony assessment (43DAA) the colony strength increased in treatment groups C and T. The mean colony strength on this day was as follows: 7405 bees/colony in C (range: 4846 to 8804) and 5665 bees/colony in treatment group T (range: 1911 to 8054). For unknown reasons, a noticeable decrease could be observed from 28DAA until the last colony assessment in replicate Ta, resulting in colony strength of 1911 bees. This replicate started as the weakest colony in treatment group T, nevertheless it was well provided with food during the course of the study and all of the brood stages were present throughout the study conduct. Mean mortality rates in Ta were also not statistically significantly higher compared to the control and therefore no test-item related adverse effects on colony strength are assumed in this replicate. In the reference item group R, the colony size decreased or stayed approximately on the same level until the last colony assessment on 43DAA (3174 bees/colony; range: 683 to 5460).

The mean colony strength values of the test item treatment group T followed a pattern of development similar to the control group C during the entire study. Therefore, no test-item related adverse effects on colony strength were observed and a clear impact in the reference item treatment R after the application was documented. A summary of the results is provided in Table B9.5.1-26 below.

Table B9.5.1-26: Colony strength during the test period.

Assessment date	Control		Reference			Treatment		
	Mean no. of bees	SD	Mean	SD	% diff. to control	Mean	SD	% diff. to control
4DBA	7217	1984	7388	2029	-2.4	7559	651	+4.7
5DAA	7508	1160	3498	1175	-53.4	7576	786	+0.9
14DAA	7661	2003	4658	1692	-39.2	8054	1840	+5.1
21DAA	7320	1875	4283	2312	-41.5	6416	1420	-12.3
28DAA	6774	1472	3396	2120	-49.9	5955	1297	-12.1
35DAA	6279	643	3481	2604	-44.6	5341	1760	-14.9
43DAA	7405	1757	3174	2535	-57.1	5665	2727	-23.5

+ Increase in in comparison to the control
- Decrease in comparison to the control

Development of the brood area

The colonies of the control C and the treatment group T showed all brood stages (eggs, larvae, capped brood) at all assessment dates during the entire observation period.

In the reference item group R, a lack of eggs was observed in Rd and no larvae were present in replicates Rb, Rc and Rd during the assessment on 5DAA. During the assessment on 14DAA, no brood stages were present in replicate Rd and a lack of pupae was observed in replicates Rb and Rc. In replicate Rd, a lack of larvae and pupae was recorded on 21DAA, no pupae were present on 28DAA, no eggs and no larvae were documented on 35DAA and on 43DAA, no larvae were present.

The mean amount of brood in the colonies (sum of cells containing eggs, larvae and pupae) assessed during the first colony assessment on 4DBA (during confinement) was 10553 cells/colony for treatment group C (range: 8820 to 12390), 9450 cells/colony for treatment group T (range: 3570 to 17430) and 10920 cells/colony for treatment group R (range: 8610 to 12600).

At the second colony assessment on 5DAA (during confinement), the abundance of brood cells decreased in all treatment groups C, T and R.

At the third colony assessment on 14DAA (at the monitoring site), the abundance of brood increased again in treatment groups C and T or stayed approximately onthe same level in reference item group R. At the following colony assessments on 21DAA and 28DAA, (at the monitoring site), the abundance of brood increased strongly in treatment groups C and T, and outreached the initial values, indicating a good colony development. The reference item group R showed the weakest recovery by not reaching the initial values. During the following assessments on 35DAA and 43DAA, the abundance of brood in treatment groups C, T and R decreased again due to the seasonal development of the honeybee colonies. On the last assessment day (43DAA) the abundance of brood in treatment groups C, T and R was 10343, 9398 and 6405 cells/colony, respectively.

Overall, the mean numbers of brood cells of the test item group showed a similar pattern of brood development compared to the corresponding values of the control group during the entire study. Therefore, no test-item related adverse effects on honeybee brood development were observed.

Development of the food storage area

At start of the test the mean number of nectar cells was 12915, 12915 and 12653 cells/colony in the control, test item and reference item treatment, respectively. The mean number of pollen cells was 5565, 5460 and 6773 cells/colony in the control, test item and reference item treatment group. The mean extent of food stores in the colonies (sum of cells containing nectar and pollen) assessed during the first colony assessment on 4DBA was 18480 cells/colony for treatment group C (range: 9450 to 27720), 18375 cells/colony in treatment group T (range: 13650 to 25620) and 19425 cells/colony for treatment group R (range: 11760 to 26250). The mean extent of food stores slightly decreased at the second colony assessment (5DAA) and then slightly increased at the third colony assessment (14DAA) in the control. In treatment group T, the mean extent of food stores approximately stayed on the same level with only slight fluctuations and in the reference group R, the mean extent of food stores decreased.

During the following assessments up to 35DAA, the mean extent of food stores slightly decreased in all treatment groups (C: to 12495 cells/colony; T: to 16065 cells/colony; R: to 11183 cells/colony). This observed decrease was most likely due to the declined seasonal food supply at the monitoring site and all of the replicates were fed according to common beekeeper practice on 26 Jun 2017 and again on 02 Jul 2017.

On the last colony assessment (43DAA) the food stores increased in all treatment groups and finally reached 14385 cells/colony in C (range: 12180 to 17640), 19425 cells/colony in T (range: 11760 to 24150) and 12600 cells/colony in R (range: 5670 to 17430).

During the course of the study the mean number of nectar cells stayed approximately at the same level or slightly increased during the tunnel phase in C and T and slightly decreased in R due to the low foraging intensity. During the monitoring phase the mean number of nectar cells decreased in all treatment groups until 35DAA. Thereafter, on 43DAA, due to the feeding, the mean number of nectar cells increased again in all treatment groups. The mean number of cells containing nectar was 9765, 15173 and 8295 cells/colony for the control, test item and reference item treatment, respectively.

In contrast the provision with pollen slightly increased in C until 14DAA, decreased thereafter until 35DAA and showed an increase again during the last colony assessment (43DAA). In T, the mean number of pollen cells decreased during the tunnel phase until 5DAA, which often can be observed during the caging phase.

During the following colony assessments until 28DAA, the provision with pollen increased, slightly decreased on 35DAA and increased again on 43DAA. In the reference item group R, the mean number of pollen cells slightly decreased until 14DAA due to the low foraging intensity, slightly increased on 21DAA and showed a decrease again until 35DAA. The provision in pollen increased on the last assessment on 43DAA. The mean numbers of pollen cells were 4620, 4253 and 4305 cells/colony in the control, the test item and reference item treatment.

Overall, the colonies were well provided with food during the course of the study and no test-item related adverse effects on the development of the food storage area were observed.

A summary of the results for brood and food area is provided in Table B9.5.1-27 and table Table B9.5.1-28 below.

Table B9.5.1-27: Development of brood area during the course of the test.

Assessment date	Control		Reference			Treatment		
	No. cells with brood (total)	SD	No. cells with brood (total)	SD	% diff. to control	No. cells with brood (total)	SD	% diff. to control
4DBA	10553	1498	10920	1917	+3.5	9450	5810	-10.5
5DAA	7875	1194	4463	1438	-43.3	8295	3182	+5.3
14DAA	9398	627	4568	1692	-51.4	9608	2445	+2.2
21DAA	12968	1575	6930	4465	-46.6	12443	2631	-4.0
28DAA	15645	1693	8400	5792	-46.3	14648	3495	-6.4
35DAA	13493	1048	6983	5985	-48.2	13283	4459	-1.6
43DAA	10343	2245	6405	4486	-38.1	9398	2767	-9.1

\+ Increase in in comparison to the control
\- Decrease in comparison to the control

Table B9.5.1-28: Development of food storage area during the course of the test.

Assessment date	Control		Reference			Treatment		
	No. cells with food (pollen + nectar)	SD	No. cells with food (pollen + nectar)	SD	% diff. to contro	No. cells with food (pollen + nectar)	SD	% diff. to contro
4DBA	18480	7519	19425	6155	+5.1	18375	5258	0.6
5DAA	15488	5651	17850	6491	+15.3	19058	4379	+23.1
14DAA	16485	6517	15173	5264	-8.0	19110	5585	+15.9
21DAA	15750	6021	14385	4845	-8.7	18218	4512	+15.7
28DAA	14490	6848	12600	4458	-13.0	17378	5646	+19.9
35DAA	12495	5394	11183	3711	-10.5	16065	5451	+28.6
43DAA	14385	2316	12600	5240	-12.4	19425	5631	+35.0

\+ Increase in in comparison to the control
\- Decrease in comparison to the control

The RMS evaluator has calculated the brood to food ratio for the observation point prior to application of the test item. This is presented in table Table B9.5.1-29 below :

Table B9.5.1-29: Brood to food ratio prior to treatment

Treatment	Brood (total)	Food (nectar and pollen)	Brood :Food ratio
Control	10553	18480	1: 1.75 (4: 7.00)
Test item	9450	18375	1:1.94 (4:7.78)
Reference item	10920	19425	1:1.78 (4:7.12)

Analytical findings

The exposure of the honeybees to the test item was confirmed by analytical measurement of the active substance isoflucypram in the spray solution samples taken from the biological assessment tunnels (replicates a-d) and the additional residue tunnels (replicates Te, Tf and Tg). The concentration of isoflucypram in both groups of tunnels was in a comparable range so that it is assumed that the exposure conditions were comparable in all tunnels treated with the test item.

In those tunnels allocated to residue determination, honeybees were used as sampling device. The concentration of isoflucypram measured in the collected pollen and nectar samples of the day of application (0DAA) and the day after (1DAA) allows for confirmation of the exposure of the bees inside the tunnels.

The following table gives an overview of the concentration of isoflucypram in the analysed sample materials after application of Isoflucypram EC 50 G with 75 g a.s./ha in 400 L water/ha. Results are shown in Table B9.5.1-30 below :

Table B9.5.1-30: Analytical determination of BCS-CN88460 in nectar, pollen and the spray solution.

Sample Material	Test Item	Sampling Day	BCS-CN88460	
			Concentration [mg/kg]	Mean Concentration [mg/kg]
Nectar	BCS-CN88460	DAA0	0.049– 0.096	0.073
		DAA1	<LOQ – 0.013	0.01
Pollen		DAA0	22 - 42	33
		DAA1	2.1 – 2.8	2.5
Spray Solution		DAA0	Ta - Td: 92 - 142	128
			Te - Tg: 118 - 144	135

LOQ = Limit of Quantification = 0.01 mg/kg (= 10 µg/kg = 10 ppb) for BCS-CN88460
LOD = Limit of Detection = 0.003 mg/kg (= 3 µg/kg = 3 ppb) for BCS-CN88460
DAA = Days after application
Ta-Td = biological assessment tunnel, Te-Tg = residue analysis tunnel

Analyte:	Final determination as:	Residues calculated as:
BCS-CN88460	BCS-CN88460	BCS-CN88460

Conclusion:

Isoflucypram EC 50 G was applied at 75 g a.s. in 400 L/ha (1.46 L product/ha) during full flowering of the surrogate crop *Phacelia tanacetifolia* and with honey bees present.
Overall, no clear adverse effects on mortality and no adverse effects on flight activity, behaviour, colony strength, the amount of brood or on the development of the food storage area were observed.
Based on the results of this study, it can be concluded that Isoflucypram EC 50 G does not adversely affect honey bee behaviour, brood development, colony strength and queen survival when applied at a rate of 75 g a.s. in 400 L/ha (1.46 L product/ha) under the above described conditions.

RMS comments

This study was conducted according to GLP and following EPPO (2010) guidance.

The analytical methods from this study have been acceptably validated (see section B5.1.2.6.5 of the CA document for further details).
The conduct of this study will be considered in the risk assessment in section B9.6.

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

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

This is considered to represent a data point for active substances according to (EU) No. 283/2013. These study summaries can therefore be found at CA B.9.3.2

B.9.5.2.2. Extended laboratory testing, aged residue studies with non-target arthropods

Report:	KCP 10.3.2.2/01; Waibel, J.; 2017; M-583441-01-1
Title:	Toxicity to the parasitoid wasp Aphidius rhopalosiphi (Hymenoptera: Braconidae) using an extended laboratory test on barley BCS-CN88460 EC 50 g/L
Report No.:	CW16/038
Document No.:	M-583441-01-1
Guideline(s):	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; MEAD-BRIGGS ET AL. (2010), CANDOLFI ET AL. (2001)
Guideline deviation(s):	none
GLP:	yes

Objective:

The objective of this extended laboratory study was to investigate the lethal and sublethal toxicity of BCS-CN88460 EC 50 on the parasitoid wasp *Aphidius rhopalosiphi* when exposed on a plant surface.

Material and methods:

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

Toxic reference item: active substance: dimethoate, applied at 4 g a.s./ha was included to indicate the relative susceptibility of the test organisms and the test system.

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

Rhopalosiphum padi (aphids used for parasitization in the reproduction assessment) were taken from the breeding of the testing facility.

The barley seedlings (variety: Marthe) were provided by the horticultural group of BAG-CS-RD-SMRWeed Control (formerly BCS-R&D-SMR Weed-Control).

Test design: Five adult female wasps per replicate (six replicates for each concentration of the test item, the control and the reference item, giving a total of 30 wasps per treatment condition) were exposed to each test concentration, control or reference item for 48 hours to assess mortality.

The test item was applied on barley seedlings (3D substrate) (*Hordeum vulgare*) at rates of 7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha in 400 L deionised water/ha and the effects on the parasitoid wasp *Aphidius rhopalosiphi* were compared to those of a deionised water treated control. A toxic reference (active substance: dimethoate) applied at 4 g a.s./ha was included to indicate the relative susceptibility of the test organisms and the test system.

Mortality and condition of the test organisms were assessed 2, 24 and 48 h after exposure started. They were classified according to the following:

- Live (alive and apparently unaffected)

- Affected (showing reduced co-ordination or any abnormal behaviour)

- Moribund (unable to walk, but still moving legs or antennae)

- Dead (no longer moving)

Repellency of the test item was assessed during the initial 3 h after the release of the females. Five separate observations were made at 30-minute intervals starting 15 - 30 minutes after the introduction of all wasps. An additional repellency assessment for the control and the 13.3, 23.7, 42.2 and 75.0 g a.s./ha rates of the test item was conducted 24 h after the release of the wasps into the exposure units.

From the water control and all test item rates,

20 impartially chosen females per treatment were each transferred to a cylinder containing untreated barley seedlings infested with *Rhopalosiphum padi* for a period of 24 h. The number of mummies was assessed 11 days later.

Endpoints: LR_{50}, ER_{50}

Test concentrations: Control, 7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha in 400 L deionised water/ha.

Application method: The suspensions for the test and reference items were prepared at the day of application and were applied to the test plants using a linear cabinet track sprayer which had been calibrated by weighing of five glass plates. Within, the first hour after application the potted seedlings were enclosed within the polyacrylic cylinder and the test organisms were introduced.

Test conditions: The mummies obtained from the breeder were distributed to several glass tubes. Each tube was inserted in a hatching cage so that the hatched wasps could move into the cage. This cage consisted of two glass plates (100 x 100 x 3 mm) and an acryl frame (inner size 92 x 92 x 14 mm) with three ventilation holes on each side. Ten holes were covered with gauze (mesh size 80 µm) which was jammed in the orifices by a piece of Teflon tubing (8 mm outer diameter). One of these orifices was left uncovered for the introduction of the wasps, another one for the insertion of a feeding tube.

Until the start of the study the mummies were stored at a temperature range of 25.0 – 26.0 °C and a relative humidity range of 60 - 73%. The insects were fed via a feeding tube filled with a solution which consisted of 3 parts of water + 1 part honey.

Two days prior to the start of the study the tubes with still unhatched mummies were inserted in empty glass cages to ensure that the newly hatched wasps are not older than 48 hours.

For the mortality assessment seven days prior to the start of the study the barley seedlings were sown (8 -10 per pot). The seedlings had a uniform height of 10 - 11 cm at the day of application. Each test unit consisted of a pot with treated barley seedlings which was enclosed within a clear polyacrylic cylinder (195 mm high and 100 mm in diameter) with a hole (approximately 5 mm in diameter) for the introduction of the parasitoids. After introduction the hole was closed by a stopper. The top of the cylinder was closed with a fine mesh gauze.

Prior to application the seedlings were sprayed with a 10% fructose solution and were left to dry for at least one hour. The sugar provides both food and a foraging stimulus for the wasps. Finally, the soil surface was covered with a thin layer of quartz sand before treatment.

For the reproduction assessment five days prior to the start of the study the barley seedlings required were sown (18 - 20 seed grains each). One day after the start of the study the seedlings were infested with *Rhopalosiphum padi*, and the soil surface of the pots was covered with quartz sand.

The climatic test conditions during the study were 19.0 - 21.0 °C temperature and 63 - 85% relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 524 - 685 Lux in the mortality phase, 2510 - 4370 Lux in the parasitisation phase and 9200 - 15960 Lux in the reproduction phase of the study.

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

The reproduction and repellency data were tested for normal distribution using the Shapiro-Wilk test and for homogeneity of variance using the Levene test.

As the repellency data in this study were not normally distributed the Wilcoxon test (one-sided with Bonferroni-Holm adjustment; $\alpha = 0.05$) was used.

As the reproduction data in this study were not normally distributed the Wilcoxon test (one-sided with Bonferroni-Holm adjustment; $\alpha = 0.05$) was used. The ER50 value was calculated using Spearman-Karber method. The computer program SAS (Version 9.4, 2002-2012) was used to perform the statistical analyses.

Findings:

Validity criteria

Validity criteria	Required	Obtained
Control mortality	≤ 13 %	0%
Toxic reference item mortality	≥ 50 %	87%

Mean number of mummies per female in control	≥ 5	31
Number of female wasps producing zero values	≤ 2	0

All validity criteria were met for the study.

Biological results

Mortality

The corrected mortality in all test item rates (7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha) was below 7%. All test item rates were not statistically significantly different compared to the control. (see Table B.9.2.2-1)

Repellency

Repellent effects of the test item (settling of the wasps on plants <30%) were observed in the first 3 h after the introduction of the wasps into the exposure units at the test item rates of 13.3, 23.7, 42.2 and 75.0 g a.s./ha (table B.9.2.2-1). No further repellent effects were observed after 24 h.

Reproduction

Reproduction was assessed for all rates of BCS-CN88460 EC 50, 7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha. The reduction in reproductive success relative to the control at the 7.5 and 13.3 g a.s./ha rate was 45.8% and 53.8%. At the higher test item rates of 23.7, 42.2 and 75.0 g a.s./ha the reproduction was reduced by 77.0%, 62.8% and 60.6%, respectively. All test item rates were statistically significantly different compared to the control.

A summary of the effects observed in this study is given in table B.9.2.2-1.

Table B.9.2.2-1: Mortality and reproductive performance of *Aphidius rhopalosiphi* following exposure to Isoflucypram EC 50, a deionised water control or a toxic reference substance (dimethoate) on a 3-Dimensional natural substrate during an extended laboratory test.

Test item		BCS-CN88460 EC 50						
Test organism		*Aphidius rhopalosiphi*						
Exposure on		Barley seedlings						
		Mortality after 48 h [%]			Reproduction			Repellency (first 3 h)
Treatment	g a.s./ha	un-corr.	corr.	P-Value (*)	Rate (mummies per female)	Reduction relative to control [%]	P-Value (#)	% Wasps on plant P- Value (#)
Control	0	0			31.2			38.7
Test item	7.5	0.0	0.0	1.000 n. sign.	16.9	45.8	0.010 sign.	34.7 0.374 n. sign.
Test item	13.3	0.0	0.0	1.000 n. sign	14.4	53.8	0.003 sign.	21.5 0.504 n. sign.
Test item	23.7	6.7	6.7	1.000 n. sign	7.2	77.0	< 0.001 sign.	19.3 0.568 n. sign.
Test item	42.2	6.7	6.7	1.000 n. sign	11.6	62.8	< 0.001 sign.	25.2 0.689 n. sign.
Test item	75.0	3.3	3.3	1.000 n. sign	12.3	60.6	< 0.001 sign.	18.2 0.591 n. sign.
Reference item	4.0	86.7	86.7		n.a.	n.a.		35.8

LR_{50}: > 75.0 g a.s./ha

ER_{50}: 17.8 g a.s./ha; 95 % Conf. Interv.: (13.5 - 23.4) (calculated with Spearman-Karber) – **note: this value has subsequently been changed by the RMS (see RMS Comments)**

* Fisher`s Exact test (one-sided), p-values are adjusted according to Bonferroni-Holm

Wilcoxon test (one-sided), p-values are adjusted according to Bonferroni-Holm

n.a. not assessed n. sign. not significant sign. significant

Conclusion:

The LR_{50} was estimated to be > 75.0 g a.s./ha. The NOER for mortality was 75.0 g a.s./ha.

The ER_{50} was calculated by the applicant to be 17.8 g a.s./ha. This has subsequently been changed by the RMS (see RMS Comments). The NOER for reproduction was < 7.5 g a.s./ha.

The figures obtained fulfil the validity criteria of the extended laboratory method (MEAD-BRIGGS ET AL., 2010).

RMS Comments

This study was evaluated according to the guidelines of Meads-Briggs *et al*. (2000). The study was compliant with GLP and all validity criteria were met.

The ER_{50} value was calculated to be 17.8 g a.s./ha following exposure to residues on a 3-Dimensional natural substrate by the study author. However, the observed reproductive effects in the study does not support the report-proposed ER_{50}, due to the wide associated confidence intervals and the observed 53.8% effects on reproduction at a tested rate below the ER_{50}. **As such the RMS is only confident to define that 50% effects on reproduction will occur at > 7.50 g a.s./ha.**

This study is considered valid and acceptable for use in the risk assessment.

Previous evaluation:	New data submitted for purpose of review

Report:	KCP 10.3.2.2/02; Waibel, J.; 2017
Title:	Toxicity to the predatory mite Typhlodromus pyri (Acari: Phytoseiidae) using a laboratory test on bean BCS-CN88460 EC 50 g/L
Report No.:	CW16/037
Document No.:	M-608958-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable BLÜMEL ET AL. (2000) CANDOLFI ET AL. (2001)
Guideline deviation(s):	none
GLP:	yes

Objective:

The objective of this extended laboratory study was to investigate the lethal and sublethal toxicity of BCS-CN88460 EC 50 to the predatory mite *Typhlodromus pyri* when exposed to treated leaf surfaces.

Material and methods:

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

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

The bean plants (*Phaseolus vulgaris*; variety: Jutta) were provided by the horticultural group of BAG-CS-RD-SMR-Weed Control.

Test design: 20 *Typhlodromus pyri* protonymphs per replicate (five replicates per treatment condition) were exposed to each test concentration (5 test concentrations), 1 deionised water control and 1 toxic reference item

(dimethoate). The study was carried out in accordance with the guidelines of Blümel et al. (2000), modified for the use of a 2-dimensional natural substrate; in this case detached leaves of bean plants.

The test item was applied onto detached bean leaves (*Phaseolus vulgaris*) at rates of 7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha in 200 L deionised water/ha using a calibrated laboratory track sprayer (mean measured application rate: 193 L/ha). The effects of the test item on the predatory mite *Typhlodromus pyri* were compared to those of a deionised water treated control. A reference item (active substance: dimethoate) applied at 20.0 g a.s./ha in 200 L deionised water/ha was included.

A treated *Phaseolus vulgaris* leaf disc was laid on a layer of wet filter paper on top of a water soaked floral foam. A circle of insect glue (ø approximately 40 mm) was formed on the leaves. Sets of such units were placed on a plastic tray such that the filter paper was constantly provided with deionised water.

Day 0: After the test units were set up the protonymphs were placed onto the exposure units by test group (within one and a half hour after application). The mites were transferred with a fine brush under a stereomicroscope and immediately afterwards examined to ensure they were undamaged and in good condition. To avoid contamination, a fresh brush was used for each treatment group. Then pollen (birch - pine mixture) was supplied as food and the units were maintained under the climatic conditions of the test (see 3.2). The water supply for the mites was ensured by sticking a pin into each of the leaves.

Day 4: The number of dead and living mites was counted. Dead mites were removed with a fine brush. The number of escaped mites was calculated. Food was replenished.

Day 7: The number of dead and living mites was counted, the dead mites were removed, and the number of escaped mites was calculated. The number of females, males, eggs and juveniles was counted. Eggs and juveniles were removed. The sex-ratio (number of females per exposure unit divided by the total number of males and females) was assessed. Food was replenished.

Day 10, 12 and 14: The number of dead and living mites was counted, the dead mites were removed, and the number of escaped mites was calculated. The number of females, males, eggs and juveniles was counted. Eggs and juveniles were removed. Food was replenished on day 10 and 12.

Endpoints: LR_{50}, ER_{50}

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

Application method: The suspensions for the test and reference item were prepared on the day of application. They were applied to the upper side of detached *Phaseolus vulgaris* leaf discs using a linear cabinet track sprayer with a spray pressure of 3.00 bar, and application speed of 2.0 km/h and a distance (nozzle – target area) of 32 cm.

In accordance with the guideline the substances were then applied with 200 L deionised water/ha. The sprayer was calibrated beforehand to deliver 200 L/ha ±10 % by spraying glass plates of known weight and area and weighing them immediately after in order to determine the actual amount of water applied. The mean measured application rate was 193 L/ha calculated based on the values from 5 weighed glass plates. The uniformity of the deposit distribution was checked visually.

Test conditions: The climatic test conditions during the study were 23.5 - 25.0 °C temperature and 60 - 72% relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 96 - 399 Lux.

Statistics: The mortality data were analysed for significance using the Fisher Exact test (one-sided with Bonferroni-Holm adjustment; $\alpha = 0.05$). The reproduction data were tested for normal distribution using the Shapiro-Wilk test ($\alpha = 0.05$) and for homogeneity of variance using the Levene test ($\alpha = 0.05$). As the reproduction data in this study were normally distributed but not homogenous, the Welch test ($\alpha = 0.05$) was used.

The non-linear analysis of the reproduction data (non-GLP) did not reveal a reliable ER50 value (effect rate causing 50% effects on reproduction). Due to a reduction of 64 % in the highest treatment rate relative to the control, an ER50 > 42.2 g a.s./ha is considered.

The computer program SAS (Version 9.4) and the software environment R (Version 3.3.2; non-GLP) was used to perform the statistical analyses.

Findings:

Validity criteria

	Required	Obtained
Mortality/Escape rate in the control group on day 7	≤ 20%	11.0%
Average corrected mortality in the reference item	≥ 50%	84.3%
Average number of eggs/females (calculated as sum of 3 assessment dates – from day 7 on) in the control group	≥ 4	4.9

The validity criteria are met.

Biological findings

Mortality

The mortality / escaping rate in the control exposure units up to day 7 after application was 11.0%.

At the rates of 7.5, 13.3 and 23.7 g a.s./ha, no statistically significantly different mortality compared to the control occurred. A statistically significant mortality was found in the group treated with 42.2 g a.s./ha (Fisher`s Exact test, one-sided, $\alpha = 0.05$). At the highest rate of 75.0 g a.s./ha, no statistically significant mortality was detected.

At the lower rates of 7.5, 13.3 and 23.7 g a.s./ha, the corrected mortality was 6.7%, 9.0% and 4.5%, respectively. At the 42.2 g a.s./ha rate, a corrected mortality of 19.9% was found. 10.1% corrected mortality were detected at the highest test item rate of 75.0 g a.s./ha.

In the reference item group, the corrected mortality was 84.3% on day 7 of the study. (see table B.9.2.2-2 for results).

Reproduction

Reproduction was assessed for all rates of BCS-CN88460 EC 50 g/L. At the lower rates of 7.5, 13.3 and 23.7 g a.s./ha, the reduction of reproduction was 44.4%, 31.3% and 19.2%, respectively, which was statistically significantly different compared to the control (Welch test, one-sided, $\alpha = 0.05$). A reduction of 21.2% was found in the 42.2 g a.s./ha rate which was not statistically significant. At the highest test item rate of 75.0 g a.s./ha, a statistically significant reduction of reproduction of 64.0% was found (Welch test, one-sided, $\alpha = 0.05$). See table B.9.2.2-2 for results.

Table B.9.2.2-2: Mortality and reproductive performance of *Typhlodromus pyri* following exposure to Isoflucypram EC 50, a deionised water control or a toxic reference substance (dimethoate) during an extended laboratory test on detached bean leaves.

Test item		BCS-CN88460 EC 50					
Test organism		*Typhlodromus pyri*					
Exposure on		Detached bean leaves					
		Mortality after 7 days [%]			Reproduction		
Treatment	g a.s./ha	uncorr.	corr.[A]	P-Value [B]	Rate (eggs per female)	Reduction relative to control [%]	P-Value (#)
Control	0	11.0			4.9		
Test item	7.5	17.0	6.7	0.308 n.sign.	2.7	44.4	0.005 sign.
Test item	13.3	19.0	9.0	0.247 n.sign.	3.4	31.3	0.004 sign.
Test item	23.7	15.0	4.5	0.308 n.sign.	4.0	19.2	0.021 sign.
Test item	42.2	28.8	19.9	0.012 sign.	3.9	21.2	0.137 sign.
Test item	75.0	20.0	10.1	0.234 n.sign.	1.8	64.0	0.002 sign.
Reference item	20.0	86.0	84.3		n.a.	n.a.	

LR_{50} > 75 g a.s./ha

ER_{50} > 42.2 g a.s./ha
[A] Corrected mortality according to SCHNEIDER-ORELLI (1947)
[B] Fisher's Exact test (one-sided, $\alpha = 0.05$)
n.a. = not assessed, n. sign = not significant, sign. = significant.

Conclusion:

The LR_{50} was estimated to be > 75.0 g a.s./ha. The NOER for mortality was ≥ 75.0 g a.s./ha.

The ER_{50} was calculated to be > 42.2 g a.s./ha. The NOER for reproduction was < 7.5 g a.s./ha.

The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates BLÜMEL ET AL., 2000).

RMS comments

This study was conducted to the guidelines of (Blümel et al. 2000) but modified as an extended laboratory study using detached bean leaves treated with the test item for the mortality exposure period. The test was compliant with GLP and all validity criteria were met.

In this study the reference item was applied at a rate of 20 mL dimethoate/ha, resulting in a mean mortality of 84.3 %. The volume recommended in the guidelines Blümel et al. 2000 is only 9-15 ml formulation/ha. However, this rate is for use on glass plates, whereas in this study a 2-Dimensional natural substrate (maize leaves) was used, which is likely to result in lower levels of bioavailable residue, hence a higher applied amount. Thus, the RMS considers the volume of reference item applied to be acceptable.

The proposed LR_{50} is >75 g a.s./ha following exposure to residues on a 2-Dimensional natural substrate. This is considered to be appropriate for use in the risk assessment by the RMS. The ER_{50} value could not be determined by the non-linear analysis of the reproduction data. However, it can however be confirmed by the RMS that **50% effects on reproductive capacity would be expected at > 42.2 g a.s./ha, following exposure to the test item on a 2-Dimensional natural substrate.**

Previous evaluation:	New data submitted for purpose of review

Report:	KCP 10.3.2.2/03; Waibel, J.; 2017;
Title:	Toxicity to the green lacewing Chrysoperla carnea (Neuroptera: Chrysopidae) using an extended laboratory test on bean - BCS-CN88460 EC 50 g/L
Report No.:	CW16/039
Document No.:	M-601137-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable VOGT ET AL. (2000) modified CANDOLFI ET AL. (2001)
Guideline deviation(s):	None
GLP:	Yes

Objective:

The purpose of this study was to investigate the lethal and sublethal toxicity of BCS-CN88460 EC 50 g/L to the green lacewing *Chrysoperla carnea* when exposed to treated leaf surfaces.

Material and methods:

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

Reference item: active substance: dimethoate at 36.0 g a.s./ha in 200 L deionised water/ha

Test species: Larvae of *Chrysoperla carnea* (2 days old) were used as test organisms. The original source of the larvae had been BioChem Agrar, Machern, Germany; the rearing in the testing facility started 2006 (rearing conditions were 23 - 27 °C, 60 - 90% rel. humidity, day length 16:8 h with a light intensity of >1000 Lux, food: eggs of *Ephestia kuehniella, Heliothis virescens or Sitotroga cerealella*).

Eggs of *Ephestia kuehniella* (food for the larvae) were supplied by Katz Biotech AG, 15837 Baruth, Germany.

The bean plants (*Phaseolus vulgaris*; variety: Jutta) were provided by the horticultural group of BAG-CSRD-SMR-Weed Control.

Test design:

Summary

The preimaginal mortality of 40 larvae (per test group), 2 days old at study start, was assessed till the hatch of the imagines up to 20 days (larvae food = UV-sterilized eggs of *Ephestia kuehniella*). The fertility and fecundity of the surviving hatched adults were then evaluated over the period of one week (adult food = artificial diet).

Preparation of test organisms

Lacewing eggs which have been laid within a 24 h - period were put into hatching boxes closed with a gauze lid. The new larvae were fed with eggs of *Ephestia kuehniella*. The eggs and larvae were maintained under the climatic conditions of the test (25 °C ±2 °C, 60 - 90% rel.humidity) until the start of the study.

Application

The suspensions for the test and reference item were prepared on the day of application. They were applied to the upper side of detached bean leaf discs using a linear cabinet track sprayer with a spray pressure of 3.00 bar, and application speed of 2.0 km/h and a distance (nozzle – target area) of 33 cm.

In accordance with the guideline the substances were then applied with 200 L deionised water/ha. The sprayer was calibrated beforehand to deliver 200 L/ha ±10 % by spraying glass plates of known weight and area and weighing them immediately after in order to determine the actual amount of water applied. The mean measured application rate was 195 L/ha calculated based on the values from 5 weighed glass plates. The uniformity of the deposit distribution was checked visually.

A treated bean leaf disc was laid on a wet cotton wool pad in a petri dish (9 cm diameter). A constant supply of deionised water from the bottom was assured. As individual test unit one steel ring (about 4.1 cm diameter and 3 cm high) was placed on the leaf. The top inner edge of the ring was coated with a lubricant (PTFE) in order to prevent the larvae from escaping. One larva (2 days old) was added to each test unit (within the first hour after application).

Assessment

All exposure units were assessed daily and the condition of the lacewings recorded. The larvae were fed with UV-sterilized eggs of *Ephestia kuehniella* ad libitum. At least once a week, old eggs from earlier feeding sessions were removed, in order to maintain a constant contact between the larvae and the treated leaf surface.

5 to 7 days after pupation the pupae of one test group were transferred into one glass jar (2.5 L) closed with a gauze lid. Emerging adults were kept in these glass jars and fed with an artificial diet (consisting of condensed milk, egg, egg yolk, honey, fructose, brewer's yeast, wheat germ and water) and supplied continuously with water through a cotton plug.

The reproduction phase started with the adult lacewings being sexed and transferred to cylinders of black paper (Ø = 10 - 12 cm; 15 - 17 cm high); all lacewings of one test group together. These were closed at both ends with gauze.

After an egg laying period of 24 h the adult lacewings were transferred into new glass jars and the cylinders with the gauze were put into plastic boxes closed with a gauze lid. UV-sterilized eggs of *Ephestia kuehniella* were provided as food for the newly hatched larvae to avoid cannibalism. For the determination of the hatching rate all eggs (hatched and non-hatched) on both the gauze and on the cylinder were counted. Two egg samples were taken within one week (e.g. from Monday to Tuesday and Thursday to Friday) and used for the fertility and fecundity assessments.

The test item was applied to detached bean leaves (*Phaseolus vulgaris*) at rates of 7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha in 200 L deionised water/ha using a calibrated laboratory track sprayer (mean measured application rate: 195 L/ha). The effects of the test item on the green lacewing *Chrysoperla carnea* were compared to those

of a deionised water treated control. A reference item (active substance: dimethoate) applied at 36 g a.s./ha in 200 L deionised water/ha was included to indicate the relative susceptibility of the test organisms and the test system.

Endpoint: LR_{50}, NOER

Test concentrations: Control, 7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha

Test conditions: The climatic test conditions during the study were 24.5 - 25.5 °C temperature and 69 - 76% relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 1770 - 3585 Lux during the mortality phase and of 2740 - 3250 Lux during the reproduction phase of the study.

Statistics: The number of dead larvae was recorded daily. The corrected mortality was obtained by comparing the values observed in the treated samples with those in the control samples, according to the formula of SCHNEIDER-ORELLI (1947). After the successful hatch of the last adult the mortality of the pupae was calculated in the same way (test organisms which died during hatching were counted as dead) and the figures added to those of the larval mortality in order to calculate the preimaginal mortality.

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

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

For each egg sampling of each test group, the number of eggs laid per female was determined by dividing the total number of eggs by the mean number of viable females, corrected for mortality during the egg laying.

Finally, the mean number of eggs laid per female per day (= fecundity) as well as the hatching rate (= fertility) for the total assessment period was calculated by averaging the respective values of the two egg samplings.

Findings:

Validity criteria

	Required	**Obtained**
Mortality in water control	≤ 20%	5.0%
Corrected mortality reference item	≥ 50%	57.9%
Mean number of eggs per female and day in water control	≥ 15	25.1
Mean hatching rate of the eggs (fertility) in water control	≥ 70%	80.7%

All validity criteria were met for the study.

Biological results

In this extended laboratory test the effects of BCS-CN88460 EC 50 g/L residues on the survival of the green lacewing *Chrysoperla carnea* were determined at the rates of 7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha applied to detached bean leaves (*Phaseolus vulgaris*).

No statistically significantly different mortality compared to the control was found in any test item rate (Fisher`s Exact test, one-sided).

Reproduction was assessed for all rates of BCS-CN88460 EC 50 g/L. There were no adverse effects of the test item on the reproductive performance. The mean number of eggs/female/day was above the lower limit given as validity criterion for the glass plate method (mean number of eggs/female/day: ≥ 15, mean hatching rate: ≥ 70%) according to the historical database of the ring testing group (VOGT ET AL., 2000). A summary of the results is provided for mortality and reproductive performance in Table B.9.2.2-3.

Table B.9.2.2-3: Mortality and reproductive performance of *Chrysoperla carnea* following exposure to Isoflucypram EC 50, a toxic reference substance (dimethoate) or a deionised water control during an extended laboratory test on detached *Phaseolus vulgaris* leaves.

Test item		BCS-CN88460 EC 50 g/L					
Test organism		*Chrysoperla carnea*					
Exposure on		Detached bean leaves					
		Preimaginal mortality [%]			Reproduction		
Treatment	g a.s./ha	Uncorrected	Corrected (*)	P-Value (**)	Eggs per female and day	Fertility [hatching rate in %]	Reduction compared to control (%) [a)]
Control	0.0	5.0			25.1	80.7	
Test item	7.5	0.0	-5.3	1.000 n.sign.	18.3	75.7	6.2
Test item	13.3	17.5	13.2	0.386 n.sign.	22.0	79.6	1.4
Test item	23.7	5.0	0.0	1.000 n.sign.	23.0	83.2	-3.1
Test item	42.2	17.5	13.2	0.386 n.sign.	21.7	79.7	1.2
Test item	75.0	15.0	10.5	0.395 n.sign.	20.0	82.7	-2.5
Reference item	36.0	60.0	57.9		n.a.	n.a.	

LR_{50}: > 75 g a.s./ha
* Corrected mortality according to SCHNEIDER-ORELLI (1947)
** Fisher`s Exact test (one-sided), p-values are adjusted according to Bonferroni-Holm
n.a. not assessed n.sign. not significant
[a)] A negative number indicates an increase relative to the control

Conclusion:

The LR_{50} was estimated to be > 75.0 g a.s./ha.

The reproductive performance was not statistically significantly affected up to and including the test item rate of 75.0 g a.s./ha. The figures obtained fulfil the validity criteria of the laboratory method for the exposure on glass plates (VOGT ET AL., 2000).

RMS Comments

This study was performed according to the guidelines of Vogt et al. (2000), modified to include the use of detached leaves as a test substrate (Candolfi et al. 2001) for mortality exposures. The study was compliant with GLP.

The concentration of the dimethoate toxic reference was 36.0 g a.s./ha (equivalent of 90 ml/ha) with 57.9% mortality resulting following exposure. A volume of 30-45 mL/ha is recommended in the Vogt et al. (2000) guidelines for a mortality of > 60 %. Although the concentration of dimethoate would ideally be lower, the validity criteria state that the mortality level in the reference treatment should be ≥ 50 and therefore this result meets the validity criteria. In addition, the use of a natural substrate, as in this study, is likely to result in lower levels of bioavailable residue, hence a higher applied amount of the reference substance is required for the same effect, and thus the RMS consider this deviation to be acceptable.

The result is considered valid for use in risk assessment:

LR_{50} > 75.0 g a.s./ha.

ER_{50} for reproductive parameters > 75.0 g a.s./ha.

Previous evaluation:	New data, submitted for purpose of review

Report:	KCP 10.3.2.2/04; Müller, R. U.; 2017;
Title:	Toxicity to the ladybird beetle Coccinella septempunctata (Coleoptera: Coccinellidae) using an extended laboratory test on bean BCS-CN88460 EC 50 g/L
Report No.:	CW17/010
Document No.:	M-608806-01-1
Guideline(s):	Schmuck et al. (2000) modified Candolfi et al. (2001)
Guideline deviation(s):	none
GLP/GEP:	yes

Objective:

The purpose of this study was to investigate the lethal and sublethal toxicity of BCS-CN88460 EC 50 g/L to the ladybird beetle *Coccinella septempunctata* when exposed to treated leaf surfaces.

Material and methods:

Test item: BCS-CN88460 EC 50, Supplier batch No: 2016-001002, Spec. no: 102000031262, analysed content of active substance isoflucypram: 5.28% w/w (51.45 g/L).

Reference item: (active substance: dimethoate) applied at 12 g a.s./ha in 200 L deionised water/ha.

Test species: Larvae of *Coccinella septempunctata* (4 days old at study start) were used as test organisms. The test organisms were supplied as eggs by Katz Biotech AG, 15837 Baruth, Germany. The original source of the beetles had been IACR Rothamsted and INRA Antibes and a wild population of Czechia. The rearing in the laboratory of Katz Biotech started in 1999, since 2001 the culture was periodically refreshed with wild beetles sampled in the region of Welzheim, Germany (rearing conditions: 22 - 28 °C, 60 - 80% rel. humidity, daylength 16:8 h with a light intensity of > 3000 Lux, food: mixture of *Acyrthosiphon pisum* and *Megoura viciae*, bee-pollen and honey).

Acyrthosiphon pisum (aphids as food for the ladybirds) was taken from the breeding of the testing facility.

The bean plants (*Phaseolus vulgaris*; variety: Jutta) were provided by the horticultural group of BAG-CS-RD-SMR-Weed Control.

Test design:

Summary

The test item was applied to detached bean leaves (*Phaseolus vulgaris*) at rates of 7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha in 200 L deionised water/ha using a calibrated laboratory track sprayer (mean measured application rate: 203 L/ha). The effects of the test item on the ladybird beetle *Coccinella septempunctata* were compared to those of a deionised water treated control. A reference item (active substance: dimethoate) applied at 12 g a.s./ha in 200 L deionised water/ha was included.

Preparation of the test organisms

The ladybird beetle eggs obtained from the breeder were put into hatching boxes closed with a gauze lid and maintained under the climatic conditions of the test (25 °C ±2 °C, 60 - 90% rel. humidity) until the start of the study. The newly hatched larvae were fed with aphids (*A. pisum*). The study was performed with 4-day old larvae.

Application

The suspensions for the test and reference item were prepared on the day of application. They were applied to the upper side of detached *Phaseolus vulgaris* leaf discs using a linear cabinet track sprayer

Exposure

A treated *Phaseolus vulgaris* leaf disc was laid on a wet cotton wool pad in a petri dish (9 cm diameter). A constant supply of deionised water from the bottom was assured. Each individual test unit was built by placing a steel ring (about 4 cm diameter and 3 cm high) on the leaf disc. The top inner edge of the rings was coated with a lubricant (PTFE) in order to prevent the larvae from escaping (except the lower 3 mm to avoid contamination of larvae which adversely affects metamorphosis). One larva (4 days old) was added to each test unit (within the first hour after application).

Assessment

The preimaginal mortality of 40 larvae, 4 days old at study start (per test group), was assessed daily until the hatch of the imagines at 15 days (food = *Acyrthosiphon pisum*). At every feeding session dead aphids and exuviae from earlier feeding sessions were removed, in order to maintain a constant contact between the larvae and the treated surface.

Once the larvae had pupated and the pupae hatched, all emerged beetles of one test group were transferred to one glass jar (25 L) closed with a gauze lid and the sex of the beetles was determined. The beetles were fed with fresh aphids, pollen and water *ad libitum.* The reproduction assessment of the surviving hatched adults started one week after the first eggs in the control could be observed. The number of fertile eggs laid per viable female was recorded over a period of two weeks. Folded sheets of black paper were offered to the beetles for the egg-laying. These sheets were checked daily and freshly laid eggs were cut out of the paper and stored in petri dishes under test conditions until larval hatch.

Mortality of the beetles during the egg laying period was recorded and the sex of dead individuals was determined.

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

Test conditions: The climatic test conditions during the study were 23.5 - 27.0 °C temperature and 60 - 75% relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 1890 - 3470 lux during the study.

Statistical analysis:

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

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

Findings:

Validity Criteria

	Validity criteria	**Finding**
Preimaginal mortality in water control	≤ 30%	15.0%
Preimaginal mortality reference item	> 40%	97.1%
Mean number of fertile eggs per female and day in water control	≥ 2	10.0

All of the validity criteria were met.

Biological results

In this extended laboratory study, the effects of BCS-CN88460 EC 50 g/L residues on the survival of the ladybird beetle *Coccinella septempunctata* were determined at the rates of 7.5, 13.3, 23.7, 42.2 and 75 g a.s./ha applied to detached bean leaves (*Phaseolus vulgaris*).

Table B.9.2.2-4: Mortality and reproductive performance of *Coccinella septempunctata* following exposure to Isoflucypram EC 50, a toxic reference substance (dimethoate) or a deionised water control during an extended laboratory test on detached bean leaves.

There were no		BCS-CN88460 EC 50 g/L				
Test organism		*Coccinella septempunctata*				
Exposure on		Detached bean leaves				
		Preimaginal mortality [%]			Reproduction	
Treatment	g a.s./ha	uncorrected	corrected (*)	P-Value (**)	Fertile eggs per female and day	Reduction compared to the control (%)
Control	0.0	15.0			10.0	
Test item	7.5	10.5	-5.3	1.000 n.sign.	8.1	19%
Test item	13.3	13.2	-2.2	1.000 n.sign.	8.8	12%
Test item	23.7	5.0	-11.8	1.000 n.sign.	7.0	30%
Test item	42.2	17.5	2.9	1.000 n.sign.	6.9	31%
Test item	75.0	12.5	-2.9	1.000 n.sign.	7.6	24%
Reference item	12.0	97.5	97.1		n.a.	

LR_{50}: > 75.0 g a.s./ha
* Corrected mortality according to SCHNEIDER-ORELLI (1947)
** Fisher`s Exact test (one-sided, a=0.05), p-values are adjusted according to Bonferroni-Holm
n.a. not assessed n.sign. not significant

Conclusion:

The LR_{50} was estimated to be > 75 g a.s./ha. The NOER for mortality was ≥ 75 g a.s./ha.

The reproductive performance was affected by the test item but the reduction in reproduction was by less than 50% for each tested rate. The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates (SCHMUCK ET AL., 2000).

RMS Comments

This study was compliant with GLP and the test design is a modified version of that detailed in Schmuck *et al* (2000).

It is noted that Schmuck *et al* (2000) recommend use of Afugan 30 EC (active ingredient: pyrazophos) for the reference item for ladybird studies. It is noted that the reference item used in this study (Dimethoate EC 400 g/L) is not one that is suggested in the guidelines. Additionally, there is no historical data reported for Dimethoate in order to assess if the sensitivity of this organism has been tested at the correct concentrations.

This study and its endpoints are considered valid and appropriate to use in risk assessment:

LR_{50} > 75 g a.s./ha.

ER_{50} for reproductive parameters > 75.0 g a.s./ha.

Previous evaluation:	New data submitted for purpose of review

Report:	KCP 10.3.2.2/05; Jans, D.; 2017;
Title:	Toxicity to the parasitoid wasp Aphidius rhopalosiphi in an extended laboratory test with aged residues on maize Isoflucypram EC 50 g/L
Report No.:	CW17/014
Document No.:	M-600692-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable MEAD-BRIGGS ET AL. (2010) modified CANDOLFI ET AL. (2001)
GLP:	yes

Objective:

The objective of this study was to investigate the lethal and sublethal toxicity of Isoflucypram EC 50 to the parasitoid wasp Aphidius rhopalosiphi when exposed to fresh and aged residues of the test item on maize.

Material and methods:

Test item: BCS-CN88460 EC 50, Supplier batch No: 2016-001002, Spec. no: 102000031262, analysed content of active substance isoflucypram: 5.28% w/w (51.45 g/L).

Reference Test Item: A reference item (active substance: dimethoate) was applied at 4 g a.s./ha in 400 L deionised water/ha on the application day of the test item on potted maize plants as well. For the further exposure dates the reference item was applied directly on detached maize leaves (with 4 g a.s./ha in 400 L deionised water/ha).

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

The bird cherry-oat aphid Rhopalosiphum padi (aphids used for parasitization in the reproduction assessment) were taken from the breeding of the testing facility.

Test design:

Number of test groups: 3 (control 1, test item 1, reference item 1)

Number of application rates: Test item: 1, Reference item: 1.

Number of replicates per group: 6 (one replicate = one pot)

Number of wasps per replicate: 5

For the mortality assessment the potted maize plants were cultivated under non-GLP conditions until the date of the application. The test item was applied on potted maize plants (Zea mays) at a rate of 75 g a.s./ha in 400 L deionised water/ha using a calibrated plot sprayer (mean measured application rate: 393 L/ha). The control plants were treated with deionised water in the same way as the test item.

Aging of the spray deposits of the test item on the potted maize plants took place under semi-field conditions with UV permeable rain protection during the whole study. Two bioassays were performed, the first started on the application day (0DAT1 = 0 days after treatment) and the last two weeks later (2DAT1).

After the application or the appropriate aging period, pieces of maize leaves (approx. 25 cm long) randomly taken from different maize plants were cut. Then one piece of leaf was transplanted in a pot (Ø100 mm with a disc of filter paper on the bottom). A wet piece of paper was wrapped around the leaf part which was put in the pot bottom. Now the pot was filled with quartz sand (up to 0.5 - 2 cm below the upper pot rim) so that the leaf stood in an upright position.

Finally, the leaf was sprayed with a 10% fructose solution and left to dry. Then the pot was filled with quartz sand completely to the pot rim.

Now each pot with one treated maize leaf was enclosed within a clear polyacrylic cylinder (195 mm high and 100 mm in diameter) with a hole (approximately 5 mm in diameter) for the introduction of the parasitoids. The

top of the cylinder was closed with fine mesh gauze. The pots were placed on a water absorbent mat to allow the pots to soak up water.

For the mortality assessment five female test organisms - impartially selected from the stock culture - were introduced per cylinder. Five healthy female wasps were gently sucked out of the hatching cage into a glass tube which was closed on both ends. From these tubes the wasps were then transferred into the cylinders by slightly blowing into the tubes. When all five females were in the exposure unit, the orifice was closed with a stopper. During the exposure phase of 2 days the test organisms had access to the sugar solution on the treated maize leaf.

Parasitoid wasps (Aphidius rhopalosiphi) were exposed to these residues on the treated leaf surfaces.

Mortality of 30 female wasps, not older than 48 h at study start (6 replicates with 5 wasps per test group), was assessed 2, 24 and 48 h after exposure in both bioassays (food = 10% fructose solution sprayed onto test plants).

Repellency of the test item was assessed during the initial 3 h after the release of the females. Five separate observations were made at 30-minute intervals starting 15 - 30 minutes after the introduction of all wasps.

The reproductive performance was assessed in both bioassays. For this 20 impartially chosen females from the water control and the test item group were each transferred to a cylinder containing untreated barley seedlings (five days prior to the start of the bioassay, the barley plants required were sown [18 - 20 seed grains each]). One day after the start of the bioassay the plants were infested with more than 100 Rhopalosiphum padi, and the soil surface of the pots was covered with quartz sand. The number of mummies (parasitized aphids in which wasp pupae subsequently develop) was assessed 12 days later in the first and 10 days later in the second bioassay.

The effects of the test item on the test organisms were compared to those of the control with suitable statistical procedures using the computer program SAS (Version 9.4).

Test concentrations: 75 g a.s./ha

Test conditions: The climatic conditions (temperature, relative humidity and light intensity) in the outdoor area were continuously recorded using a data logger (ELPRO Messtechnik GmbH). The temperature ranged from 3.5 to 32.5 °C and the relative humidity from 17% to 100% during the aging time of the maize plants.

The laboratory phase for each exposure date was performed in a controlled environment room (target range 20 ± 2 °C and 60 - 90% relative humidity). Short deviations of the test conditions (less than 2 h; e.g. due to handling of the test system) are considered being without consequence to the study outcome and were not reported. During the reproduction phase of the first bioassay the temperature increased on two days to max. 23 °C for a total duration of 10 hours.

Temperature and relative humidity were continuously recorded with data logger (ELPRO Messtechnik GmbH). The light intensity was measured once per phase for each bioassay using a Luxmeter (RS Components GmbH).

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

The reproduction data were tested for normal distribution using the Shapiro-Wilk test and for homogeneity of variance using the Levene test.

As the repellency data in the first bioassay were normally distributed and homogenous one-way ANOVA and the Dunnet test (one-sided) were used.

As the repellency data in the second bioassay were not normally distributed the Wilcoxon test (one-sided with Bonferroni-Holm adjustment) was used.

As the reproduction data in this study were normally distributed and homogenous one-way ANOVA and the Dunnet test (one-sided) were used.

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

Findings:

Validity criteria:

	Validity criteria	Findings	
		Start of bioassay	
		0DAT1[a]	14DAT1[a]
Mortality in control treatment	≤ 10%	0.0%	0.0%
Corrected mortality in reference item treatment	≥ 50%	100.0%	100.0%
Mean number of mummies per surviving female wasp in control treatment	≥ 5	16.8	28.3
Number of surviving female wasps in control treatment producing zero values for reproduction	≤ 2	0	0

[a] DAT = Days After Treatment

All of the validity criteria were met.

Biological results

The bioassays were started on the application day of the test item (0DAT1) and 14 days later (14DAT1). These bioassays resulted for the test item group in 3.3% mortality in the first and no mortality in the second bioassay. All data for the test item group were not statistically significantly different compared to the control group (Fisher´s Exact test, one-sided).

In both bioassays the exposure to the reference item resulted in 100% mortality of the test organism after 48 h of exposure.

In the first bioassay a mean of 54.0% of the wasps settled on the leaves in the control group within the first 3 h after the release of the females. In the test item group, a mean of 32.8% of the wasps were found on the leaves, indicating a statistically significant repellent effect (Dunnett test, one-sided). In the reference item group 50.2% of the wasps settled on the leaves.

In the second bioassay a mean of 54.2% of the wasps settled on the leaves in the control group within the first 3 h; this compared to 46.7% in the test item group and was not statistically significantly different (Wilcoxon test, one-sided). In the reference item group 37.3% of the wasps were found on the leaves.

The reproduction was assessed in both bioassays. A statistically significant reduction in reproductive success relative to the control of 44.7% was found in the first bioassay (one-way ANOVA, Dunnett test, one-sided). In the second bioassay a reduction in reproduction of 13.8% was observed which was not statistically significantly different (Dunnett test, one-sided).

Table B.9.2.2-5 shows the biological results of the two bioassays.

Table B.9.2.2-5: biological results of two bioassays of *A.rhopalopsiphi* on maize leaves (mortality assessment) and barley plants (reproductive assessment).

Test item	**Isoflucypram EC 50 g/L**	
Application	**75 g a.s./ha**	
Test organism	***Aphidius rhopalosiphi***	
Exposure on	**Dried spray deposits on maize leaves (from treated maize plants)**	
Start bioassay	**0DAT1[a]**	**14DAT1[a]**
	Mortality (%) after 48 h	
Control:	0.0	0.0
Test item:	3.3	0.0
Reference item:	100.0	100.0
	Corrected Mortality (%)	
Test item:	**3.3** (p-value 0.500, not significant[b])	**0.0** (p-value 1.000, not significant[b])
Reference item:	100.0	100.0
	Repellency (mean values)	
	% Wasps on plant	
Control:	54.0	54.2
Test item:	**32.8** (p-value 0.005, significant[c])	**46.7** (p-value 0.188, not significant[d])
Reference item:	50.2	37.3
	Reproduction	
	Mean number of mummies per female wasp	
Control:	16.8	28.3
Test item:	9.3	24.4
	Reduction rel. to control (%)	
Test item:	**44.7** (p-value 0.017, significant[c])	**13.8** (p-value 0.205, not significant[c])

[a] DAT = days after treatment, **[b]** Fisher´s Exact test (one-sided), **[c]** one-way ANOVA, Dunnett test (one-sided)
[d] Wilcoxon test (one-sided)

Conclusion:

Both bioassays (started on 0DAT1 and 14DAT1) resulted in a corrected mortality of < 50% as well a reduction of reproduction of < 50%. However, a statistically significant effect on both reproduction and repellence occurred in the 0DAT1 bioassay. The figures obtained fulfil the validity criteria of the extended laboratory method (MEAD-BRIGGS ET AL., 2010).

RMS Comments

It is noted that application of the test item occurred on actively growing plants from which leaves were selected for use in the exposure phase of the bioassay. Given that application occurred via spray apparatus the distribution of the test item on the leaf surface cannot be confirmed. Whilst this reflects the proposed use it adds additional uncertainty regarding the exposure of the test organisms. Additionally, leaves were randomly selected from the whole plant for use in the test arenas, during the exposure phase. Ideally leaves would have been randomly obtained from a constrained portion of the canopy to minimise the impact of the location in the canopy on the amount of test item residues.

The number of mummies (parasitized aphids in which wasp pupae subsequently develop) was assessed 12 days later in the first and 10 days later in the second bioassay. While the exposure to the text item would be expected to be less in the second bioassay, it is noted that the minimum number of mummies per female (of 5) was

exceeded in both. Additionally, 10-12 days is an appropriate length of time, proposed by the test guidelines. Therefore, the reproduction results are considered valid.

While there were <50% adverse effects on reproduction at 0DAT1, this is coupled with a significant repellency effect of the test item. As such, the results from the 0DAT1 bioassay should be treated with caution.

However, in the 14DAT1 bioassay, there was no significant effect on repellency, nor on measured endpoint parameters, so we can say with much higher confidence that <50% toxic effects occurred following exposure to 2 week-aged residues of the test item.

Furthermore, all validity criteria were met in the controls and the endpoints are considered valid for use in the risk assessment.

B.9.6. RISK ASSESSMENT FOR ARTHROPODS

B.9.6.1. Risk assessment for bees

The risk assessment has been performed according to the existing guidance in force at the time of the preparation and submission of this dossier namely the EU Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2) and EPPO Standard PP 3/10 Environmental Risk Assessment Scheme for Plant Protection Products – Chapter 10: Honeybees.

Regulations (EU) 283/2013 and 284/2013 require, where bees are likely to be exposed, testing of both acute (oral and contact) and chronic toxicity, including sub-lethal effects. Consequently, in addition to the standard toxicity studies performed with adult bees (OECD 213 and 214) studies that describe the intrinsic chronic toxicity to adult honeybees and honeybee larvae were performed, which are provided in the Vol 3CA dossier in section B9.5.1

Further data on honeybees was generated under semi-field conditions with the representative formulation Isoflucypram EC 50, which is described in detail in this section. These tunnel tests with the representative formulation Isoflucypram EC 50 were conducted according to OECD GD 75 and EPPO 170 and are presented in the Vol 3CP dossier in section B9.5.1.

Acute bee endpoints

The following table (table B9.6-1) summarise the available valid acute toxicity endpoints for bees:

Table B9.6-1: Toxicity of isoflucypram (technical and formulated products) to bees

Test substance	Test species/ study type	Endpoint	References
Isoflucypram tech.	Honeybee, 48 h	LD_{50} – oral > 106.3 µg a.s./bee LD_{50} – contact > 100 µg a.s./bee	Schmitzer, S.; 2014; M-503824-01-1 KCA 8.3.1.1.1/01 KCA 8.3.1.1.2/01
	Honeybee, 48 h	LD_{50} – oral > 109.5 µg a.s./bee LD_{50} – contact > 100 µg a.s./bee	Schmitzer, S.; Haupt, S.; 2013; M-472468-01-1 KCA 8.3.1.1.1/02 KCA 8.3.1.1.2/02*
	Bumble bee, 48 h	LD_{50} – oral > 200.2 µg a.s./bumble bee	Taenzler, V.; 2015; M-542774-01-1 KCA 8.3.1.1.1/03
	Bumble bee, 48 h	LD_{50} – contact > 100 µg a.s./bumble bee	Haupt, S.; 2015; M-509048-01-1 KCA 8.3.1.1.2/03
Isoflucypram EC 50	Honeybee, 72 h 96 h	LD_{50} – oral 69.1 µg a.s./bee LD_{50} - contact 14.1 µg a.s./bee	Ehmke, A.; 2016; M-571280-01-1 KCP 10.3.1.1.1/01

*Study conducted with a batch of unknown impurity profile and not used to inform the risk assessment.

Active substance toxicity endpoints: For the active substance, oral and contact exposure studies are available for both honey bees and bumble bees. All studies were considered valid for use in the risk assessment. Two studies are available with honey bees testing oral and contact toxicity of the active substance; these studies are both valid but were conducted with different batches of the active substance. The acute/contact toxicity study conducted by Schmitzer, S. and Haupt, S. (2013) was conducted with a batch of unknown impurity profile (See Volume 4); as this study did not indicate adverse toxicity it was not used to inform the risk assessment and the study conducted by Schmitzer, S (2014) was used in the risk assessment. There is currently no noted risk assessment for bumble bees, however it would appear on the basis of the active substance data, bumblebees in the form of *Bombus terrestris* are less sensitive than *Apis mellifera*, therefore there are no formulation data.

Formulation toxicity endpoints: One study was submitted testing the acute oral and contact toxicity of the formulated product to honey bees. It is noted that the formulated product is more toxic to *Apis mellifera* than the active substance, having a lower endpoint for acute oral and contact toxicity when expressed on the basis of the active substance content. In the oral test, behavioural abnormalities were observed at doses of 90.7, 81.1 and 39.8 µg a.s./bee four hours after treatment and at doses of 90.7 and 81.1 µg a.s./bee 24 hours after treatment. In the contact test, behavioural abnormalities were observed up to 96 hours after treatment, with behavioural effects observed at all test concentrations with the exception of the lowest test concentration. The study was considered valid for use in the risk assessment.

Acute bee risk assessment

Assessment of the acute risk of the active substance isoflucypram to bees has been conducted in accordance with Regulation (EC) No. 1107/2009, and the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002).

The risk assessment for bees for isoflucypram is based on the application rates of 1.5 L prod/ha corresponding to 75 g a.s./ha for applications in cereals using the endpoints (LD_{50} values) for the formulation Isoflucypram EC 50 and the active substance isoflucypram.

Hazard Quotients
The risk assessment is based on Hazard Quotient approach (Q_H) by calculating the ratio between the application rate (expressed in g a.s./ha) and the laboratory contact and oral LD_{50} (expressed in µg a.s./bee).

Q_H values are calculated using data from the studies performed with the active substance and with the formulation. Q_H values higher than 50 indicate the need of higher tiered activities to clarify the actual risk to honeybees.

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

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

Table B9.6-2: Hazard quotients for bees – oral exposure

Compound	Oral LD_{50} [µg a.s./bee]	Max. application rate [g a.s./ha]	Hazard quotient Q_{HO}	Trigger	Acceptable risk for adult bees
Isoflucypram	> 106.3	75	< 0.71	50	yes
Isoflucypram EC	69.1	75	1.09	50	yes

The hazard quotients for oral exposure are below the validated trigger value for higher tier testing (i.e. $Q_{HO} < 50$).

Table B9.6-3: Hazard quotients for bees – contact exposure

Compound	Contact LD_{50} [µg a.s./bee]	Max. application rate [g a.s./ha]	Hazard quotient Q_{HC}	Trigger	Acceptable risk for adult bees
Isoflucypram	> 100.0	75	< 0.75	50	yes
Isoflucypram EC	14.1	75	5.32	50	yes

The hazard quotients for contact exposure are below the validated trigger value for higher tier testing (i.e. Q_{HC} < 50).

Chronic bee endpoints

The following table (table B9.6-4) summarises the available valid chronic toxicity endpoints for bees.

Table B9.6-4: Chronic toxicity of isoflucypram (technical) to bees

Test substance	Test species/ study type	Endpoint		References
Isoflucypram SC 200	Honeybee, 10 day chronic adult feeding study	LDD_{50} NOEDD	> 89.7 µg a.s./bee/day 89.7 µg a.s./bee/day (equivalent to 3333 mg a.s./kg diet)	Gossmann, A.; 2015; M-540173-01-1 KCA 8.3.1.2/01

Chronic adult toxicity endpoint: To satisfy point 8.3.1.2 of the data requirements according to Commission Regulation (EU) 283/2013, a chronic adult honey bee study was submitted. The active substance was not used in this test due to solubility issues; instead the formulation BCS-CN88460 SC 200 has been used. This is not the representative formulation and no comparison has been made to the active substance or the representative formulation, and no justification given why the formulation tested is representative, noting that this is also a different formulation type (SC) in comparison to the representative formulation (EC). As such, there is uncertainty as to the effects of co-formulants on the toxicity of the tested formulation in comparison to the active substance and representative formulation. However, as the inclusion of co-formulants is likely to increase the toxicity of the formulation to bees, this is considered acceptable by the RMS and the study considered valid.

Effects on honeybee development and other life stages: To satisfy point 8.3.1.3 of the data requirements according to Commission Regulation (EU) 283/2013, a repeated dose larval toxicity was submitted testing the active substance.The validity criteria for this study were not met; according to OECD 239, the validity criteria must be met across all replicates; in this study the validity criteria for larval mortality and mean adult emergence were not met across all replicates in the solvent control, where 18.8% mortality was observed in replicate 1 on day 8, and emergence rate in replicate 3 was 56.3%. However, overall the study indicated a potential low toxicity with a NOED of 62.5 µg a.s./larva per developmental period (equivalent to 406 mg a.s./kg). In addition the applicant submitted four semi-field studies which were conducted to OECD 75 and hence there was an assessment of honeybee development. These studies are considered in detail below.

Chronic bee risk assessment

There is currently no agreed chronic risk assessment; therefore the RMS has not performed one. However as the chronic adult bee study endpoint is presented in terms of concentration in diet it is possible to see how this endpoint compares to what the bees may be exposed to. Vallon (2017) and Schmitzer (2017) measured the residue levels of isoflucypram in pollen and nectar. Maximum residue levels were recorded in Vallon (2017) 0DAA; these values are 0.073 mg a.s/kg and 33 mg a.s./kg in nectar and pollen respectively. If a comparison is made between the chronic endpoint based on concentration in food and the maximum concentrations of isoflucypram in nectar and pollen, a margin of safety of between 45658 and 101 is demonstrated. This provides some indication that the chronic risk to bees is acceptable, however it should be noted that there are semi-field and field studies presented below that will also provide further information regarding the chronic risk to bees. Furthermore it should be noted that residues in pollen and nectar were collected on the day of application and the day after application only so only represent intial residue values.

Semi-field bee studies

Four semi-field tunnel studies were submitted by the applicant; although the acute oral and contact hazard quotients are less than the trigger value of 50 and hence indicate an acceptable risk, as indicated above these studies address the effect of isoflucypram on honeybee development and provide further information regarding effects on flight intensity. These studies are summarised in Table B.9.6-5 below.

Table B9.6-5 : Summary of semi-field bee tunnel tests

Test substance	Test species/system	Effects observed	Reference	Comments

BCS-CN88460 EC 50 G	Species: *Apis mellifera* Test type: Semi-field study, tunnel test EPPO (2010) OECD 75 7 day exposure 21 day monitoring period Application rate: 1 x 75 g a.s./ha (1.512 mL product/ha) Spray volume: 400 L water/ha Crop: *Phacelia tanacetifolia* at BBCH 65 (area of 85 m^2) Colony size: 6000 bees/colony Country: Germany	*Mortality* No statistically significant effects on worker bee or pupal/larval mortality. *Flight activity* Flight activity prior to application higher in control and test item groups. When comparing flight activity pre and post exposure, reductions of 80.7, 34.7 and 31.3% were observed 1, 2 and 3 hours after application. *Brood effects* No statistically significant effects on colony condition, brood termination rate, brood index or compensation index. *Behaviour* Intensive cleaning, coordination problems and apathy were noticed on the day of application.	KCP 10.3.1.5/01; Peters, B.; Rohland, A.; 2016	• Regular flight activity and mortality assessments on non-woven sheets could not be performed on DAT -4 and DAT -3, due to a severe thunderstorm on DAT -5 This adds some uncertainty to the pre-treatment assessments, which should be sufficient to demonstrate stable background mortality and that bees are actively foraging on the crop. However as pre-treatment mortality and flight activity could be assessed on the other days pre-exposure, this is considered sufficient to demonstrate bee foraging behaviour and mortality prior to exposure. • The brood food ratio prior to exposure was in excess of that recommended by OECD 75 (4:1); ranging from 4:1.84 – 4:2.19). • Flight activity was reduced by up to 82.3% 1 hour after application; however statistical analysis was only performed over the the whole exposure period so it is not known if this was statistically significant. • Reductions in flight activity of 98.0% and 99.3% 1 and 5DAT were observed in comparison to the pre-treatment period; however similar reductions observed in the control. • During the exposure period 100% cloud cover was recorded most days during the mortality and flight activity assessments. • Maximum temperatures were above 30°C on most days during the pre-exposure and post-exposure periods, reaching 52.2°C 15DAT. According to the study report this was due to the

Test substance	Test species/system	Effects observed	Reference	Comments
				inappropriate location of the data loggers. • The mortality data in the report is based on the sum of dead bees on gauze and dead bee traps rather than these separately. Therefore, the statistical analysis does not distinguish between mortality occurring in the colony and during foraging. • Flower meadow located in the vicinity of the bee hives during the post-exposure monitoring period.

Test substance	Test species/system	Effects observed	Reference	Comments
BCS-CN88460 EC 50 G	Species: *Apis mellifera* Test type: Semi-field study, tunnel test EPPO (2010) OECD 75 7 day exposure 36 day monitoring period Application rate: 1 x 75 g a.s./ha (1447.7 g product/ha) Spray volume: 400 L water/ha Crop: *Phacelia tanacetifolia* at BBCH 65 (area of 87.72 m^2) Colony size: 3445 – 7150 bees/colony Country: Germany	*Mortality* Statistically significant increase 0DBA (54.5 bees in control; 63.5 bees in treatment group) Statistically significant increase 14DAA (3.3 bees in control; 8.3 bees in treatment group). *Flight activity* Flight activity prior to application similar to after application in treatment group (15.1 bees/m^2 4DBA-0DBA; 14.7bees/m^2 0DAA); flight activity then increased 1 and 2DAA, decreased 13DAA and then increased 4 -7DAA. The control group followed a similar pattern however flight activity was lower prior to application in the control. When comparing the flight activity of the test item group on 0DBA to that during the exposure period, a reduction in flight activity of 42.3, 19.7 and 8.5% was observed 15, 30 and 45 minutes after application of the test item. After this, foraging activity increased. *Brood effects* No statistically significant effects on brood termination rate, brood index or compensation index. Reductions of 13.3, 17.0, 10.1 and 15.7% 9, 15, 30 and 43DAA in colony strength in comparison to control. Reductions of ≥13.1% 4DAA – 36DAA; this reduction was 4.8% at 43DAA. However brood area also 12.1% lower than control prior to application 0DBA. *Behavioural effects* 150 bees were recorded clustering at the trap of replicate Td, 6hours after application 0DAA.	KCP 10.3.1.5/02; Hein, R.; 2017	• During the exposure period >50% cloud cover was recorded most days during the mortality and flight activity assessments. • During the exposure period precipitation of a total of 63mm was recorded between 2DAA and 5DAA. • The brood to food ratio was 4: 2.88 – 5.99 prior to exposure in this study, rather than the 4 :1 specified in OECD 75 .

<table>
<tr><th>Test substance</th><th>Test species/system</th><th>Effects observed</th><th>Reference</th><th>Comments</th></tr>
<tr>
<td>BCS-CN88460 EC 50 G</td>
<td>Species: Apis mellifera

Test type: Semi-field study, tunnel test
EPPO (2010)

7 day exposure
22 day monitoring period

Application rate: 1 x 75 g a.s./ha (1.42 kg product/ha)
Spray volume: 400 L water/ha

Crop: Phacelia tanacetifolia at BBCH 65 (area of 80 m^2)

Colony size: 4770 – 6233 bees/colony

Country: Germany</td>
<td>Mortality
A statistically significant difference in adult bee mortality between the control (12.8 bees) and treated groups (27.8 bees) was found on 18DAA but not at any other point during the test following exposure.

Flight activity
When comparing the pre-exposure flight activity and flight activity during exposure, no decreases were observed, with the exception of 6DAA.

Brood effects
No statistically significant effects on colony strength or development of the brood area.

Behavioural effects
None.

Residues
Residues of up to 13.1 mg a.s./kg detected in nectar and pollen.
<table>
<tr><th>Sample Material</th><th>Sampling Day</th><th>Mean Concentration [mg/kg]</th></tr>
<tr><td rowspan="2">Nectar</td><td>DAA0</td><td>0.0206</td></tr>
<tr><td>DAA1</td><td>0.00859</td></tr>
<tr><td rowspan="2">Pollen</td><td>DAA0</td><td>13.1</td></tr>
<tr><td>DAA1</td><td>1.08</td></tr>
</table>
</td>
<td>KCP 10.3.1.5/03; Schmitzer, S.; 2017</td>
<td>

A significant amount of precipitation fell from 8DAA onwards (total 201 mm).
23mm of rain fell 3DBA.
This study has low power to detect statistical significance; there was an increase in mortality of >219% during exposure in the reference item group in comparison to the control yet this difference was not statistically significant.
High maximum temperatures (>30°C, reaching a maximum of 47 °C 40DAA) were recorded from 3DAA until the end of the test on most days.
It is noted that several pesticides were applied up to 12 months prior to the start of the test.
Cloud cover was not recorded apart from during application.
Pollen and nectar levels were only measured on 0DAA and 1DAA and not at any other point in the exposure period, so only represent initial residues.

</td>
</tr>
</table>

<table>
<tr><th>Test substance</th><th>Test species/system</th><th>Effects observed</th><th>Reference</th><th>Comments</th></tr>
<tr>
<td>BCS-CN88460 EC 50 G</td>
<td>Species: Apis mellifera

Test type: Semi-field study, tunnel test
EPPO (2010)

7 day exposure
36 day monitoring period

Application rate: 1 x 75 g a.s./ha (1447.7 g product/ha) Spray volume: 400 L water/ha

Colony size: 4436 – 9214 bees/colony

Crop: Phacelia tanacetifolia at BBCH 65 (area of 82.72 m^2)

Country: Spain</td>
<td>Mortality
Statistically significant increase 0DAA (11.5 bees in control; 17.5 bees in treatment group).
Mean mortality also higher at start in treatment group, but not statistically significant (17.0 bees in control; 38.5 bees in treatment group).
Increase in mortality 0 – 7DAA (14.8 bees in control; 24.1 bees in treatment group) but not statistically significant.
Mortality following exposure was lower than that pre-exposure in the test item group in the hours following exposure; after this (from 1DAA) mortality in the treatment group increased, however the control mortality also increased at this point.

Flight activity
When comparing the pre-exposure flight activity and flight activity during exposure, no decreases in flight activity were observed.

Brood effects
No statistically significant effects on colony strength and development of brood area were observed.
Reductions of ≥12.1% in colony strength from 21DAA to 43DAA observed in comparison to control.

Behavioural effects
None.

Residues
Residues of up to 33 mg a.s./kg detected in nectar and pollen.
<table>
<tr><th>Sample Material</th><th>Sampling Day</th><th>Mean Concentration [mg/kg]</th></tr>
<tr><td rowspan="2">Nectar</td><td>DAA0</td><td>0.073</td></tr>
<tr><td>DAA1</td><td>0.01</td></tr>
<tr><td rowspan="2">Pollen</td><td>DAA0</td><td>33</td></tr>
<tr><td>DAA1</td><td>2.5</td></tr>
</table>
</td>
<td>KCP 10.3.1.5/04; Vallon, A.; 2017</td>
<td>

Deviations to the target spray volume of ±10% were met for all replicates with the exception of Tc where a deviation of +12.78% was recorded.
During the monitoring phase, it is stated that bees were moved to areas where there were no crops attractive to bees in the vicinity. However, lavender/citrus crops were planted in the vicinity, which are attractive to bees.
The brood to food ratio was 4: 7.00 – 7.78 prior to exposure in this study, rather than the 4 :1 specified in OECD 75.
Pollen and nectar levels were only measured on 0DAA and 1DAA and not at any other point in the exposure period, so only represent initial residues.

</td>
</tr>
</table>

Higher tier bee risk assessment

In order to interpret and hence use the above studies in a regulatory decision, there are several issues that need to be considered, namely – application rate, study design (including alternative forage and consideration of exposure), crops, climatic conditions, mortality, flight activity and bee behaviour. These factors are considered below.

Formulation used

All studies used the same formulation of Isoflucypram EC50.

Application rate

All studies applied the tested formulation at an application rate of 1 x 75 g a.s./ha in a volume of 400 L/ha water. This is the same as the proposed GAP. The spray apparatus was calibrated prior to use and was within ±10% of nominal in all studies with the exception of one of the treatment group replicates in Vallon (2017) where it was +12.78%; as this would result in higher exposure it is considered acceptable by the RMS.

Study design

All studies followed EPPO 170 (2010) and Peters and Rohland (2016) Hein (2017) also followed OECD 75.

Exposure

Climatic conditions

Peters and Rohland (2016): Maximum temperatures were above 30°C on most days during the pre-exposure and post-exposure periods, reaching 52.2°C 15DAT; temperatures of >30°C in the daytime may stop nectar secretion. However it is noted that the high temperatures were due to inappropriate location of the data loggers in direct sunlight. During the exposure period 100% cloud cover was recorded most days during the mortality and flight activity assessments. This could have affected the flight activity results ; however it is noted that where cloud cover was 0% in the pre-exposure period 3DBA, flight activity was lower than at other time points where cloud cover was higher, indicating that this did not have a significant impact. Minimum flight activity of 10 bees/m^2 was also met prior to exposure. Significant precipitation occurred prior to the application of the test item; a total of 45mm fell 5 days prior to application, whilst this precipitation occurred prior to exposure and so would not have had an impact on flight activity on the day of exposure (and met the requirements of EPPO 170 as no precipitation fell 2 hours after application of the test item), this potentially makes it difficult to determine what pre-exposure flight activity is for subsequent comparison following application of the test item. Precipitation of 4.6 mm and 12.1mm was recorded 1DAT and 5DAT respectively; this is likely to have reduced the exposure post treatment, hence oral exposure is not worst case. A reduction in flight activity was observed in the test item group at these time points but also in the control, therefore the precipitation also affected flight activity However pupal mortality, brood development and flight activity was significantly affected by the reference item, indicating that exposure did occur.

Hein (2017): During the exposure period >50% cloud cover was recorded most days during the mortality and flight activity assessments. This could have affected the flight activity results ; however it is noted that where cloud cover was 0% in the pre-exposure period 1DBA, flight activity was lower than at other time points where cloud cover was higher, indicating that this did not have a significant impact. Minimum flight activity of 10 bees/m^2 was also met prior to exposure. During the exposure period precipitation of a total of 63mm was recorded between 2DAA and 5DAA; this is likely to have reduced the exposure post treatment, hence oral exposure is not worst case, although the requirements of EPPO 170 were met as no precipitation fell 2 hours after application of the test item. However pupal mortality was significantly affected by the reference item, indicating that exposure did occur, noting that it is not known what levels of exposure cause mortality following exposure to the test item.

Schmitzer (2017): Cloud cover was not recorded apart from during application. As this is known to affect foraging activity this adds uncertainty to the flight activity results, noting that the minimum of 5 bees/m^2 according to EPPO 170 was met. 23mm of rain fell 3DBA, which could have affected flight activity recorded at this point, giving a lower starting flight activity. Whilst this precipitation occurred prior to exposure and so would not have had an impact on flight activity on the day of exposure (and met the requirements of EPPO 170 as no precipitation fell 2 hours after application of the test item), this potentially makes it difficult to determine what pre-exposure flight

activity is for subsequent comparison following application of the test item. Relatively high maximum temperatures (>30°C, reaching a maximum of 47 °C 40DAA) were recorded from 3DAA until the end of the test on most days. This could have affected the nectar levels measured in the test, affecting the residue levels measured; it is noted that the residue levels in nectar in this study are lower than those in the study conducted by Vallon (2017). However worker bee mortality, colony strength and flight activity was significantly affected by the reference item, indicating that exposure did occur.

Vallon (2017): Cloud cover was 40 -70% 1DBA; this could have affected flight activity results at this point. However mean flight activity was above the minimum recommended in EPPO (2010) at this point; as such this is not considered to have significantly affected flight activity.

Overall conclusion regarding climatic conditions: Whilst some significant precipitation was recorded in several of the studies, much of this was prior to exposure and whilst this could have affected the starting flight activity for subsequent comparison following exposure, flight activity was still above the minimum recommended in EPPO (2010). The precipitation recorded after application of the test item in Peters and Rohland (2016) and Hein (2017) is likely to have reduced the exposure post treatment, hence oral exposure is not worst case, which adds some uncertainty to the results, although it is noted that conditions were dry on the day of application when the majority of effects in bees were noted. Overall the RMS considers the climatic conditions recorded in these studies to not significantly undermine the reliability of the results observed.

Residue levels in nectar and pollen
In **Vallon (2017)** and **Schmitzer (2017)** residue analysis of nectar and pollen was conducted which confirmed that honey bees had foraged the treated crop and ingested the test item. However it is noted that pollen and nectar analysis was only conducted on the day of application and one day after application and thus only represent initial residue values.

Post-exposure monitoring period

Surrounding crops
All studies state that no flowering crops attractive to bees were located in the surrounding area during the monitoring period. However in **Peters and Rohland (2016)** a flower meadow was located in the vicinity of the bee hives and in **Vallon (2017)** citrus crops and lavender were located near to the bee hives. The surrounding flora was potentially attractive to honeybees, thus providing alternative forage and potentially reducing exposure of the honey bees to the test item, as there may have been a decrease in feeding on stored food contaminated during the exposure phase. In the other studies (**Hein, 2017 and Schmitzer, 2017**), details of the surrounding crops are not given in the study report. Furthermore, there was no pollen identification conducted to clarify where the honey bees were foraging.

Brood:food ratio
In **Peters and Rohland (2016), Hein (2017) and Vallon (2017),** the brood to food ratio prior to the test was in excess of that recommended in OECD 75, which recommends a ratio of 4:1 in order to ensure exposure to the test item occurs. It was not possible to calculate the brood:food ratio for **Schmitzer (2017)** as the results for brood and food were displayed in percentages. As bees were confined to tunnels during the exposure period, this adds confidence to bees being appropriately exposed, however a large amount of stored food prior to exposure could mean that bees and brood could have been fed with uncontaminated food, instead of nectar and pollen contaminated with the test item. A summary of the brood to food ratios for the studies is presented below :

Table B9.6-6 : Brood to food ratio in the bee semi-field tests

Study	Brood to food ratio		
	Control	Treatment	Reference
Peters and Rohland (2016)	4:1.88	4:1.84	4:2.19
Hein (2017)	4:5.99	4:5.20	4:2.88
Vallon (2017)	4:7.00	4:7.88	4:7.12

Exposure period
In all studies the exposure period was 7 days, however the post-exposure monitoring period varied between studies, ranging from a minimum of 21 days in Peters and Rohland (2016) to a maximum of 36 days in Hein (2017) and Vallon (2017). This meets the minimum prescribed time according to OECD 75 and EPPO 170.

Overall conclusion regarding study design – The studies discussed were well conducted, followed guidance appropriately and adhered to GLP. However, there are certain issues that make interpretation and hence use

uncertain; predominantly issues regarding a potential reduction in exposure due to stored food above that recommended in OECD 75, variability in climatic conditions potentially reducing exposure via run-off and through a reduction in foraging activity, and potentially attractive crops in the surrounding area during the monitoring phase. The response to the reference item indicates in general that bees were appropriately exposed and residues of the test item were recorded in nectar and pollen. As such it can be concluded that exposure to the test item did occur.

Mortality

According to the first tier data for both the active substance and the formulated product and the resulting hazard quotients, no mortality would not be expected, noting that the studies conducted with the formulated product raised concerns regarding delayed effects as mortality and behavioural effects increased between 24 and 48 hours, hence the studies were extended to 96 hours . Each study is considered in more detail below:

Peters and Rohland (2016): No statistically significant effects on mortality occurred at any point during the test. It is noted that there were increases in mortality of worker bees of up to 40.5% after exposure; however as this only equates to a difference of 1.7 bees and mortality was low (5.9 bees at this point), it is not considered to be related to the test item.

Hein (2017): A statistically significant increase in mortality occurred 14DAA; however overall mortality was low (3.3 bees in control vs.8.3 bees in the test item group at this point). Furthermore mortality was higher prior to application of the test item and a reduction in mortality had occurred at 14DAA in comparison to that recorded on the previous days (60.3 and 55.5 bees 0 - 7DAA in control and treatment group respectively). Therefore there were no clear effects of the test item on mortality in this study.

Schmitzer (2017): A statistically significant increase in mortality occurred 18DAA in the test item group in comparison to the control but not at any other point during the test. Clear effects of the reference item on bee mortality were observed on the day of application (1287.5 bees) and during the exposure period (246.1 bees); as the number of dead bees 18DAA was relatively low (27.8 bees) in the test item group in comparison to the clear and immediate effects of the reference item, this reduction is not considered to be related to the test item. Furthermore, the mean number of 27.8 dead bees/day/colony is comparable to the control values among the phase outside the tunnels and within biological variance.

Vallon (2017): A statistically significant increase in mortality was observed 0DAA in the test item group in comparison to the control. There was also an increase in mortality 0 – 7DAA (14.8 bees in control; 24.1 bees in treatment group) but this was not statistically significant. The mortality pre-exposure was relatively high in all groups; therefore a comparison of pre and post exposure mortality for each test group was made. Mortality following exposure was lower than that pre-exposure in the test item group in the hours following exposure; after this (from 1DAA) mortality in the treatment group increased, however the control mortality also increased at this point, as such this is not considered to be related to the test item and no adverse effects of the test item on mortality are apparent.

Overall conclusion regarding mortality: Although some significant mortality was observed in the test item groups, it did not follow a clear pattern across the studies and overall mortality was low. Furthermore as mortality was higher prior to application in some cases, subsequent decreases are difficult to attribute to the effect of the test item. Clear effects of the reference item on mortality in each study were observed, indicating that exposure did occur. After consideration of the studies presented, it is concluded that Isoflucypram EC50 sprayed at the proposed rate of 1 x 75 g a.s./ha does not result in an increase in mortality compared to a comparable control**.**

Flight activity

In all of the studies it is noted that cloud cover was relatively high during the flight activity period or not recorded, however despite this the flight activity was above the minimum recommended in EPPO (2010). Flight activity in the treatment group was compared to that in the control by the applicant; however as the difference between the pre-exposure and exposure period is more relevant in terms of a change in flight activity, a comparison was also made of the flight activity just before application and after application of the test item. This accounts for differences in colony size betweeen the different treatment groups. Comparisons of individual measurements of flight activity were made in order to detect effects immediately following application of the test item, rather than over the whole exposure period.

Peters and Rohland (2016): When looking at the individual measurements of flight activity following application of the test item, reductions of 80.7, 34.7 and 31.3% were observed 1, 2 and 3 hours after application in comparison to that shortly before application of the test item.
Significant precipitation fell prior to application; however flight activity in all groups was higher at this point, therefore it is not considered to have led to a lower starting flight activity. 1DAT and 5DAT flight activity was reduced in all groups, including the untreated control, which may have been due to the high preciptation observed at these time points. Furthermore the minimum and maxiumum temperatures were also lower at these time points, which may have affected the flight activity.

Hein (2017): When comparing the foraging activity of the test item group on 0DBA to that during the exposure period, a reduction in flight activity of 42.3, 19.7 and 8.5% was observed 15, 30 and 45 minutes after application of the test item. After this, foraging activity increased. This indicates that flight activity was affected by the test item in the period shortly after application of the test item, after this there was a return to pre-treatment levels and hence the decreases observed 2DAA and 0 – 7DAA in comparison to the control are not considered to be related to the test item. There was no significant effect of the reference item on flight activity when comparing pre and post-exposure flight activity. During the exposure period precipitation of a total of 63mm was recorded between 2DAA and 5DAA; however flight activity at this point was higher than in the pre-exposure period where there was no precipitation and hence flight activity appears unaffected by this parameter.

Schmitzer (2017): When comparing the pre-exposure flight activity and flight activity during exposure, no decreases were observed, with the exception of 6DAA where a decrease in the control was observed, indicating that this reduction was not related to the test item.

Vallon (2017): When comparing the pre-exposure flight activity and flight activity during exposure, no decreases in flight activity were observed, indicating that the test item did not affect flight activity in this study.

Overall conclusion regarding flight activity: In two of the studies (Schmitzer, 2017 ; Vallon, 2017), there were no decreases in flight activity following application of the test item. Effects of the test item on flight activity were observed in Peters and Rohland (2017) and Hein (2017), where decreases in flight activity were observed in the hours following application, noting that the reference item (fenoxycarb) did not show clear effects on flight activity in these tests. Overall it is considered that Isoflucypram EC50 sprayed at the proposed rate of 1 x 75 g a.s./ha causes no adverse effects, or only transient effects on flight activity shortly after application**.**

Brood effects

Two of the studies measured brood termination rate, brood index and compensation index; all studies measured colony strength and development of the brood area. This is considered in more detail below.

Peters and Rohland (2016): There were no statistically significant effects on brood index, brood termination rate or compensation index. Furthermore colony strength and development of the brood areas showed no decreases in comparison to the control. In contrast, the reference item showed significant effects on brood index, brood termination rate and compensation index.

Hein (2017): There were no statistically significant effects on brood index, brood termination rate or compensation index in the treatment group. However there were reductions in colony strength of 13.3, 17.0, 10.1 and 15.7% 9, 15, 30 and 43DAA in the treatment group in comparison the control. Regarding development of the brood area, there were reductions of 13.1% 4DAA – 36DAA in the test item group in comparison to the control, however the starting brood area was 12.1% lower than the control; as such this cannot be attributed to the test item. The effects of the reference item on colony strength and development of the brood area were larger in magnitude than those seen in the test item group and the starting brood area was 17.6% higher than the control, indicating a clearer effect of the reference item on this parameter. However the reference item did not have significant effects on brood index, brood termination rate or compensation index.

Schmitzer (2017): There were no statistically significant effects on colony strength and development of the brood area in the treatment group in comparison to the control. However a statistically significant reduction in colony strength was observed 7DAA and 24DAA in the reference group in comparison to the control.

Vallon (2017): There were no statistically significant effects of the test item on colony strength, however reductions of ≥12.1% were observed from 21DAA to 43DAA. In the reference item group no statistically significant effects were observed, but colony strength and development of the brood area was reduced by up to 53.4% and 51.4 following application of the test item until test termination.

Overall conclusion regarding brood effects: No clear adverse brood effects of the test item were observed. Although some decreases in brood area were observed during the monitoring period, the starting brood area was also lower in the treatment group in comparison to the control.

Behavioural effects

Peters and Rohland (2016): Intensive cleaning, coordination problems and apathy were noticed on the day of application in the test item group.

Hein (2017): In the test item group, 150 bees were recorded clustering at the trap of replicate Td, 6hours after application 0DAA. The study report stated that temperatures were particularly high this day; however this effect was not observed in the control groups.

Schmitzer (2017): No behavioural effects were observed in the test item group.

Vallon (2017): No behavioural effects were observed in the test item group.

Overall conclusion regarding behavioural effects: In two of the studies behavioural effects were observed shortly after application of the test item but the effects were noted to be transient.

Conclusion regarding the risk to honey bees from the use of Isoflucypram EC50

Whilst the acute oral and contact hazard quotients do not raise concerns, i.e. the hazard quotients are less than 50 and the chronic assessment indicates an acceptable risk based on the ratio between the endpoint (expressed as mg/kg food) and levels of isoflucypram in pollen and nectar; the applicant has carried out 4 additional semi-field tunnel tests. It should be noted that there are general concerns regarding the overall sensitivity of the studies to detect effects – see above for further details – and hence these studies are really only capable of detecting gross effects, such as large scale mortality. The RMS is of the view that these studies confirm that the acute risk to bees is low, as under the conditions of the study, the mortality of foragers was not increased as a result of the treatment. Furthermore, no concerns are raised regarding developmental effects from these studies, hence the RMS considers the risk to larval bees to be addressed despite the lack of valid larval laboratory study.

It should be noted that there are uncertainties raised regarding the high temperatures and cloud cover observed in the majority of the studies and the significant precipitation in the days following application in some of the studies. Furthermore, residue levels in pollen and nectar were only measured in two of the studies and in the studies where the residues were measured, these were only recorded on 2 days and are not linked to real levels of exposure. Hence there are some uncertainties regarding if bees were sufficiently exposed to the test item. However, overall the studies do not raise concerns.Three of the studies were conducted in the Central Zone and one in the Southern Zone are are therefore considered representative of different climatic conditions between Member States, noting that only one study was conducted in the Southern Zone and as such the conclusions reached are limited based on the limited dataset. Member states should consider the representativeness of these studies to their national conditions.

The available data do indicate that exposure to isoflucypram could result in behavioural effects and effects on flight activity, although these effects are limited to shortly following application. There were no clear effects of the test item on brood. On the basis of the above, the RMS considers that:

- the acute oral and contact hazard quotients are less than 50,
- there is margin of safety between the concentrations of isoflucypram in pollen and nectar and endpoints for chronic toxicity (noting that a data gap was identified for larval toxicity).
- semi-field studies indicate no gross effects on colony strength or development of the brood area.

The RMS does note the behavioural effects, however as these are inconsistent in that they were not observed in all of the studies, and did not lead to clear adverse effects at the colony level, then they are considered acceptable.

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

The risk assessment was performed according to Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) and to the Guidance Document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods (ESCORT 2, Candolfi et al. 2000[3]).

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

Test species, Dossier-file-No. Reference	**Tested Formulation, study type, exposure**	**Ecotoxicological Endpoint**
Aphidius rhopalosiphi Waibel, J.; 2017; M-593743-01-1 Rep.No: CW16/036 KCP 10.3.2.1/01	Isoflucypram EC 50 Laboratory, glass plates 7.5 g a.s./ha 13.3 g a.s./ha 23.7 g a.s./ha 42.2 g a.s./ha 75.0 g a.s./ha	LR_{50} 14.13 g a.s./ha Corr. Mortality [%] 10.2 32.2 100.0 100.0 100.0
Typhlodromus pyri Waibel, J.; 2017; M-593747-01-1 Rep.No: CW16/035 KCP 10.3.2.1/02	Isoflucypram EC 50 Laboratory, glass plates 7.5 g a.s./ha 13.3 g a.s./ha 23.7 g a.s./ha 42.2 g a.s./ha 75.0 g a.s./ha	LR_{50} 30.6 g a.s./ha Corr. Mortality [%] 2.3 -6.9[A] 27.6 80.5 96.6
Aphidius rhopalosiphi Waibel, J.; 2017; M-583441-01-1 Rep.No: CW16/038 KCP 10.3.2.2/01	Isoflucypram EC 50 Extended Lab., exposure on barley seedlings 7.5 g a.s./ha 13.3 g a.s./ha 23.7 g a.s./ha 42.2 g a.s./ha 75 g a.s./ha	LR_{50} > 75 g a.s./ha; ER_{50} > 7.5 g a.s./ha Corr.Mortality [%] / Effect on Reproduction [%] / Wasps on plants [%] 0 / 45.8 / 34.7 0 / 53.8 / 21.5 6.7 / 77.0 / 19.3 6.7 / 62.8 / 25.2 3.3 / 60.6 / 18.2
Typhlodromus pyri Waibel, J.; 2017; M-608958-01-1 Rep.No: CW16/037 KCP 10.3.2.2/02	Isoflucypram EC 50 Extended Lab., exposure on bean leaves 7.5 g a.s./ha 13.3 g a.s./ha 23.7 g a.s./ha 42.2 g a.s./ha 75 g a.s./ha	LR_{50} > 75 g a.s./ha; ER_{50} > 42.2 g a.s./ha Corr. Mortality [%] / Effect on Reproduction [%] 6.7 / 44.4 9.0 / 31.3 4.5 / 19.2 19.9 / 21.2 10.1 / 64.0
Chrysoperla carnea Waibel, J.; 2017; M-601137-01-1 Rep.No: CW16/039 KCP 10.3.2.2/03	Isoflucypram EC 50 Extended Lab., exposure on detached bean leaves 7.5 g a.s./ha 13.3 g a.s./ha 23.7 g a.s./ha 42.2 g a.s./ha 75 g a.s./ha	LR_{50} > 75 g a.s./ha; ER_{50} > 75.0 g a.s./ha Corr. Mortality [%] / Effect on Reproduction [%] -5.3 / 6.2 13.2 / 1.4 0.0 / -3.1 13.2 / 1.2 10.5 / -2.5

[3] Candolfi et al.: Guidance Document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods; ESCORT 2 workshop (European Standard Characteristics Of Non-Target Arthropod Regulatory Testing), Wageningen, NL, March 21-23, 2000, SETAC Europe; SETAC publication August 2001

Test species, Dossier-file-No. Reference	**Tested Formulation, study type, exposure**	**Ecotoxicological Endpoint**		
Coccinella septempunctata Müller, R. U.; 2017; M-608806-01-1 Rep.No: CW17/010 KCP 10.3.2.2/04	Isoflucypram EC 50 Extended Lab., exposure on detached bean leaves	LR_{50} > 75 g a.s./ha; ER_{50} > 75.0 g a.s./ha		
		Corr. Mortality [%]	Effect on Reproduction [%]	
	7.5 g a.s./ha	-5.3	19.0	
	13.3 g a.s./ha	-2.2	12.0	
	23.7 g a.s./ha	-11..8	30.0	
	42.2 g a.s./ha	2.9	31.0	
	75 g a.s./ha	-2.9	24.0	
Aphidius rhopalosiphi Jans, D.; 2017; M-600692-01-1 Rep.No: CW17/014 KCP 10.3.2.2/05	Isoflucypram EC 50 aged residues spray deposits on maize plants, 1 appl. of 75 g a.s./ha,	LR_{50} > 75 g a.s./ha; ER_{50} > 75.0 g a.s./ha		
		Corr. Mortality [%]	Effect on Reproduction [%]	Wasps on plants [%]
	residues aged for 0 d:	3.3	44.7	32.8 sign.
	residues aged for 14 d:	0.0	13.8	46.7 n. sign.

[A] A negative value indicates a lower mortality in the treatment than in the control
sign.: statistically significant at 5%-level.
n.sign.: not statistically significant.

The product 'Isoflucypram EC 50' is intended to be applied in the field once at a maximum rate of 1.5 L product/ha which is equivalent to 75 g isoflucypram/ha.

According to ESCORT 2 and the Terrestrial Guidance Document (SANCO/10329/2002) the exposure is calculated as:

in-field: Application rate × MAF

off-field: Max. single application rate × MAF × drift factor/VDF × correction factor / LR_{50}

MAF = multiple application factor

Drift factor = i.e. 0.0277, 90th percentile for one application (according to Ganzelmeier)

VDF = vegetation distribution factor = 10

Correction factor = 10 (Tier 1 test, *Aphidius*, *Typhlodromus*)

5 (Tier 2 test, *Aphidius*, *Typhlodromus*, *Chrysoperla*, *Coccinella*)

The risk is considered acceptable if the calculated HQ is < 2

For the current risk assessment, the application rate is 1.5 L product/ha (= 75 g a.s./ha) and the MAF (multiple application factor) = 1.0 (1 application). The in-field and off-field exposure (Predicted Exposure Rate, PER) of the representative formulation to non-target arthropods is shown in Table B.9.6.2-2:

Table B.9.6.2-2: Exposure calculation for Tier I assessment

In-field / Off-field exposure	Crop / no. of applications	Appl. rate [g a.s./ha]	MAF	Drift [%]	VDF	Correction factor	PER [g a.s./ha]
In-field	Cereals / 1	75	1.0	N/A	N/A	N/A	75.0
Off-field				2.77	10	10	2.08

Risk assessment for non-target arthropods

The risk assessment was performed according to Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) and to the Guidance Document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods (ESCORT 2, Candolfi et al. 2000[4]).

Tier 1 in-field risk assessment for non-target arthropods

Table B.9.6.2-3: Tier 1 in-field risk assessment for non-target arthropods

Crop	Species	In-field PER [g a.s./ha]	LR_{50} [g a.s./ha]	HQ	Trigger
Cereals	*A. rhopalosiphi*	75	14.13	**5.3**	2
	T. pyri		30.6	**2.5**	2

For the standard species, the in-field scenario is above the trigger of concern.

Tier 1 off-field risk assessment for non-target arthropods

Table B.9.6.2-4: Tier 1 off-field risk assessment for non-target arthropods

Crop	**Species**	**Off-field PER [g a.s./ha]**	**LR_{50} [g a.s./ha]**	**HQ**	**Trigger**
Cereals	*A. rhopalosiphi*	2.08	14.13	0.15	2
	T. pyri		30.6	0.07	2

For the standard species, the off-field HQ values are below the trigger of concern, indicating an acceptable risk for non-target arthropods.

Tier 2 in-field risk assessment for non-target arthropods

For the standard species, the in-field scenario is above the trigger of concern in the Tier 1 risk assessment Therefore, a Tier 2 risk assessment is presented with the two standard species and the two additional species *Chrysoperla carnea* and *Coccinella septempunctata.*

Table B.9.6.2-5: Tier 2 in-field risk assessment for non-target arthropods

Crop	**Species**	**In-field PER [g a.s./ha]**	**ER_{50} [g a.s./ha]**	**Risk acceptable if:**	**Refined assessment required?**
Cereals	*A. rhopalosiphi*	75	> 7.50	Effects are <50%	**Yes**
	T. pyri		> 42.2	Effects are <50%	**Yes**
	C. carnea		> 75	Effects are <50%	No
	C. septempunctata		> 75	Effects are <50%	No

For the standard species *A. rhopalosiphi* and *Typhlodromus pyri* at Tier 2, the in-field risk assessment reveals effects >50% at the in-field rate of 75 g a.s./ha. Therefore, further refinements are necessary.

Refined in-field risk assessment for *Aphidius rhopalosiphi*

Aphidius rhopalosiphi is the most senstitive species based on glass plate and extended laboratory studies. The extended aged residue laboratory study, performed with *Aphidius rhopalosiphi* is therefore considered sufficient to cover the in-field risk for both species with outstanding risk from the Tier 2 risk assessment (from Table B.9.6.2-

[4] Candolfi et al.: Guidance Document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods; ESCORT 2 workshop (European Standard Characteristics Of Non-Target Arthropod Regulatory Testing), Wageningen, NL, March 21-23, 2000, SETAC Europe; SETAC publication August 2001

5). Isoflucypram EC 50 was applied to potted maize plants at a rate of 1.46 L product/ha. An application rate of 1.46 L product/ha is equivalent to 75 g a.s./ha under the conditions of the test.
The exposure of the test organisms to fresh residues (0DAT1) resulted in a mortality of 3.3%. No mortality occurred when test organisms were exposed to aged residues (14DAT1).
A statistically significant reduction in reproductive success relative to the control of 44.7% was found after exposure to fresh residues (0DAT1). Although these effects were <50%, they were coupled with a significant repellence effect. This creates uncertainty over the results being representative of true exposure to the test item.

After exposure to aged residues (14DAT1) a reduction in reproduction of 13.8% was observed which was not statistically significantly different to the control. This indicates that recolonization can begin to occur within 2 weeks. This is supported by a low off-field risk to non-target arthropods at tier 1, which indicates recolonization populations are likely to be available. Furthermore, at the 14DAT1 bioassay, there was no significant repellence effect, therefore the reproduction results can be used with more confidence. Therefore, no unacceptable effects on non-target arthropods in the in-field area are expected from the intended use of isoflucypram EC 50.

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

B.9.7.1. Earthworms

Previous evaluation:	New data submitted for purpose of review

Report:	KCP 10.4.1.1/01; Frommholz, U.; 2016
Title:	BCS-CN88460 EC 50 G: Effects on survival, growth and reproduction of the earthworm Eisenia fetida tested in artificial soil
Report No.:	E 312 04951-5
Guideline(s):	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP Not Applicable
GLP/GEP:	Yes

Material and methods

Test item:	BCS-CN88460 EC 50 G; 5.18 % w/w equivalent to 50.46 g/L; supplier batch code: 2016-001002; Spec. no.: 102000031262; sample description: TOX20246-00; density: 0.974 g/mL.
Reference item :	Carbendazim 360 g a.s./L (test conducted Aug 25 – Nov 19 2015, non-GLP).
Test organism:	Adult *Eisenia fetida (Eisenia fetida andrei*; strain of Prof. Graff, Forschungsanstalt für Landwirtschaft, 38104 Braunschweig, Germany*)*; mean bodyweight at the start of the test ranged from 0.34 to 0.43 g/worm.
Age of test organisms:	Approximately 7 months old (synchronised culture of earthworms). In order to obtain a synchronised culture of earthworms 30 adult worms were kept in a closed rearing vessel with substrate and food for a period of 4 weeks. After this time, the adult worms were selected out of the substrate and the offspring hatched from the cocoons. The age of the worms from the synchronised culture differed by less than 4 weeks.
Acclimation:	<1 day prior to test initiation, the test organisms were acclimated to the artificial soil and test temperature.
Test duration:	8 weeks (4 weeks exposure, 4 week reproductive phase).
Test concentrations :	*Test item*: 10, 18, 32, 56, 100, 180, 320 and 560 mg formulation/kg dws *Reference item:* 0, 1.25, 2.50, 5.00 mg carbendazim/kg dws (conducted at a different time to the main test) *Controls:* Water control.

Preparation and application of the test item:	All application solutions were prepared freshly on the day of application. Prior to the start of the test, the application solution of the highest concentration of the test item was prepared. The test item was mixed into deionised water as homogeneously as possible. The application solution of the highest test concentration was further diluted with deionised water to obtain the desired test concentrations. Uniform volumes of the application solutions were used for all replicates of the test concentrations. They were thoroughly mixed into the artificial soil of each replicate using a laboratory mixer in the order lowest test concentration to highest test concentration. The control group was treated first in the same way as described above but with deionised water only.
No. of individuals per replicate:	10
No. of replicates:	8 per control and solvent control and 4 for the test item.
Test units :	Non-re-usable plastic boxes (length x width x height *ca*. 16.5 cm x 12 cm x 6 cm, area approximately 200 cm^2) were used as test vessels. Each test vessel contained an amount of approximately 500 g artificial soil (dry weight) to obtain a depth of approximately 5 cm soil in the test vessels. The test vessels were covered with transparent lids to prevent earthworms from escaping and to reduce evaporation during the test period. The lids were perforated to allow air exchange.
Test conditions :	pH: 5.86 – 6.14 (min – max across all test vessels) WHC: 49.14 – 60.82 (min – max across all test vessels) Light intensity: 533 – 632 lux Temperature: 19.5 – 22.9°C Duration: light : dark = 16 : 8
Feeding:	One day after the application (day 1), an amount of approximately 5 g finely ground, air dried animal manure was evenly distributed over the surface of the artificial soil in each test vessel. The food was moistened with deionised water (approximately 6 mL per 5 g food). The adult earthworms were fed once per week during the test period with approximately 5 g food/vessel. If food consumption was low (determined by visual inspection), the additional amount of food was reduced accordingly. The offspring were fed once at the start of the second 4 weeks exposure period by mixing the food into the soil. The surface of the artificial soil was moistened with deionised water once per week in order to compensate evaporation.
Artificial soil:	The artificial soil was prepared with the following composition (percentage distribution on dry weight basis): • Sphagnum peat (shredded): 10 % • Kaolinite clay (content of Kaolinite = 30.2 %) 20 % • Industrial quartz sand (Particle size: 0.20 mm – 0.05 mm = 91.35 %): 70 % Calcium carbonate for the adjustment to pH 6.0 ± 0.5 (CaCO3) was added. The artificial soil was prepared by mixing the dry components intensely in a laboratory mixer. Then, the soil was pre-moistened with 5.0 L deionised water per 25 kg dry weight artificial soil.
Test procedure:	Directly after application the individually weighed adult earthworms were exposed to the treated artificial soil. Before weighing, the earthworms were quickly washed with water and surplus water was absorbed on filter paper.

	The sequence of inserting the test organisms into the test vessels followed a computer-generated random design. Only healthy animals were used. After a period of 4 weeks the adult earthworms were removed from the test vessels, the survivors were counted, and their fresh weight was measured. From these data mortality and biomass effects were determined. The cocoons and juvenile earthworms remained in the vessels for additional four weeks. After this additional test period the reproduction was determined by counting the number of offspring hatched from the cocoons per test vessel.
Observations:	*Mortality:* After 4 weeks of exposure, the content of each test vessel was emptied, and the living adult earthworms were counted and checked for any abnormal behaviour or other adverse effects (e.g. lack of movement, rigidity etc.). Those earthworms, which did not move after gentle mechanical stimulus, were considered to be dead. Also missing earthworms (compared to the number of initially placed test organisms) were considered to have died. Mortality of the adult earthworms in comparison to the initial placed test organisms was determined and expressed in % mortality. *Growth:* At the beginning (prior to exposure) and at the end of the 4 weeks of exposure, the adult test organisms of each vessel were weighed (at the start each individually, at the end together of each test vessel). Changes in body weight values of the surviving test organisms of the treatment groups during the test period were compared to the values of the solvent control group. *Reproduction:* At the end of the test after 8 weeks, the number of surviving juveniles per test vessel was determined. The test vessels were placed in a water bath at 50 - 60 °C. By this treatment, alive juvenile earthworms rose to the soil surface. The emerging earthworms were removed and counted. Afterwards the content of each test vessel was checked additionally by carefully stirring up the artificial soil with the help of tweezers. The reproduction of the surviving test organisms per test vessel at the end of the study was compared to the solvent control. Water holding capacity (WHC) of the soil was determined prior to test initiation, at day 0 and day 56. pH of the soil was determined prior to the test and at test termination. Temperature was measured daily and light intensity measured on day 0, 28 and 56.
Statistical analysis:	The calculation of mean, SD and percentage mortality of the control and treatment groups was conducted in Excel. The software used to perform the statistical analysis was ToxRat Professional 3.2.1. Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's-Test and Cochran's-Test ($\alpha = 0.05$) respectively. These conditions were met, therefore William's t-test, onesided- smaller, $\alpha = 0.05$ was used to determine the NOEC.

Results

Validity criteria:

All validity criteria were met.

Validity criteria according to OECD 222 (13 April 2004)	**Obtained in this study**
Mortality of the adults in the control should be ≤ 10%	0 %
Number of juveniles (earthworms per control vessel) should be ≥ 30	103 to 197
Coefficient of variation of reproduction in the control should be ≤ 30 %	24.8 %

Reference test:

The number of juveniles per test vessel of the two highest test concentrations of 2.50 and 5.00 mg a.s./kg dry weight artificial soil were statistically significant reduced in comparison to the control (results of a Williams multiple sequential t-test, one-sided smaller, $\alpha = 0.05$).

According to the guideline significant effects should be observed between 1 and 5 mg a.s./kg dry weight artificial soil. Thus, the results of this reference test indicated that the test system was sensitive to the reference test item.

Biological results:

Effects on mortality and growth of the adults after an exposure period of 28 days and the number of offspring per test vessel after 56 days are shown in the following table (table x; values in this table are rounded values). After 28 days of exposure, no mortality in the control group was observed. No statistically significant effects up to and including 560 mg test item/kg dry weight artificial soil (the highest test concentration) were observed (Fisher`s exact binominal test, one-sided greater, $\alpha = 0.05$). No statistically significant effects for the growth relative to the control were observed in any test item concentration (William's t-test, two-sided, $\alpha = 0.05$).

No statistically significant differences concerning the number of juveniles relative to the control were observed in any test item concentration up to and including 560 mg test item /kg dry weight artificial soil (William's t-test, one-sided smaller, $\alpha = 0.05$).

Due to the lack of a clear concentration-response relationship no reliable $EC_{10/20}$ calculation was possible.

Table B9.7.1-1 : Mortality, body weight change and reproductive output of earthworms exposed to BCS-CN88460 EC 50 G

Test object	***Eisenia fetida***								
Test item	**BCS-CN88460 EC 50 G**								
mg test item/kg dry weight artificial soil	**Control**	**10**	**18**	**32**	**56**	**100**	**180**	**320**	**560**
Mortality of adult earthworms [%] after 28 days	0	0	0	7.5	0	0	0	0	0
Significance (Mortality*)	-	-	-	-	-	-	-	-	-
Mean change of body fresh weight of the adults from day 0 to day 28 [%]	43.6	43.9	45.6	45.7	44.6	45.5	40.2	36.3	42.1
Standard Deviation	8.1	8.2	4.6	5.3	10.4	16.0	6.6	6.6	3.4
Significance (body fresh weight)**	-	-	-	-	-	-	-	-	-
Mean number of offspring per test vessel after 56 days	138.9	143.0	118.8	132.0	128.0	132.3	151.8	127.8	124.3
Standard Deviation	34.5	33.4	18.0	30.1	30.6	39.4	14.2	3.9	8.5
% of control	-	103.0	85.5	95.0	92.2	95.2	109.3	92.0	89.5
Coefficient of variance (%)	24.8	23.4	15.1	22.8	23.9	29.8	9.4	3.1	6.9
Significance (reproduction)***	-	-	-	-	-	-	-	-	-
	Adult mortality			**Growth**			**Reproduction**		
NOEC [mg test item/kg dry weight soil]	≥ 560			≥ 560			560		
EC_{10} (mg test item/kg dry weight artificial soil)							n.d.		
95% confidence limits							(n.d.)		
EC_{20} (mg test item/kg dry weight artificial soil)							n.d.		
95% confidence limits							(n.d.)		

* (Fisher`s Exact Binominal Test, one-sided greater, $\alpha = 0.05$), + significant, - not significant
** (William's t-test, two-sided, $\alpha = 0.05$), + = significant, - = not significant
*** (William's t-test, one-sided smaller, $\alpha = 0.05$), + significant, - not significant
n.d. – could not be determined, see observations and conclusions.

Conclusion:

Based on the effects observed on mortality, growth and reproduction, it is concluded, that the overall NOEC for the study is determined to be 560 mg formulation/kg dry weight soil.

RMS comments

This study was conducted according to GLP and following OECD 222 guidelines.

The following was noted by the RMS :

The temperature during the test was outside the stated range in the test guidelines (20±2°C), reaching 22.9 °C. However, as the controls performed well, meeting all validity criteria, this is not considered to invalidate the test.

The WHC of the soil varied by >10% in the control (11.24%) and in vessels at concentrations 180, 320 and 560 mg formulation/kg soil (11.69, 14.10 and 11.75%). This is a deviation to OECD 222, which states that the WHC of the soil should be maintained within 10% at the start and end of the test. As the controls performed adequately, meeting all validity criteria and the concentrations at which the WHC varied by >10% did not appear to be affected by this deviation. As such, the RMS considers the deviation to be acceptable.

Whilst not statistically significant, it is noted that a reduction in juvenile number of 14.5% and 10.5% occurred at test concentrations 18 and 560 mg formulation/kg dws respectively. The RMS does not consider the reduction in reproductive output to be related to the test item as no clear dose-response was observed at the other tested concentrations.

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

NOEC (reproductive output) = 560 mg formulation/kg dws

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

Report:	KCP 10.4.2.1/01; Larnaudie Lopez, M. I.; 2017
Title:	BCS-CN88460 EC 50 G: Influence on mortality and reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No.:	E 314 05007-0
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable
Guideline deviation(s):	none
GLP/GEP:	Yes

Material and methods:

Test item:	BCS-CN88460 EC 50 G (analytical findings: 5.18% w/w BCS-CN88460 (Isoflucypram) equivalent to 50.46 g/L, density: 0.974 g/mL (20°C), supplier batch no.: 2016-001002, sample description: TOX20246-00, specification no.: 102000031262, sample ID: M16001677001)
Reference item :	Boric acid (test conducted March 2016, non-GLP).
Test organism:	*Folsomia candida* (Collembolan, Isotomidae) bred at Bayer CropScience since September 2015. The strain was originally obtained from BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany.
Age of test organisms:	10 – 12 days old from a synchronised culture. Twelve days before starting the study, egg clusters from the breeding containers were transferred to fresh breeding substrate to obtain collembolans of a uniform age (10 - 12 days old at test start). After 2 days the egg clusters were removed and the remaining collembolans hatched from the eggs were fed with granulated dry yeast.

Breeding of the test organism:	The collembolans were bred on a mixture of Plaster of Paris and activated charcoal and demineralised water (10:1.25:12.5 w/w). Plastic vessels (9.5 cm diameter) were filled up to a height of approximately 1 cm with this mixture. The vessels, closed with plastic lids, were moistened and aerated regularly twice a week. Breeding vessels were kept at room temperature in permanent darkness and fed once per week with dried bakers yeast.
Test duration:	28 days
Test concentrations :	*Test item*: 18, 32, 56, 100, 178, 316, 562 and 1000 mg formulation/kg dws *Reference item:* 44, 67, 100, 150 and 225 mg Boric acid/kg dws (conducted at a different time to the main test) *Controls:* Water control.
Preparation and application of the test item:	All test item solutions were prepared freshly on the day of the application. Uniform volumes (50 mL) were used for all application solutions (starting with the lowest application rate and ending with the highest application rate). The test item was thoroughly mixed into the pre-moistened 500 g dry weight artificial soil of each application rate using a laboratory mixer. The control group was treated first in the same way as described above but with 50 mL deionized water only. Afterwards the treated artificial soil of each application rate and the control was portioned out. Each test vessel of the 8 control replicates and the 4 treatment replicates of each concentration plus the one for measurement purpose was filled up with 30±1 g dry weight artificial soil avoiding compression of the artificial soil.
No. of individuals per replicate:	10
No. of replicates:	8 per control and 4 for the test item.
Test units :	Test containers were reusable glass vessels (volume 140 mL, diameter 5 cm). Each test vessel contained 30±1 g wet weight artificial soil. The test vessels were covered with glass lids to prevent the collembolans from escaping but allowing aeration during the test period.
Feeding and water:	Directly after the addition of the collembolans, they were fed with granulated dry yeast. Feeding was also done 14 days after test start. Approximately 2-10 mg (one spatula tip) per test vessel was added per feeding date. At test start each test vessel was weighed for the determination of water loss. After 14 days the loss of water was determined by reweighing the test vessels. The vessels were rewetted with the approximately 2-fold amount of the missing water.
Artificial soil:	The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): - 75 % fine quartz sand (sort F 36, particle size 0.2 – 0.05 mm = 90.8 %) - 5 % Sphagnum peat, air dried and finely ground - 20 % Kaolin clay (content of Kaolinite: 30.2 %) - Calcium carbonate (CaCO3) for the adjustment to pH to 6.0 ± 0.5 The artificial soil was prepared by mixing the dry components in a laboratory mixer. Four days before test start the dry artificial soil was pre-moistened with 66 mL deionized water per 500 g dry weight artificial soil. During the application of the test item the water content was adjusted to a final water content of approximately 50 % of the maximum water holding capacity.
Test procedure:	Directly after application the 10-12 days old collembolans from the synchronised cohort were collected with an exhauster into small glass tubes. They were counted to ensure that 10 non-damaged individuals were introduced. Then the 10 collembolans were placed on the soil surface of the treated soil and the untreated control respectively. The sequence of inserting the test organisms into the test vessels followed a computer-

	generated random design. The test vessels were set up randomised in a climatic test room. After 7, 14 and 21 days the test vessels were re-randomised. After 28 days, the soil of each replicate was transferred into a plastic vessel (volume: 200 mL; surface: 75 cm^2). Each portion was stirred up with 80 mL of deionized water and the collembolans drifted to the surface. The water was coloured with 10 mL black ink in order to increase the contrast between the water and the white collembolans. From each vessel a digital image was taken and checked by visual inspection. The adult collembolans were visually counted and marked on the digital image, followed by confirmation of the automatically generated juvenile count, which was manually corrected if necessary. The extraction efficiency of this method was determined to be 98 % in a separate extraction run in vessels containing a known number of adult and juvenile collembolans kept in untreated artificial soil.
Observations:	Mortality and reproduction was recorded at test termination. Missing adults (compared to the number of initially placed test organisms) were considered to be dead Water holding capacity (WHC) of the soil was determined at day 0 and day 28. pH of the soil was determined at test initiation and at test termination. Temperature was measured continuously and light intensity measured on day 0, 14 and 28.
Statistical analysis:	The calculation of mean, SD and percentage mortality of the control and treatment groups was conducted in Excel. Data of reproduction were tested for normal distribution and homogeneity of variance using Shapiro-Wilks-Test and Levene-Test (α = 0.05) respectively. Data of reproduction were normally distributed but homogeneity of variances was not given. Therefore Bonferroni-Welsh-t-test (one-sided-smaller, α = 0.05) was used to determine NOEC and LOEC values. The software used to perform the statistical analysis was ToxRat Professional 3.2.1.

Results

Validity criteria:

Validity criteria according to OECD 232 (2016)	**Obtained in this study**
Mean adult mortality ≤ 20%	8.8%
Mean number of juveniles per replicate ≥ 100	1145
Coefficient of variation calculated for the number of juveniles per replicate ≤ 30%	13.4%

All validity criteria were met in this study.

Toxic reference test:

The most recent non-GLP-test (LAR-Coll-Ref-28/16, Maria Ivonne Larnaudie Lopez, November 16, 2016) with Boric acid showed an EC_{50} of 82 mg test item/kg dry weight artificial soil (95% confidence limits from 57 mg to 112 mg Boric acid/kg dry weight artificial soil) for reproduction according Probit analysis using linear maximum likelihood regression. The result is in the recommended range of the guideline (about 100 mg Boric acid/kg artificial soil dry weight).

Biological results:

Mortality

Concerning the mortality of the adult test organisms statistical analysis (Fisher's Exact Binominal Test with Bonferroni Correction, one-sided-greater, α = 0.05) revealed no significant difference between control and any treatment group up to and including 316 mg test item/kg dry weight artificial soil. Therefore the No-Observed-Effect-Concentration (NOEC) for mortality is 316 mg test item/kg dry weight artificial soil.

The LC_{10} and LC_{20} values for mortality were calculated to be 230 mg test item/kg soil dry weight (95% confidence limits: 131 - 290) and 277 mg test item/kg soil dry weight (95% confidence limits: 184 - 336), respectively.

Reproduction

Concerning the number of juveniles statistical analysis (Bonferroni-Welsh-t-test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and any treatment group up to and including 100 mg test item/kg dry weight artificial soil.

Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 100 mg test item/kg dry weight artificial soil.

The EC_{10} and EC_{20} values for reproduction were calculated to be 98 mg test item/kg soil dry weight (95% confidence limits: 66 - 122) and 127 mg test item/kg soil dry weight (95% confidence limits: 95 - 151), respectively.

A summary of the test results is presented in Table B9.7.2-1 below :

Table B9.7.2-1: Effects of BCS-CN88460 EC 50 G on mortality and reproductive output of *Folsomia candida*

Test item **Test object** **Exposure**	**BCS-CN88460 EC 50 G** ***Folsomia candida*** **Artificial soil**				
mg test item/kg dry weight artificial soil (nominal concentrations)	**Adult mortality (%)**	**Significance (*)**	**Mean number of juveniles per test vessel ± standard deviation**	**Reproduction (% of control)**	**Significance (**)**
Control	8.8	--	1145.0 ± 153.7	--	--
18[2)]	6.7	-	1282.7 ± 96.9	112.0	-
32	5.0	-	1275.8 ± 87.5	111.4	-
56	7.5	-	1193.0 ± 52.2	104.2	-
100	7.5	-	1090.3 ± 142.1	95.2	-
178	20.0	-	614.8 ± 98.2	53.7	+
316	25.0	-	356.0 ± 106.4	31.1	+
562	100.0	+	0.0 ± 0.0	0.0	+
1000	100.0	+	0.0 ± 0.0	0.0	+
				Mortality	Reproduction
NOEC (mg test item/kg dry weight artificial soil)				316	100
				Mortality	Reproduction
LC_{10}/EC_{10} (mg test item/kg dry weight artificial soil)[1] 95% confidence limits				230 (131 – 290)	98 (66 – 122)
LC_{20}/EC_{20} (mg test item/kg dry weight artificial soil)[1] 95% confidence limits				277 (184 – 336)	127 (95 – 151)

The calculations were performed with unrounded values

(*) = Fisher's Exact Binomial Test with Bonferroni Correction, one-sided-greater, $\alpha = 0.05$, + = significant, - = not significant)

(**) = (Bonferroni-Welsh-t-test; t-test, one-sided smaller, $\alpha = 0.05$, + = significant, - = not significant)

1) Mortality = Weibull analysis; reproduction = Probit analysis

2) = Evaluation with 3 replicates

Experimental conditions:

pH, water content and WHC during at test initiation and test termination are reported in Table B9.7.2-2 below :

Table B9.7.2-2: pH, water content and WHC of the soil at test initiation and test termination

Test item concentration[1)]	pH		Water content (%)		WHC_{max}[2)]	
	Start	End	Start	End	Start	End
control	5.68	5.50	19.62	19.39	50.11	49.37
18	5.79	5.51	18.73	19.70	47.30	50.36
32	5.77	5.50	18.79	19.21	47.49	48.80
56	5.76	5.52	20.18	19.64	51.90	50.16
100	5.70	5.52	18.81	18.87	47.55	47.75
178	5.60	5.50	18.68	19.34	47.16	49.23
316	5.67	5.50	18.63	18.94	46.99	47.97
562	5.57	5.49	18.64	18.98	47.03	48.09
1000	5.68	5.55	19.06	19.00	48.35	48.15

[1)] mg test item/kg dry weight artificial soil

[2)] % WHC_{max} = percent of maximum water holding capacity of 48.71 g water per 100 g dry weight artificial soil

Conclusions:

$NOEC_{mortality}$: 316 mg test item/kg dry weight artificial soil

$NOEC_{reproduction}$: 100 mg test item/kg dry weight artificial soil

$EC_{10\text{-}reproduction}$: 98 mg test item/kg dry weight artificial soil

$EC_{20\text{-}reproduction}$: 127 mg test item/kg dry weight artificial soil

RMS comments

This study was conducted according to GLP and following OECD 232 (2016) test guidelines. The following was noted by the RMS :

Collembolans were bred under complete darkness, rather than the 12 :12 hour light :dark cycle recommended in the test guidelines. As sufficient synchronised collembolans were bred for the test using this method, this is not considered to be an issue by the RMS.

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

$NOEC_{reproduction}$: 100 mg test item/kg dry weight artificial soil

$EC_{10\text{-}reproduction}$: 98 mg test item/kg dry weight artificial soil

Previous evaluation:	New data, submitted for purpose of review

Report:	KCP 10.4.2.1/02; Larnaudie Lopez, M. I.; 2017
Title:	BCS-CN88460 EC 50 G: Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No.:	E 428 05008-7
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP: Not Applicable
Guideline deviation(s):	none
GLP/GEP:	Yes

Material and methods

Test item:	BCS-CN88460 EC 50 G, batch ID: 2016-001002; sample description: TOX20246-00; specification no.: 102000031262; sample ID: M16000677001; (analytical findings: 5.18% w/w (BCS-CN88460) equivalent to 50.46 g/L; density: 0.974 g/mL (20 °C)).
Reference item :	Dimethoate (non-GLP, conducted August 09 2016).
Test organism:	*Hypoaspis aculeifer* bred at Bayer CropScience AG since 2017. The strain was originally obtained from ECT Oekotoxikologie GmbH, 65439 Flörsheim a. M.
Age of test organisms:	28 days old (adult females) from a synchronised culture. In order to obtain adult, female *Hypoaspis aculeifer* of a uniform age on 2015-01-05, 2 x 300 adult, female *Hypoaspis aculeifer* were transferred to fresh breeding vessels. On 2017-01-16, after three days of egg laying, these females were removed. The *Hypoaspis aculeifer* hatched from the eggs were fed with *Panagrellus redivivus.*
Breeding of the test organism:	The mites were bred on a mixture of Plaster of Paris and activated charcoal and demineralised water (10:1.25:12.5 w/w). Plastic vessels (9.5 cm diameter) were filled up to a height of approximately 1 cm with this mixture and are closed with lids. Mites were fed with *Panagrellus redivivus* (nematodes) which were bred on watered oak flakes.

	The breeding culture was kept under the following conditions: Temperature: room temperature Light cycle: permanent dark
Test duration:	14 days (plus 2 days for extraction of mites)
Test concentrations :	*Test item*: 18, 32, 56, 100, 178, 316, 562 and 1000 mg formulation/kg dws *Reference item:* 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dws (conducted at a different time to the main test) *Controls:* Water
Preparation and application of the test item:	All test item solutions were prepared freshly on the day of the application. A uniform volume of 50 mL was used for all application solutions (starting with the lowest application rate and ending with the highest application rate). The test item was thoroughly mixed into 500 g artificial dry weight artificial soil of each application rate using a laboratory mixer. The control group was treated first in the same way as described above but with 50 mL deionised water only. Afterwards the treated artificial soil of each application rate and the control was portioned out. Each test vessel of the 8 control replicates and the 4 treatment replicates of each concentration plus the one for measurement purpose was filled up with 20±1 g dry weight artificial soil avoiding compression of the artificial soil.
No. of individuals per replicate:	10
No. of replicates:	8 per control and 4 for the test item. There was one additional vessel for each applicationrate for measurement of pH value and moisture of the artificial soil at the end of the test not loaded with *Hypoaspis aculeifer*.
Test units :	Test containers were reusable glass vessels (Weck Mini-Sturzglas, volume 140 mL, diameter 5 cm at the bottom, height 7 cm). Each test vessel contained 20 g dry weight artificial soil (height of artificial soil layer approximately 1.5 cm). The test vessels were covered with glass lids to prevent *Hypoaspis aculeifer* from escaping but allowing aeration during the test period.
Feeding and water:	Directly after the addition of the *Hypoaspis aculeifer*, they were fed with nematodes (*Panagrellus redivivus*). Nematodes were bred on watered oak flakes in the laboratory. During the continuation of the test the soil mites were fed 3, 7 and 10 days after test start with the nematodes. Between 52 and 109 mg food per test vessel was added. Each test vessel was weighed for the determination of water loss. Seven days after test start water loss was determined and replenished.
Artificial soil:	The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): - 75 % fine quartz sand (sort F 36, particle size 0.2 – 0.05 mm = 91.35 %) - 5 % Sphagnum peat, air dried and finely ground - 20 % Kaolin clay (content of Kaolinite: = 30.2 %) - Calcium carbonate (CaCO3) for the adjustment to pH to 6.0 ± 0.5 The artificial soil was prepared by mixing the dry components in a laboratory mixer. Two days before test start the dry artificial soil was pre-moistened with 66 mL deionised water per 500 g dry weight artificial soil. During the application of the test item the water content was adjusted to a final water content of approximately 50 % of the maximum water holding capacity by mixing 50 mL deionised water into 500 g dry weight artificial soil for the control group and 50 mL test item solution for the treatment groups.

Test conditions:	Water content: test start:18.72 – 19.36 (equivalent to 47.28 – 48.92 % of WHC); test end: 18.34 – 19.14 (equivalent to 46.12 – 48.60 % of WHC). pH: test start: 5.68 – 5.73; test end: 5.68 – 5.74. Temperature: 20 ± 2 °C. Light intensity: 532 – 651 lux, 16:8 hour light:dark cycle.
Test procedure:	Directly after application of the test item, the adult, fertilized, females (28 days after start of egg laying for three days) were exposed to the control and treatment vessels. This was achieved by putting 10 females individually onto the surface of the artificial soil using a fine brush. The transfer of the test animals was finished within two hours after the application of the test item. After a period of 14 days, the surviving adults and the living juveniles per test vessel were extracted, applying a temperature gradient. The content of each test vessel was carefully transferred to sieve vessels (mesh size approximately 0.8 mm). The vessels were positioned in MacFadyen- Extractor. The temperature was increased from approximately 25 to 40 °C within two days. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L). The extracted mites in the fixing solution were stored in a refrigerator until the start of the counting of surviving adults and juveniles. All *Hypoaspis aculeifer* (adult females and juveniles) were counted under a binocular. The extraction efficiency of this method was determined to be 91 % in a separate extraction run in vessels containing a known number of adult and juvenile mites kept in untreated artificial soil.
Observations:	Mortality and reproduction was recorded at test termination. Missing adults (compared to the number of initially placed test organisms) were considered to be dead. Water holding capacity (WHC) of the soil and pH was determined at day 0 and day 14. Light intensity was measured at test initiation and after each feeding of the test organisms. Temperature was measured continuously.
Statistical analysis:	The statistical analysis was performed with the software ToxRat Professional 2.10 (Ratte 2010). For the determination of normal distribution and homogeneity of variance Kolmogorov-Smirnov Test and Cochran's Test ($\alpha = 0.05$), respectively were used. These conditions were met; therefore William's-t test (one-sided-smaller, $\alpha = 0.05$) was used to determine NOEC values.

Results

Validity criteria:

Validity criteria according to OECD 226 (2016)	**Obtained in this study**
Mean adult mortality ≤ 20%	3.8%
Mean number of juveniles per replicate ≥ 50	312.9
Coefficient of variation calculated for the number of juveniles per replicate ≤ 30%	11.4%

All validity criteria were met in this study.

Toxic reference test:

Dimethoate EC 400 G showed an EC_{50} of 5.4 mg a.s./kg dry weight artificial soil (95% confidence limits from 5.3 mg a.s./kg to 5.5 mg a.s./kg) for reproduction according Probit analysis using maximum likelihood regression.

This is in the recommended range of the guideline, indicating that an EC_{50} based on the number of juveniles of 3.0 – 7.0 mg a.s./kg dry weight artificial soil shows that the test organisms are sufficiently sensitive.

Biological results**:**

Mortality

Concerning the mortality of the adult test organisms statistical analysis (Fisher´s Exact Binomial Test with Bonferroni Correction, one-sided greater, α = 0.05) revealed no significant difference between control and any treatment group up and including 1000 mg test item/kg dry weight artificial soil.

Therefore the No-Observed-Effect-Concentration (NOEC) for mortality is ≥1000 mg test item/kg dry weight artificial soil.

Reproduction

Concerning the number of juveniles statistical analysis (William's t-test, one-sided smaller, α = 0.05) revealed no significant difference between control and any treatment group up and including 316 mg test item/kg dry weight artificial soil.

Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 316 mg test item/kg dry weight artificial soil.

The EC_{10} and EC_{20} values for reproduction were calculated to be 362 mg (95% confidence limits: 349-375) and 422 mg (95% confidence limits: 410-432) test item/kg soil dry weight, respectively.

A summary of the results is provided in the below table (Table B9.7.2-3).

Table B9.7.2-3: The effect of BCS-CN88460 EC 50 G on mortality and reproductive output of on mortality and reproductive output of *Hyposapis aculeifer* following exposure in artificial soil.

Test item test object exposure	**BCS-CN88460 EC 50 G *Hypoaspis aculeifer* artificial soil**					
mg test item/kg dry weight artificial soil	**adult mortality (%)**	**significance (*)**	**mean number of juveniles per test vessel ± standard dev.**		**reproduction (% of control)**	**significance (**)**
Control	3.8	--	312.9 ±	35.7	--	--
18	15.0	-	311.0 ±	60.9	99.4	-
32	5.0	-	352.8 ±	20.1	112.7	-
56	5.0	-	350.8 ±	25.6	112.1	-
100	0.0	-	347.5 ±	28.6	111.1	-
178	0.0	-	345.8 ±	26.9	110.5	-
316	0.0	-	302.0 ±	29.4	96.5	-
562	7.5	-	155.5 ±	32.4	49.7	+
1000	12.5	-	18.5 ±	10.3	5.9	+

	adult mortality	reproduction
NOEC (mg test item/kg dry weight artificial soil)	≥1000	316
EC_{10} mg test item/kg dry weight artificial soil1) (95% confidence limits) EC_{20} mg test item/kg dry weight artificial soil1) (95% confidence limits)		362 (349 – 375) 422 (410 – 432)

Calculations were done with un-rounded values.

(*) = Fisher´s exact Binomial Test with Bonferroni Correction, one-sided greater, α=0.05, "-": non-significant; "+": significant

(**) = William's t-test, one sided smaller; α=0.05; "-": non-significant; "+": significant

1) = Probit analysis

Experimental conditions:

pH and water content/WHC of the soil during the test is shown in Table B9.7.2-4 below:

Table B9.7.2-4: pH, water content and water holding capacity of the test soil

Test item concentration [1]	pH		Water content (%)		WHC_{max}	
	Start	End	Start	End	Start	End
Control	5.68	5.72	19.36	19.14	49.82	48.60
18	5.70	5.71	19.04	18.85	48.29	47.69
32	5.70	5.74	19.07	18.74	48.36	47.34
56	5.70	5.74	18.73	18.99	47.32	48.11
100	5.69	5.71	19.39	18.87	49.37	47.75
178	5.69	5.70	18.72	19.09	47.28	48.45
316	5.70	5.70	19.14	18.34	48.60	46.12
562	5.72	5.69	18.83	18.73	47.62	47.30
1000	5.73	5.68	19.40	18.77	49.42	47.43

[1] mg/kg soil dry weight

Conclusion:

$NOEC_{adult\ mortality}$: ≥1000 mg test item/kg dry weight artificial soil

$NOEC_{reproduction}$: 316 mg test item/kg dry weight artificial soil

$EC_{10\text{-}reproduction}$: 362 mg test item/kg dry weight artificial soil

$EC_{20\text{-}reproduction}$: 422 mg test item/kg dry weight artificial soil

RMS comments

This study was conducted according to GLP and following OECD 226 (2008) guidance.

All validity criteria were met and the study is considered acceptable for use in the risk assessment. The endpoints are confirmed as follows :

$NOEC_{reproduction}$: 316 mg test item/kg dry weight artificial soil

$EC_{10\text{-}reproduction}$: 362 mg test item/kg dry weight artificial soil

B.9.8. RISK ASSESSMENT FOR NON-TARGET SOIL MESO- AND MACROFAUNA

Earthworms

Toxicity

For the earthworm studies EC_{10} values were not calculable as explained in the study summaries and the risk assessment is based on the NOEC values from the studies.

The risk assessment (calculation of TER values) was based on the NOEC values calculated from the studies performed with the product, the active substance or the metabolite.

The endpoints considered in the risk assessment are provided in Table B9.8-1 below

Table B9.8-1: Endpoints used in risk assessment

Test item	Test species, test design	Ecotoxicological endpoint		Reference
Isoflucypram EC 50	*Eisenia fetida* reproduction 56 d, mixed	NOEC	560 mg formulation/ kg d.w.soil 29 mg a.s./ kg d.w.soil#	Frommholz, U.; 2016; M-574897-01-1 KCP 10.4.1.1/01
		$NOEC_{corr}$*	**280 mg formulation/ kg d.w.soil** **14.5 mg a.s./ kg d.w.soil#**	
Isoflucypram	*Eisenia fetida* reproduction 56 d, mixed	NOEC	56 mg a.s./ kg d.w.soil	Frommholz, U.; 2016; M-548749-01-1 KCA 8.4.1/01
		$NOEC_{corr}$*	**28 mg a.s./ kg d.w.soil**	
BCS-CN88460-carboxylic acid (M12)	*Eisenia fetida* reproduction 56 d, mixed	NOEC	100 mg p.m./kg dws	Frommholz, U.; 2017; M-579263-01-1 KCA 8.4.1/02
		$NOEC_{corr}$*	**50 mg p.m./kg dws**	

dws = dry weight soil; a.s. = active substance; p.m. = pure metabolite,
*Endpoint corrected due to lipophilic substance (log P_{OW} > 2)
Endpoint calculated on the basis of analysed isoflucypram content in the formulation (5.18% w/w; as given in study report)
Endpoints in **Bold** used in risk assessment

Exposure

Estimates of the maximum predicted environmental concentrations in soil (PEC_S) of isoflucypram, its relevant soil metabolite and the formulation, 'Isoflucypram EC 50', have been established in Section B.8 of this assessment report by the Environmental Fate and Behaviour specialist.

The relevant predicted environmental concentrations (PEC) values considered for toxicity exposure ratio (TER) calculations based on the proposed use in cereals are summarised in Table B9.8-2 below. Maximum values are used for risk assessments.

Table B9.8-2: Max PECsoil values

Compound	Cereals BBCH 30 – 69 1 x 75 g a.s./ha		
	PECsoil, ini* [mg/kg]	PECsoil, accu* [mg/kg]	PECsoil, max* [mg/kg]
Isoflucypram	0.0200	0.0616	**0.0616**
BCS-CN88460-carboxylic acid (M12)	<0.0001	0.0001	**0.0001**

Bold values: worst case considered in risk assessment
*Based on worst-case values following early application to cereals

Risk assessment for earthworms

Earthworm (*Eisenia fetida*) toxicity studies have been submitted in line with the reporting requirements in Commission Regulation (EU) No 283/2013. The studies investigate the impact of the active substance isoflucypram as the formulated product 'Isoflucypram EC50' and the metabolite BCS-CN88460-carboxylic acid on earthworms.

All studies were deemed valid for regulatory purposes with no significant deviations from the study guidelines.

The risk assessment was conducted according to the SANCO/10329/2002 guidance on Terrestrial Ecotoxicology for the proposed application rate of 'Isoflucypram EC 50'.

Calculation of TERs

Risk is assessed in terms of Toxicity Exposure Ratios (TERS), using the endpoints from Table 9.8-1 above and is calculated using the following equation:

TER = Study endpoint / PEC_{soil}

As the log P_{ow} values for isoflucypram and its metabolite is >2, correction of the study endpoints is required to account for differences in the organic matter content of the test soil in comparison to artificial soils.

The risk is considered acceptable if the TER_{LT} is >5.

The resulting TERs for earthworms are summarised in Table B9.8-3 below.

Table B9.8-3: TER calculations for earthworms for isoflucypram EC50, isoflucypram and its metabolite BCS-CN88460-carboxylic acid (M12)

Compound	**Species, study type**	**Endpoint [mg a.s./kg]**		**$PEC_{soil,max}$ [mg a.s./kg]**	**TER_{LT}**	**Trigger**
Isoflucypram EC 50	Earthworm, reproduction	NOEC	14.5*	0.0616	235	5
Isoflucypram	Earthworm, reproduction	NOEC	28	0.0616	435	5
BCS-CN88460-carboxylic acid (M12)	Earthworm, reproduction	NOEC	50	0.0001	500000	5

*Expressed as a.s. content of the formulation

Conclusion: The TER values exceed the trigger value of 5 indicating that no unacceptable adverse effects on earthworms are to be expected from the intended use of Isoflucypram EC 50 in cereals at the proposed application rate.

Non-target soil meso- and macrofauna (other than earthworms)

Toxicity

A summary of the endpoints used in the risk assessment is provided in Table B9.8-4 below.

Table B9.8-4: Endpoints used in risk assessment

Test substance	Test species, test design	Ecotoxicological endpoint		Reference
Collembola, reproduction				
Isoflucypram EC 50	*Folsomia candida* reproduction 28 d, mixed	NOEC	100 mg prod./kg dws 5.18 mg a.s./kg dws#	Larnaudie Lopez, M. I.; 2017; M-591834-01-1 KCP 10.4.2.1/01
		$NOEC_{corr}$*	**50 mg prod./kg dws** **2.59 mg a.s./kg dws#**	
		EC_{10}	98 mg prod./kg dws 5.08 mg a.s./kg dws#	
		EC_{10corr}*	49 mg prod./kg dws 2.54 mg a.s./kg dws#	
		EC_{20}	127 mg prod./kg dws 6.58 mg a.s./kg dws#	
		EC_{20corr}*	63.5 mg prod./kg dws 3.29 mg a.s./kg dws#	
Isoflucypram	*Folsomia candida* reproduction 28 d, mixed	NOEC	99 mg a.s./kg dws	Frommholz, U.; 2015; M-522863-01-1 KCA 8.4.2.1/01
		$NOEC_{corr}$*	**49.5 mg a.s./kg dws***	
BCS-CN88460-carboxylic acid (M12)	*Folsomia candida* reproduction 28 d, mixed	NOEC	18 mg p.m./kg dws	Friedrich, S.; 2017; M-587760-01-1 KCA 8.4.2.1/02
		$NOEC_{corr}$*	**9 mg p.m./kg dws**	
		EC_{10}	13 mg p.m./kg dws	
		EC_{10corr}*	6.5 mg p.m./kg dws	
		EC_{20}	20 mg p.m./kg dws	
		EC_{20corr}*	10 mg p.m./kg dws	
Soil mites, reproduction				
Isoflucypram EC 50	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC	316 mg prod./kg dws 16.37 mg a.s./kg dws#	Larnaudie Lopez, M. I.; 2017; M-592571-01-1 KCP 10.4.2.1/02

		$NOEC_{corr}$*	**158 mg prod./kg dws** **8.18 mg a.s./kg dws#**	
		EC_{10}	362 mg prod./kg dws 18.75 mg a.s./kg dws#	
		EC_{10corr}*	181 mg prod./kg dws 9.38 mg a.s./kg dws#	
		EC_{20}	422 mg prod./kg dws 21.86 mg a.s./kg dws#	
		EC_{20corr}*	211 mg prod./kg dws 10.93 mg a.s./kg dws#	
Isoflucypram	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC **$NOEC_{corr}$***	990 mg a.s./kg dws 495 mg a.s./kg dws	Larnaudie-Lopez, M.; 2015; M-528194-01-1 KCA 8.4.2.1/03
BCS-CN88460-carboxylic acid (M12)	*Hypoaspis aculeifer* reproduction 14-d, mixed	NOEC **$NOEC_{corr}$***	990 mg a.s./kg dws 495 mg a.s./kg dws	Larnaudie-Lopez, M. I.; 2015; M-524464-01-1 KCA 8.4.2.1/04

dws = dry weight soil, a.s. = active substance, p.m. = pure metabolite

* Endpoint corrected due to lipophilic substance (log P_{OW} > 2)

Endpoint calculated on the basis of analysed isoflucypram content in the formulation (5.18% w/w; as given in study report)

Exposure

Estimates of the maximum predicted environmental concentrations in soil (PEC_S) of isoflucypram, its relevant soil metabolite and the formulation, 'Isoflucypram EC 50', have been established in Section B.8 of this assessment report by the Environmental Fate and Behaviour specialist and provided in Table 9.8-2 of this document for reference. These exposure estimates have been used to conduct the risk assessment.

Risk assessment for non-target soil meso- and macrofauna (other than earthworms)

Studies with *Hypoaspis aculeifer* and *Folsomia candida* have been submitted in line with the reporting requirements in Commission Regulation (EU) No 283/2013. The studies investigate the impact of the active substance isoflucypram as the formulated product 'Isoflucypram EC50' and the metabolite BCS-CN88460-carboxylic acid on soil meso- and macrofauna.

All studies were deemed valid for regulatory purposes with no significant deviations from the study guidelines.

The risk assessment was conducted according to the SANCO/10329/2002 guidance on Terrestrial Ecotoxicology for the proposed application rate of 'Isoflucypram EC 50'.

Calculation of TERs

Risk is assessed in terms of Toxicity Exposure Ratios (TERS), using the endpoints from Table B9.8-4 above and is calculated using the following equation:

TER = Study endpoint / PEC_{soil}

As the log P_{ow} values for isoflucypram and its metabolite is >2, correction of the study endpoints is required to account for differences in the organic matter content of the test soil in comparison to artificial soils.

The risk is considered acceptable if the TER_{LT} is >5.

The resulting TERs for *F.candida* and *H.aculeifer* are summarised in Table B9.8-5 below.

Table B9.8-5: TER calculations for Isoflucypram EC50, Isoflucypram and its metabolite BCS-CN88460-carboxylic acid (M12) for other non-target soil meso- and macrofauna

Compound	**Species**	**Endpoint [mg a.s./kg]**		**$PEC_{soil,max}$ [mg a.s./kg]**	**TER_{LT}**	**Trigger**
Isoflucypram EC 50	*Folsomia candida*	NOEC	2.59*	0.0616	42	5
Isoflucypram, a.s.	*Folsomia candida*	NOEC	49.5	0.0616	804	5
BCS-CN88460-carboxylic acid (M12)	*Folsomia candida*	NOEC	9.0	0.0001	90000	5
Isoflucypram EC 50	*Hypoaspis aculeifer*	NOEC	8.18*	0.0616	133	5
Isoflucypram, a.s.	*Hypoaspis aculeifer*	NOEC	495	0.0616	8036	5
BCS-CN88460-carboxylic acid (M12)	*Hypoaspis aculeifer*	NOEC	495	0.0001	4950000	5

*Expressed as a.s. content of the formulation

Conclusion: All TER values exceed the trigger value of 5 indicating that no unacceptable adverse effects on soil macro-organisms are to be expected from the intended use of Isoflucypram EC 50 in cereals.

B.9.9. EFFECTS ON SOIL NITROGEN TRANSFORMATION

Previous evaluation:	New data, submitted for purpose of review

Report:	KCP 10.5/01; Schulz, L.; 2017
Title:	Amendment no. 1 to the final report - BCS-CN88460 EC 50 G: Effects on the activity of soil microflora (Nitrogen transformation test) - Final report
Report No.:	16 10 48 062 N
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 (2009) US EPA OCSPP Not Applicable
Guideline deviation(s):	none
GLP/GEP:	**yes**

Material and methods:

Test item:	BCS-CN88460 EC 50 G, Supplier batch No.: 2016-001002, Sample description: TOX20246-00, Specification No.: 102000031262, analytical findings: 5.18% w/w (50.46 g/L) BCS-CN88460, Density (20 °C): 0.974 g/mL, water solubility: dispersible.
Reference item :	Dinoterb (conducted from 2016-01-06 to 2016-02-08)
Origin of soil used in test:	Origin: Wassergut Canitz Country: Germany Federal state: Saxony Municipality: Canitz Field name: Schlag 34/3 Land owner: Wassergut Canitz GPS-position 12.694435960 degrees East, 51.403774567 degrees North No fertilisers applied since 2003 and no plant protection products since 1990. The soil was removed to a depth of 20 cm as a mixed sample. Afterwards, the soil was dried at room temperature, passed through a 2 mm mesh sieve and then stored at a temperature of approx. 4°C in containers under aerobic conditions in the dark. The soil was adapated to test conditions prior to application of the test item.
Soil characteristics:	pH: 6.5 Organic carbon: 1.43 % Humus content: 2.46 % Carbon content of microbial biomass: 37.99 mg C/100 g soil d.w = 2.66 % of organic carbon content N_{min}: 1.08 mg/100g soil d.w. Total N = 0.14 % Soil type: Loamy sand (according to DIN 11277); 10.3 % clay; 38.3 % silt; 51.4 % sand Water holding capacity: 40.56 g/100g soil d.w. Water content: 11.60 g/100g soil d.w. Cation exchange capacity: 8.5 $cmol^+$/kg soil
Test duration:	28 days
Test concentrations :	*Test item*: Application rates: 75g a.s./ha, corresponding to 1.5 L test item/ha; 375 g a.s./ha, corresponding to 7.5 L test item/ha Test concentrations: 0.1 mg a.s kg soil dry weight corresponding to 1.95 mg test item/kg soil dry weight; 0.5 mg a.s kg soil dry weight corresponding to 9.74 mg test item/kg soil dry weight

	Reference item: 6.80 mg, 16.00 mg and 27.00 mg Dinoterb/kg dws (conducted at a different time to the test) *Controls:* Untreated
Application and incubation of the test soil:	200 g soil dry weight (= one sub-sample) per test vessel was weighed. The soil was mixed with 0.5 % (i.e. 1.0 g/200 g soil d.w.) lucerne meal by means of a hand-stirrer (the C/N ratio of the lucerne meal was 13.2/1). One additional soil sample (without lucerne meal) was used for determination of the initial NO3-N-content. The initial NO3-N-content was 2.29 mg /100 g soil d.w. The test item was mixed with deionised water and the test solution was subsequently mixed with the soil by means of a hand-stirrer. Water was added to the soil to achieve a water content of approximately 45 % of WHC.
No. of replicates:	3
Test units :	The incubation of the prepared soil was carried out in wide mouth glass flasks (500 mL) with screw caps to permit air exchange.
Test conditions:	Water content: test start:17.86 – 18.35 g/100 g soil d.w.; test end: 17.58 – 18.04 g/100 g soil d.w pH: test start: 6.2 – 6.3; test end: 6.2 – 6.3. Temperature: 19.7 – 21.0 °C.
Sampling and analysis:	Soil samples (10 g soil d.w. per replicate) were taken at intervals of 3 hours, 7, 14 and 28 days after application and the NH4-N-, NO3-N- and NO2-N-contents were determined. Soil was extracted by adding 50 mL 1 M KCl solution to the equivalent of 10 g soil d.w. and mixing on a rotator at 150 rpm for 60 minutes. The mixtures were centrifuged and stored deep-frozen prior to analysis at -20 ± 5 °C. For the quantitative determination of the mineralized part of nitrogen the Autoanalyzer was used. The Autoanalyzer is a continuous flow analysis system. Ammonium reacts with salicylate and dichloroisocyanuric acid to form an indophenoleblue compound. The intensity of the formed compound is colorimetrically measured at a wavelength of 625 nm. Nitrate is reduced to nitrite by hydrazinesulphate. The nitrite reacts with sulphanilamide in an acidic solution to form a diazocompound. The diazotized product is then coupled with naphthylamine. The intensity of the formed azodye, which is proportional to the sum of the nitrate and nitrite originally present in the sample, is colorimetrically measured at a wavelength of 525 nm. Thee autoanalyzer was calibrated before each measurement series by establishing a calibration curve.
Observations:	A sample of each replicate of each treatment was taken at intervals of 3 hours, 7, 14 and 28 days and the mineral nitrogen content of the soil was determined. The water content of the soil in each test vessel was determined at test start (after application) and adjusted once a week to the required range of 40 - 50 % of WHC. The pH-values of the soil used in the test were measured at test start (after application) and at the final sampling on day 28.
Statistical analysis:	A statistical evaluation of the test results was performed by means of a 2-sided Student-t-test (for homogeneous variances at 5 % significance level).

Results

Validity criteria:

All validity criteria were met in this study.

Validity criteria according to OECD 216 (2000)	Obtained in this study
The coefficient of variation in the control for NO_3-N ≤ 15%	3.9%

Reference test:

In a separate study the reference item Dinoterb caused an inhibition of - 37.0% and a stimulation of nitrogen transformation of + 37.6% at 6.80 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application (time interval 14-28).

Biological results

The test item BCS-CN88460 EC 50 G (Isoflucypram EC 50) caused temporary stimulation of the daily nitrate rate at the tested concentration of 0.1 mg a.s./kg soil dry weight at time interval 7-14 days after application.

No adverse effects of BCS-CN88460 EC 50 G on nitrogen transformation in soil could be observed at both tested concentrations at the end of the test, 28 days after application (time interval 14-28). Differences from the control of + 6.3% (test concentration 0.1 mg test item/kg soil d.w.) and -9.7% (test concentration 0.5 mg a.s./kg soil d.w.) were measured at the end of the 28-day incubation period (time interval 14-28) as shown in Table B9.9-1 below.

Table B9.9-1: Effects on nitrogen transformation in soil after treatment with BCS-CN88460 EC 50 G

Time interval (days)	Control			0.1 mg a.s./kg soil dry weight equivalent to 75 g a.s./ha				0.5 mg a.s./kg soil dry weight equivalent to 375 g a.s./ha			
	Nitrate-N [1]			Nitrate-N [1]			% difference to control	Nitrate-N [1]			% difference to control
0-7	4.60	±	0.11	4.31	±	0.16	- 6.2 [n.s.]	4.49	±	0.26	- 2.4 [n.s.]
7-14	1.50	±	0.12	1.87	±	0.17	+ 25.2 [n.s.]	1.80	±	0.41	+ 20.4 [n.s.]
14-28	1.51	±	0.08	1.61	±	0.19	+ 6.3 [n.s.]	1.37	±	0.17	- 9.7 [n.s.]

The calculations were performed with unrounded values.

[1] Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation

[n.s.] No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, p ≤ 0.05)

[*s] Statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, p ≤ 0.05)

Conclusion:

BCS-CN88460 EC 50 G (Isoflucypram EC 50) caused no adverse effects (difference to control < 25%, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production rate) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.5 mg a.s./kg soil dry weight (9.74 mg prod./kg), which are equivalent to application rates up to 375 g a.s./ha. (7.5 L prod/ha).

RMS comments

This study was conducted according to GLP and following OECD 216 (2000) guidance.

All validity criteria were met.

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

<25% effects on nitrogen transformation at up to 375 g a.s./ha. (7.5 L prod/ha).

B.9.10. RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION

Toxicity

The below table (table B9.10-1) displays the available endpoints for the effects of isoflucypram, the representative formulation 'Isoflucypram EC 50' and the metabolite BCS-CN88460-carboxylic acid (M12).

Table B9.10-1: Endpoints used in risk assessment

Test substance	Test species, test design	Ecotoxicological endpoint	Reference
N-transformation			
Isoflucypram EC 50	Study duration, 28 days	no unacceptable effects at a rate of 9.74 mg prod./kg (7.5 L prod./ha) equivalent to 0.5 mg a.s./kg soil (375 g a.s./ha)	Schulz, L.; 2016; M-574633-02-1 KCP 10.5/01
Isoflucypram	Study duration, 28 days	no unacceptable effects at an application rate of 0.53 mg a.s./kg soil (375 g a.s./ha)	Schulz, L.; 2015; M-532055-01-1 KCA 8.5/01
BCS-CN88460-carboxylic acid (M12)	Study duration, 28 days	no unacceptable effects at an application rate of 0.54 mg p.m./kg soil (403 g p.m./ha)	Schulz, L.; 2015; M-538059-01-1 KCA 8.5/02

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

Exposure

Estimates of the maximum predicted environmental concentrations in soil (PEC_S) of isoflucypram, its relevant soil metabolite and the formulation, 'Isoflucypram EC 50', have been established in Section B.8 of this assessment report by the Environmental Fate and Behaviour specialist.

The relevant predicted environmental concentrations (PEC) values considered for toxicity exposure ratio (TER) calculations based on the proposed use in cereals are summarised in Table B9.10-2 below. Maximum values are used for risk assessments.

Table B9.10.-2: Max PECsoil values

Compound	Cereals BBCH 30 – 69 1 x 75 g a.s./ha		
	PECsoil, ini* [mg/kg]	PECsoil, accu* [mg/kg]	PECsoil, max* [mg/kg]
Isoflucypram	0.0200	0.0616	**0.0616**
BCS-CN88460-carboxylic acid (M12)	<0.0001	0.0001	**0.0001**

Bold values: worst case considered in risk assessment

*Based on worst-case values following early application to cereals

Risk assessment for Soil Nitrogen Transformation

According to regulatory requirements the risk is acceptable if the effect on nitrogen transformation at the maximum PEC_{soil} values is < 25% after 100 days. As such, a comparison has been made of the endpoints from each study and the $PEC_{soil\ max}$ values in the following table.

Table B9.10-3: Risk Assessment for Isoflucypram EC50, Isoflucypram and its metabolite BCS-CN88460-carboxylic acid for soil micro-organisms

Compound	**Species**	**Endpoint [mg a.s./kg]**	**$PEC_{soil,max}$ [mg/kg]**	**Refinement required**
Isoflucypram EC50	Soil micro-organisms	0.50*	0.0616	No
Isoflucypram	Soil micro-organisms	0.53	0.0616	No
BCS-CN88460-carboxylic acid (M12)	Soil micro-organisms	0.54	0.0001	No

*Expressed as a.s. content of the formulation

Conclusion: According to regulatory requirements the risk is acceptable, if the effect on nitrogen transformation at the maximum PEC_{soil} values is < 25% after 100 days. In no case, deviations from the control exceeded 25% after 28 days, indicating low risk to soil micro-organisms.

B.9.11. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

B.9.11.1. Testing on non-target plants

Previous evaluation:	New data, submitted for purpose of review

Report:	KCP 10.6.2/01; Koehler, P.; 2017;
Title:	BCS-CN88460 EC 50 g/L - Effects on the vegetative vigour of ten species of non-target terrestrial plants (Tier 2)
Report No.:	VV17/001
Document No.:	M-589028-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP 850.4150 OECD 227 Vegetative vigour
Guideline deviation(s):	none
GLP/GEP:	yes

Objective:

The objective of this specific study was to evaluate the effect of BCS-CN88460 EC 50 g/L on the vegetative vigour of ten non-target terrestrial plant species following a post-emergence application of the test item onto the foliage of plants at the 2-4 leaf stage.

Material and methods:

Test item: BCS-CN88460 EC 50 g/L, sample description: TOX 20246-01, 5.28 % w/w (51.45 g/L). Supplier batch no: 2016-001002, specification no.: 102000031262. Appearance: Light brown turbid liquid.

Test species: A total of 10 species, 6 dicotyledonous and 4 monocotyledonous species were tested in this vegetative vigour test representing 8 plant families (*Beta vulgaris, Brassica napus, Cucumis sativus, Glycine max, Helianthus annus, Solanum lycopersicum, Allium cepa, Avena sativa, Lolium perenne* and *Zea mays*. The plants were grown in a greenhouse in 15 cm pots (filled with approx. 1.2 L soil). Routine germination tests were carried out on the commercially-sources seeds to ensure their viability. Details of the plant species used in the test are provided in Table B.9.11.1-1 below. Seeds were stored in the dark in plastic boxes in a refrigerator (0 to 15 º C).

Table B.9.11.1-1: plant species tested and historical germination results.

Monocot/ Dicot (M/D)	Species name	Common name	Family	Historical germination
D	*Beta vulgaris*	Sugar beet	Chenopodiaceae	85%*
D	*Brassica napus*	Oilseed rape winter	Brassicaceae	100%*
D	*Cucumis sativus*	Cucumber	Cucurbitaceae	100%*
D	*Glycine max*	Soybean	Fabaceae	95%#
D	*Helianthus annuus*	Sunflower	Asteraceae	85% *
D	*Solanum lycopersicum*	Tomato	Solanaceae	95%*
M	*Allium cepa*	Onion	Amaryllidaceae	95%*
M	*Avena sativa*	Oat	Poaceae	100%*
M	*Lolium perenne*	Ryegrass	Poaceae	90%*
M	*Zea mays*	Corn	Poaceae	100%*

* Germination tests conducted in petri dishes throughout 2016.
# Germination tests conducted in petri dishes throughout 2017.

Test design: Planting density included 2 or 4 plants per 15cm diameter pot with 16 or 8 replicate pots, respectively, for a total of 32 plants per treatment level. The pots were filled with approximately 1.2 L of a silt loam soil.

The plants were treated at the 2-4 leaf stage with 5 test item rates and a water control. The stock and application solutions were prepared in the laboratory and transported to the application site immediately before application. Serial dilutions of BCS-CN88460 EC 50 g/L were sprayed onto the foliage of plants using a calibrated laboratory track sprayer at a volume rate of 200 L/ha. Control pots were sprayed with 200 L/ha of deionized water.

After application, the plants were transferred back to the greenhouse and placed on the tables in a randomized design.

Assessments were made 7, 14 and 21 days after application. On day 7 and 14, only plant survival and visual phytotoxicity were recorded. Final assessments were made for plant survival, visual phytotoxicity, plant growth stage, shoot length and shoot dry weight.

Test concentrations: Control (deionised water), 4.7, 9.4, 18.8, 37.5 and 75.0 g a.s./ha.

Test conditions: Following application, the pots with plants were maintained under greenhouse conditions, natural daylight was supplemented by artificial lighting. The temperature was regulated to maintain 19°C to 31°C during the light cycle (16 h) and 14°C to 26°C during the dark cycle (8 h). The relative humidity was regulated to maintain 55-85 %. The light intensity was measured at 188.6 – 559.4 µmol/m2/sec.

The soil composition is as follows: clay: 17.1%, silt: 51.6%, sand: 31.3%. Organic carbon content: 0.72%, pH: 6.92.

After application, bottom watering was performed with saucers standing below each pot throughout the study. Water was given and retained within the saucer according to the need of the plants to maintain an optimal water supply for plant growth. Watering was checked daily.

During the course of the study, liquid fertilizer (Wuxal Universaldünger) was added into the saucers during watering for all plant species.

Statistical analysis: Statistical analyses of the data were performed using ToxRat statistical software.

Results:

Validity criteria (OECD 227)

Criteria	Required	Observed
Seedling emergence	≥70%	≥85%
Phytotoxic effects in the control plants	None.	None.
Mean plant survival in the controls	≥90%	100%

The germination rate of the seeds used in this study was ≥ 70%.

All plant species in this study met the validity criterion for survival in the controls (at least 90%). In accordance with US EPA guideline (OCSPP 850.4150) and OECD guideline (OECD 227), there was no visible phytotoxicity, and normal growth occurred in the controls of the ten species tested. The control plants of each species showed normal variation in growth, plant development and morphology. The environmental conditions during the test time were kept identical within each species. The pots used for all species of this study were filled with the same soil.

The analysis of BCS-CN88460 content in the initial test item stock solution revealed measured concentrations of 114 % of nominal.

Biological results

Survival: No effects on any plant species was observed up to the highest test item rate tested, there was 100% survival of all species for all rates. See table B.9.11.1-2

Table B.9.11.1-2: Survival

Plant Species	ER_{50} (g a.s./ha)	95% Confidence Limits	
		lower	upper
Beta vulgaris	>75 [a]	n.d.	n.d.
Brassica napus	>75 [a]	n.d.	n.d.
Cucumis sativus	>75 [a]	n.d.	n.d.
Glycine max	>75 [a]	n.d.	n.d.
Helianthus annuus	>75 [a]	n.d.	n.d.
Solanum lycopersicum	>75 [a]	n.d.	n.d.
Allium cepa	>75 [a]	n.d.	n.d.
Avena sativa	>75 [a]	n.d.	n.d.
Lolium perenne	>75 [a]	n.d.	n.d.
Zea mays	>75 [a]	n.d.	n.d.

n.d.: Confidence limits not determined (outside the range tested)
[a]: No effects were observed up to the highest concentration tested.

Shoot length: No statistically significant effects on shoot length was observed at any test rate, except for on *Zea mays* at the highest tested rate (3.7% inhibition). See Table B.9.11.1-3 for IR_{50} values for shoot length.

Table B.9.11.1-3: Shoot length

Plant Species	IR_{50}* (g a.s./ha)	95% Confidence Limits	
		lower	upper
Beta vulgaris	>75 [b]	n.d.	n.d.
Brassica napus	>75 [b]	n.d.	n.d.
Cucumis sativus	>75 [b]	n.d.	n.d.
Glycine max	>75 [b]	n.d.	n.d.
Helianthus annuus	>75 [b]	n.d.	n.d.
Solanum lycopersicum	>75 [b]	n.d.	n.d.
Allium cepa	>75 [b]	n.d.	n.d.
Avena sativa	>75 [b]	n.d.	n.d.
Lolium perenne	>75 [b]	n.d.	n.d.
Zea mays	>75 [b]	n.d.	n.d.

*: IR_{50} corresponds to ER_{50}.
n.d.: Confidence limits not determined (outside the range tested)
[b]: Not calculated (outside the range tested).

Shoot Dry Weight: Inhibition of shoot dry weight was statistically significant in a number of plant species. However, the inhibition was always <50% (see Table B.9.11.1-4).

Table B.9.11.1-4: Shoot dry weight

Plant Species	Highest inhibition (%) and rate (g.a.s./ha)	IR_{50}* (g a.s./ha)	95% Confidence Limits	
			lower	upper
Beta vulgaris	8.3% at 75.0	>75 [b]	n.d.	n.d.
Brassica napus	None	>75 [b]	n.d.	n.d.
Cucumis sativus	None	>75 [b]	n.d.	n.d.
Glycine max	11.2% at 75.0	>75 [b]	n.d.	n.d.
Helianthus annuus	16.0% at 75.0	>75 [b]	n.d.	n.d.
Solanum lycopersicum	8.6% at 75.0	>75 [b]	n.d.	n.d.
Allium cepa	None	>75 [c]	n.d.	n.d.
Avena sativa	None	>75 [b]	n.d.	n.d.
Lolium perenne	None	>75 [b]	n.d.	n.d.
Zea mays	14.9% at 75.0	>75 [b]	n.d.	n.d.

*: IR_{50} corresponds to ER_{50}.
n.d.: Confidence limits not determined (outside the range tested)
b: Not calculated (outside the range tested)
c: Not calculated (No statistically significant rate response was found).

Growth stage: Table B.9.11.1-5 shows the minimum – maximum growth stage of each plant species at the final assessment.

Table B.9.11.1-5: Growth stage (BBCH) Min-Max at test item rates (in g a.s./ha) at the final assessment

Plant species	Control	4.7	9.4	18.8	37.5	75
Beta vulgaris	19	19	19	19	19	19
Brassica napus	30	30	30	30	30	30
Cucumis sativus	69	69	69	69	69	69
Glycine max	63-65	63-65	63-65	63-65	63-65	63-65
Helianthus annuus	51	51	51	51	51	51
Solanum lycopersicum	51-63	51-62	51-62	51-63	51-62	51-61
Allium cepa	14-41	41	41	14-41	14-41	41
Avena sativa	32-33	32-33	32-33	32-33	32-33	32-33
Lolium perenne	22-26	22-28	13-29	23-29	21-28	22-29
Zea mays	31-33	31-33	32-33	31-33	32-33	31-33

Phytotoxicity

The symptoms observed at the final assessment (on day 21 after application) in vegetative vigour testing include chlorosis, necrosis, deformation and stunting of the plants. In this study, the severity and occurrence of phytotoxic symptoms differed among species and test item rates and was slight. Table B.9.11.1-6 shows the observed phytotoxicity symptoms.

Table B.9.11.1-6: Phytotoxicity summary (mean damage in %) at test item rates (in g a.s./ha) at the final assessment

Plant species	Control	4.7	9.4	18.8	37.5	75
Beta vulgaris	0.0	0.0	0.0	0.6 e	0.6 e	10.0 be
Brassica napus	0.0	0.6 b	0.0	1.3 b	7.5 d	10.6 bd
Cucumis sativus	0.0	0.0	1.9 ae	1.9 ab	1.3 ae	6.9 abe
Glycine max	0.0	0.6 ad	0.6 e	1.9 bd	3.1 abd	7.5 bde
Helianthus annuus	0.0	0.0	1.3 ab	1.3 be	1.3 ab	13.1 abde
Solanum	0.0	2.5 abe	1.3 abe	2.5 e	1.3 be	5.6 be
Allium cepa	0.0	0.0	0.0	0.0	0.0	0.0
Avena sativa	0.0	1.3 e	5.0 e	1.3 e	1.3 e	8.8 abe
Lolium perenne	0.0	0.0	1.3 e	1.3 de	6.3 de	8.8 bde
Zea mays	0.0	0.0	0.6 e	0.6 e	1.9 bde	6.3 bde

Codes for phytotoxic symptoms:
a: chlorosis (yellowing of green shoot tissue)
b: necrosis (e.g. brown shoot tissue, parts of the plant die)
d: deformation (e.g. leaf curl, abnormal leaf shape, abnormal plant habitus)
e: stunting (e.g. plant height reduced with shorter internode length, plant growth reduction)

Conclusion:

This vegetative vigour and growth study, in which the effect of BCS-CN88460 EC 50 g/L on ten non-target terrestrial plant species was tested under greenhouse conditions, resulted in no adverse effects on survival, visual phytotoxicity, growth stage development, shoot length and shoot dry weight above the 50% effect level.

RMS Comments

This study was conducted according to OECD 227 (2006) and EPA 850.4150 (2012) and was GLP compliant. Some minor deviations were noted in the study methodology:

The light intensity was measured at 188.6 – 559.4 µmol/m2/sec. EPA 850.4150 (2012) and OECD 208 (2006) state that the light intensity should be 350 ± 50 µmol/m2/sec at the top of the canopy. Therefore, the lower end of the measured range is lower than that recommended in the guidelines. However, the survival and growth of all

plants by the end of the test was satisfactory so this deviation is not considered to have affected the results of the test.

The OECD guidelines state that a sandy loam, loamy sand or sandy clay loam soil should be used; however, a silt loam was used in this study. Whilst this is a deviation from the study guidelines, the soil met the requirements for up to 1.5 % organic carbon and was sieved to a particle size of 2 mm, and thus the RMS considers this to be a minor issue.

The method used in support of the analytical phase of this study (analysis of the spray solution samples) is method number AM027115MF1. This method has not been submitted for consideration, but a later version of this method, AM027115MF2, has been submitted as the method for the analysis of the formulation and has been acceptably validated.

The applicant has confirmed that methods AM027115MF1 and AM027115MF2 are different versions of the same method and that the later version was created to "correct typing errors" relating to the chromatographic conditions. They have confirmed that "all testing (including accelerated and cold storage stability) have been performed according to the validated parameters in AM027115MF2".

The differences between the validated method and the method used in this study appear to be minor and limited to the rinsing flow rate of the HPLC system and the amount of test item analysed. The only significant impact of these changes is in relation to the final extract concentration, which, for this analysis now falls outside of the validated linear range. Linearity data were not included in this report as concentrations were measured against a single point recovery determination (thus assuming linearity between the measured concentrations). While this is not ideal, it is noted that the proposed endpoint is a greater-than value therefore this data can be used as an indicator of spray concentration.

The proposed endpoint is an ER_{50} value of > 75.0 g a.s./ha as no adverse effects on survival, shoot height, phytotoxicity or dry shoot weight reached or exceeded the 50 % effect level in any species of plant tested. The RMS considers this value acceptable for use in the risk assessment.

Previous evaluation:	New data, submitted for purpose of review

Report:	KCP 10.6.2/02; Koehler, P.; 2017; M-596298-01-1
Title:	BCS-CN88460 EC 50 g/L - Effects on the seedling emergence and growth of ten species of non-target terrestrial plants (Tier 1) - Final report -
Report No.:	SE17/008
Document No.:	M-596298-01-1
Guideline(s):	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4100; OECD 208 Seedling Emergence
Guideline deviation(s):	none
GLP/GEP:	yes

Objective:

The objective of this specific study was to evaluate the potential effect of BCS-CN88460 EC 50 g/L on the seedling emergence and growth of ten species of non-target terrestrial plants following a pre-emergence application of the product to the soil surface.

Material and methods:

Test item: BCS-CN88460 EC 50 g/L, sample description: TOX 20246-01, 5.28% w/w (51.45 g/L). Supplier batch no: 2016-001002, specification no.: 102000031262. Appearance: Light brown turbid liquid.

Test species: A total of 10 species, 6 dicotyledonous and 4 monocotyledonous species were tested in this seedling emergence test representing 8 plant families. Table B.9.11.1-7 shows the species tested at 75 g a.s./ha.

Table B.9.11.1-7: species tested with 75 g a.s./ha isoflucypram.

Species name	EPPO CODE	Common name	Test item rates in g a.s./ha
			75
Beta vulgaris	BEAVA	Sugar beet	X
Brassica napus	BRSNW	Oilseed rape (winter)	X
Cucumis sativus	CUMSA	Cucumber	X
Glycine max	GLXMA	Soybean	X
Helianthus annuus	HELAN	Sunflower	X
Solanum lycopersicum	LYPES	Tomato	X
Allium cepa	ALLCE	Onion	X
Avena sativa	AVESA	Oat	X
Lolium perenne	LOLPE	Ryegrass	X
Zea mays	ZEAMA	Corn	X

X: Plant species tested with test item rate.

Test design: The seeds were sown on the day of the application of the test item to the soil surface in 15 cm pots (filled with approx. 1.2 L soil). The used soil was a silt loam (17.1% clay, 51.6% silt and 31.3% sand, 0.72% organic carbon content, pH: 6.0 – 7.5).

Planting density included 2 or 4 seeds per pot, with 20 or 10 replicate pots, respectively, for a total of 40 seeds (20 seeds) per treatment level.

The test was conducted as a limit test, the sown seeds of the plant species were treated with a single test item application rate and a water control.

The stock and application solutions were prepared in the laboratory and transported to the application site immediately before application.

The single rate 75 g a.s./ha of BCS-CN88460 EC 50 g/L was applied once at test initiation to the soil surface using a calibrated laboratory track sprayer at a volume rate of 200 L/ha. The analysis of BCS-CN88460 content in the initial test item stock solution revealed measured concentrations of 113 % of nominal.

Control pots were sprayed with 200 L/ha of deionized water.

After application, the pots with seeds were transferred back to the greenhouse and placed on the tables in a randomized design. During the course of the experimental study part the pots of each plant species were rearranged within each species plot.

The control pots of each species were observed daily for the number of seedlings emerged until 50% of the seedlings had emerged (= day 0). Assessments were made individually for each species on this day (= day 0) and 7, 14 and 21 days post emergence of 50 % of the control seedlings. On day 0, 7 and 14, only plant emergence, survival and visual phytotoxicity were recorded.

Final assessments (21 days post emergence of 50 % of the control seedlings) were made for emergence, plant survival, visual phytotoxicity, plant growth stage, shoot length and shoot dry weight.

Test conditions: Following application, the pots with plants were maintained under greenhouse conditions, natural daylight was supplemented by artificial lighting. The temperature was regulated to maintain 19°C to 31°C during the light cycle (16 h) and 14°C to 26°C during the dark cycle (8 h).The relative humidity was regulated to maintain 55 to 85%. The light intensity was measured between 200.1 and 868.2 µmol/m^2/sec.

After application bottom watering was performed with saucers standing below each pot throughout the study. Water was given and retained within the saucer according to the need of the plants to maintain an optimal water supply for plant growth. The top soil was kept moist until the controls reached 50% of seedling emergence. Watering was checked daily

Statistical analysis: Statistical analysis of emergence, survival, shoot length and shoot dry weight data was carried out with the Mann-Whitney-U-Test (one sided smaller; $p \leq 0.05$), included in ToxRat statistics.

Results:

Validation criteria (OECD 208)

Criteria	Required	Observed
Seedling emergence	≥70%	≥87.5%
Phytotoxic effects in the control plants	None.	None
Plant survival in the controls	≥90%	≥ 90.9 % (*A. cepa*) lowest species survival.

All species in this study met the validity criteria for seedling emergence (at least 70 %) and survival (at least 90 %) in the controls. In accordance with OECD guideline (OECD 208) and US EPA guideline (OCSPP 850.4100), there was no visible phytotoxicity, and normal growth occurred in the controls of the ten species tested. The control plants of each species showed normal variation in growth, plant development and morphology. The environmental conditions during the test time were kept identical within each species. The pots used for all species of this study were filled with the same quantity of the same soil.

Biological results

Growth stage

As a result of this seedling emergence and growth study, in which BCS-CN88460 EC 50 g/L was tested on 10 species of non-target terrestrial plants with the test item rate of 75 g a.s./ha, minor effects on the growth stage of *Beta vulgaris, Helianthus annuus* and *Zea mays* were seen at the final assessment. For all other plant species tested no adverse effects on the growth stage development were found in comparison to the control. *Brassica napus, Cucumis sativus, Glycine max, Solanum lycopersicum, Allium cepa, Avena sativa* and *Lolium perenne* exhibited normal variation in the growth stage development compared to the control.

Phytotoxic symptoms

At the final assessment, no phytotoxic symptoms were observed for *Beta vulgaris, Glycine max, Helianthus annuus, Solanum lycopersicum, Allium cepa, Avena sativa, Lolium perenne* and *Zea mays* at the test item rate of 75 g a.s./ha. Slight phytotoxic symptoms were observed in a few cases for *Brassica napus* (1.5 %, stunting) and *Cucumis sativus* (1.0 %, chlorosis, necrosis).

Emergence

At the test item rate of 75 g a.s./ha emergence for *Lolium perenne* was reduced by 11.1 %, compared to the water treated controls. This reduction was not statistically significant. There was no negative effect on emergence of the other species tested (*Beta vulgaris, Brassica napus, Cucumis sativus, Glycine max, Helianthus annuus, Solanum lycopersicum, Allium cepa, Avena sativa and Zea mays)*.

Survival

There was no negative effect on survival at the test item rate of 75 g a.s./ha for any species tested at the final assessment.

Shoot length

Compared to the control plants Shoot length was reduced by 4.0 % for *Beta vulgaris* and for *Brassica napus* by 6.2 % at the test item rate of 75 g a.s./ha. This reduction was statistically significant. The reduction of shoot length for *Glycine max* (1.0 %), *Helianthus annuus* (1.5 %), *Allium cepa* (7.4 %) and *Zea mays* (2.5 %) was not statistically significant. There was no reduction of shoot length observed for *Cucumis sativus, Solanum lycopersicum, Avena sativa* and *Lolium perenne*, compared to the control plants.

Shoot dry weight

At the test item rate of 75 g a.s./ha compared to the control plants shoot dry weight for *Beta vulgaris, Glycine max,* and *Allium cepa* was statistical significantly reduced by 7.3 %, 1.9 % and 19.4 % , respectively. For *Helianthus annuus* and *Zea mays* shoot dry weight was not statistical significantly reduced by 3.0 % and 9.7 %, respectively. For all other plants tested (*Brassica napus, Cucumis sativus, Solanum lycopersicum, Avena sativa* and *Lolium perenne*) no reduction was observed compared to the control plants.

Table B.9.11.1-8 summarises percent inhibition of emergence, survival, shoot dry weight and shoot length as calculated for the final assessment (21 days after 50 % emergence of the control seedlings). In addition, ratings of phytotoxicity and growth stage (BBCH) are provided for all species tested.

Table B.9.11.1-8: Biological effects of plant species treated with BCS-CN88460 (equivalent to 75 g a.s./ha)

Plant Species	Observations at the test item rate of 75 g a.s./ha					BBCH control min - max	BBCH treated min - max
	Emergence (% inhibition)*	Survival (% inhibition)*	Shoot dry weight (%)	Shoot length (%)	Phyto-toxicity (%)		
Beta vulgaris	0.0	0.0	7.3	4.0	0	16-19	16-18
Brassica napus	-2.9	0.0	-6.8	6.2	1.5 [e]	30-31	30-31
Cucumis sativus	0.0	-2.6	-4.4	-5.7	1.0 [a, b]	51-55	51-56
Glycine max	0.0	0.0	1.9	1.0	0	51-59	51-59
Helianthus annuus	0.0	2.6	3.0	1.5	0	32-33	31-33
Solanum lycopersicum	0.0	0.0	-7.2	-9.9	0	14-51	15-51
Allium cepa	-18.2	-7.2	19.4	7.4	0	12-14	12-14
Avena sativa	-2.6	0.0	-1.8	-5.8	0	14-33	14-33

Lolium perenne	11.1	0.0	-13.0	-3.1	0	13-25	13-27
Zea mays	-5.4	0.0	9.7	2.5	0	16-32	15-32

*****A negative value indicates an increase compared to the control
Bold figures are statistically significant (Pairwise Mann-Whitney-U-test, one sided smaller; $p \leq 0.05$).
Codes for phytotoxic symptoms: a: chlorosis (yellowing of green shoot tissue), b: necrosis (e.g. brown shoot tissue, parts of the plant die), e: stunting (e.g. plant height reduced with shorter internode length, plant growth reduction)

Conclusion:

This Tier 1 seedling emergence and growth study in which the effect of BCS-CN88460 EC 50 g/L on ten non-target terrestrial plant species was tested under greenhouse conditions resulted in no adverse effects on emergence, survival, shoot length and shoot dry weight above the 50 % effect level at the test item rate of 75 g a.s./ha.

RMS Comments

This study was conducted according to OECD 208 (2006) and EPA 850.4100 (2012) and was GLP compliant. Some minor deviations were noted in the study methodology:

The light intensity was measured at 188.6 – 559.4 µmol/m2/sec. EPA 850.4150 (2012) and OECD 208 (2006) state that the light intensity should be 350 ± 50 µmol/m2/sec at the top of the canopy. Therefore, the lower end of the measured range is lower than that recommended in the guidelines. However, the survival and growth of all plants by the end of the test was satisfactory so this deviation is not considered to have affected the results of the test.

The OECD guidelines state that a sandy loam, loamy sand or sandy clay loam soil should be used; however, a silt loam was used in this study. Whilst this is a deviation from the study guidelines, the soil met the requirements for up to 1.5 % organic carbon and was sieved to a particle size of 2 mm, and thus the RMS considers this to be a minor issue.

The method used in support of the analytical phase of this study (analysis of the spray solution samples) is method number AM027115MF1. This method has not been submitted for consideration, but a later version of this method, AM027115MF2, has been submitted as the method for the analysis of the formulation and has been acceptably validated.

The applicant has confirmed that methods AM027115MF1 and AM027115MF2 are different versions of the same method and that the later version was created to "correct typing errors" relating to the chromatographic conditions. They have confirmed that "all testing (including accelerated and cold storage stability) have been performed according to the validated parameters in AM027115MF2".

The differences between the validated method and the method used in this study appear to be minor and limited to the rinsing flow rate of the HPLC system and the amount of test item analysed. The only significant impact of these changes is in relation to the final extract concentration, which, for this analysis now falls outside of the validated linear range. Linearity data were not included in this report as concentrations were measured against a single point recovery determination (thus assuming linearity between the measured concentrations). While this is not ideal, it is noted that the proposed endpoint is a greater-than value therefore this data can be used as an indicator of spray concentration.

The proposed endpoint is an ER_{50} value of > 75.0 g a.s./ha as no adverse effects on survival, shoot height or dry shoot weight reached or exceeded the 50 % effect level in any species of plant tested. The RMS considers this value acceptable for use in the risk assessment.

Previous evaluation:	New data, submitted for purpose of review

Report:	KCP 10.6.2/03; Köhler, P.; 2017;
Title:	Effects on the seedling emergence and growth of four species of non-target terrestrial plants (Tier 2) Isoflucypram EC 50 g/L
Report No.:	SE17/056
Document No.:	M-607264-01-1
Guideline(s):	US EPA OCSPP 850.4100 (2012) OECD 208 (2006)
Guideline deviation(s):	none
GLP/GEP:	yes

Objective:

The objective of this specific study was to evaluate the potential effect of BCS-CN88460 EC 50 g/L on the seedling emergence and growth of four species of non-target terrestrial plants following a pre-emergence application of the product to the soil surface.

Material and methods:

Test item: BCS-CN88460 EC 50 g/L, sample description: TOX 20246-01, 5.28% w/w (51.45 g/L). Supplier batch no: 2016-001002, specification no.: 102000031262. Appearance: Brown light turbid liquid.

Test species: Four crop species, 3 dicotyledonous and 1 monocotyledonous species were tested in this seedling emergence test representing 4 different plant species. *Beta vulgaris, Brassica, Glycine max, Alium cepa.*

Test design: The seeds were sown on the day of application of the test item to the soil surface in 15 cm pots (filled with approximately 1.2 L soil). The soil used was a silt loam (17.1% clay, 51.6% silt and 31.3% sand, 0.72% organic carbon content, pH: 6.92).

Planting density included 2 or 4 seeds per pot, with 20 or 10 replicate pots, respectively, for a total of 40 seeds per treatment level (test item rates, water control).

The sown seeds of each of the plant species were treated with 5 test item rates or a water control.

The stock and application solutions were prepared in the laboratory and transported to the application site immediately before application.

Serial dilutions of isoflucypram EC 50 g/L were sprayed to the soil surface using a calibrated laboratory track sprayer at a volume rate of 200 L/ha. Details of the range of test item rates per species are summarized in table B.9.11.1-9.

Table B.9.11.1-9: Test item rates per species

Species name	EPPO CODE	Common name	Test item rates in g a.s./ha				
			4.7	9.4	18.8	37.5	75
Beta vulgaris	BEAVA	Sugar beet	X	X	X	X	X
Brassica napus	BRSNW	Oilseed rape winter	X	X	X	X	X
Glycine max	GLXMA	Soybean	X	X	X	X	X
Allium cepa	ALLCE	Onion	X	X	X	X	X

X: Test item rate tested

Control pots were sprayed with 200 L/ha of deionized water. After application, the pots with seeds were transferred back to the greenhouse and placed on the tables in a randomized design with all pots of one species arranged together in a species plot. During the course of the experimental study part, the pots of each plant species were rearranged within each species plot.

Following application, the pots with plants were maintained under greenhouse conditions and natural daylight was supplemented by artificial lighting. The temperature was regulated to maintain 19°C to 31°C during the light cycle (16 h) and 14°C to 26°C during the dark cycle (8 h). The relative humidity was regulated to maintain 55 to 85% during dark and light cycle. The light intensity was measured between 130.44 and 578.5 µmol/m^2/sec.

The control pots of each species were observed daily for the number of seedlings emerged until 50% of the seedlings had emerged (= day 0). Assessments were made individually for each species on this day (= day 0) and 7, 14 and 21 days post emergence of 50% of the control seedlings. On day 0, 7 and 14, only plant emergence, survival and visual phytotoxicity were recorded.

Final assessments were made for emergence, plant survival, visual phytotoxicity, plant growth stage, shoot length and shoot dry weight 21 days post emergence of 50% of the control seedlings.

Statistical analysis of the data was performed to obtain ER_{50} (Effect Rate) for emergence, survival, and IR_{50} (Inhibition Rate) for shoot length and shoot dry weight, using ToxRat statistical software.

Results:

Validity criteria

Control plants showed no signs of phytotoxicity and environmental conditions were identical for all species during the test. Emergence of control seedlings was ≥ 95.0 % for each species and survival of control seedlings was 100.0 % for each species for the duration of the study, as detailed in Table B.9.11.1-10 below. Therefore, all validity criteria were met for the study.

Table B.9.11.1-10: Validity criteria

Plant Species	Emergence (% of sown)	Survival (% of emerged seedlings)
	≥ 70 % required	≥ 90 % required
Dicotyledoneae		
Beta vulgaris	95.0	100.0
Brassica napus	95.0	100.0
Glycine max	95.0	100.0
Monocotyledoneae		
Allium cepa	95.0	100.0

In accordance with OECD guideline (OECD 208) and US EPA guideline (OCSPP 850.4100), there was no visible phytotoxicity, and normal growth occurred in the controls of the four species tested. The control plants of each species showed normal variation in growth, plant development and morphology. The environmental conditions during the test time were kept identical within species.

The pots used for all species of this study were filled in equal manner with the same soil (see point 3.2).

The analysis of isoflucypram (BCS-CN88460) content in the initial test item stock solution revealed measured concentrations of 108% of nominal.

Symptoms observed at the final assessment (day 21 after 50% control seedling emergence) in seedling emergence testing include chlorosis, necrosis, deformation and stunting of the seedlings. In this study, the severity and occurrence of phytotoxic symptoms differed among species and test item rates and was mainly slight and sporadically.

The ER_{50} for emergence and survival and IR_{50} values for shoot length and shoot dry weight expressed in g a.s./ha are summarized for each of the plant species in the tables B.9.11.1-11 – table B.9.11.1-14 for the final assessment (21 days after 50% emergence of the control seedlings). Table B.9.11.1-15 shows the observed phytotoxic effects of the test item at final assessment.

Table B.9.11.1-11: ER_{50} for emergence

Emergence			
Plant species	ER_{50} (g a.s./ha)	95% Confidence limits	
		lower	upper
Beta vulgaris	>75[a]	n.d.	n.d.
Brassica napus	>75[a]	n.d.	n.d.

Glycine max	>75[a]	n.d.	n.d.
Allium cepa	>75[a]	n.d.	n.d.

n.d.: Confidence limits not determined (outside the range tested)
[a]: Not calculated (outside the range tested).

Table B.9.11.1-12: ER_{50} for survival

Survival			
Plant species	ER_{50} (g a.s./ha)	95% Confidence limits	
		lower	upper
Beta vulgaris	>75[b]	n.d.	n.d.
Brassica napus	>75[b]	n.d.	n.d.
Glycine max	>75[a]	n.d.	n.d.
Allium cepa	>75[a]	n.d.	n.d.

n.d.: Confidence limits not determined (outside the range tested)
[a]: Not calculated (outside the range tested).
[b]: Not calculated (no effect observed).

Table B.9.11.1-13: IR_{50} for shoot length

Shoot length			
Plant species	IR_{50}* (g a.s./ha)	95% Confidence limits	
		lower	upper
Beta vulgaris	>75[a]	n.d.	n.d.
Brassica napus	>75[a]	n.d.	n.d.
Glycine max	>75[a]	n.d.	n.d.
Allium cepa	>75[a]	n.d.	n.d.

*: IR corresponds to ER.
n.d.: Confidence limits not determined (outside the range tested)
[a]: Not calculated (outside the range tested).

Table B.9.11.1-14: IR_{50} for shoot dry weight

Shoot dry weight			
Plant species	IR_{50}* (g a.s./ha)	95% Confidence limits	
		lower	upper
Beta vulgaris	>75[a]	n.d.	n.d.
Brassica napus	>75[a]	n.d.	n.d.
Glycine max	>75[a]	n.d.	n.d.
Allium cepa	>75[a]	n.d.	n.d.

*: IR corresponds to ER.
n.d.: Confidence limits not determined (outside the range tested)
[a]: Not calculated (outside the range tested).

Table B.9.11.1-15: Phytotoxic effects of the test item at the final assessment

Plant species	Phytotoxicity summary (mean damage in %) at test item rates (in g a.s./ha) at the final assessment					
	Control	4.7	9.4	18.8	37.5	75
Beta vulgaris	0.0	1.0 e	1.5 e	3.0 e	2.0 e	2.5 e
Brassica napus	0.0	0.0	1.5 e	1.5 e	4.2 e	1.0 e
Glycine max	0.0	3.0 ade	4.0 ade	1.5 de	2.0 abe	2.5 abe

Allium cepa	0.0	0.0	3.0 e	4.0 e	3.0 e	3.0 e

Codes for phytotoxic symptoms:
a: chlorosis (yellowing of green shoot tissue)
b: necrosis (e.g. brown shoot tissue, parts of the plant die)
c: bleaching (e.g. shoot tissue without pigmentation)
d: deformation (e.g. leaf curl, abnormal leaf shape, abnormal plant habitus)
e: stunting (e.g. plant height reduced with shorter internode length, plant growth reduction)
f: reddening (reddening of green shoot tissue)
Any plant considered as being dead was not rated for phytotoxicity.

Table B.9.11.1-16 shows the growth stages at the final assessment of each species.

Table B.9.11.1-16: Growth stages of test species at the final assessment.

Plant species	Growth stage (BBCH) Min-Max at test item rates (in g a.s./ha) at the final assessment					
	Control	4.7	9.4	18.8	37.5	75
Beta vulgaris	15-16	15-17	15-16	14-17	15-17	15-16
Brassica napus	16-17	16-17	15-17	15-17	13-17	16-17
Glycine max	13-21	21	11-21	21-22	21	12-21
Allium cepa	12-13	12-13	11-13	12-13	12-13	12-13

Conclusion:

This seedling emergence and growth study, in which the effect of Isoflucypram EC 50 g/L on four non-target terrestrial plant species was tested under greenhouse conditions, resulted in no adverse effects on emergence, survival, shoot length, shoot dry weight, growth stage development or visual phytotoxicity above the 50% effect level.

For all measurements and species the ER_{50}/IR_{50} values for all species tested were higher than the highest test item rate and are therefore reported as > 75 g a.s./ha.

RMS Comments

This study was conducted according to OECD 208 (2006) and EPA 850.4100 (2012) and was GLP compliant. Some minor deviations were noted in the study methodology:

The light intensity was measured at 130.44 and 578.5 µmol/m^2/sec. EPA 850.4150 (2012) and OECD 208 (2006) state that the light intensity should be 350 ± 50 µmol/m2/sec at the top of the canopy. Therefore, the lower end of the measured range is lower than that recommended in the guidelines and the higher end is higher. However, the survival and growth of all plants by the end of the test was satisfactory so this deviation is not considered to have affected the results of the test.

The OECD guidelines state that a sandy loam, loamy sand or sandy clay loam soil should be used; however, a silt loam was used in this study. Whilst this is a deviation from the study guidelines, the soil met the requirements for up to 1.5 % organic carbon and was sieved to a particle size of 2 mm, and thus the RMS considers this to be a minor issue.

The method used in support of the analytical phase of this study (analysis of the spray solution samples) is method number AM027115MF1. This method has not been submitted for consideration, but a later version of this method, AM027115MF2, has been submitted as the method for the analysis of the formulation and has been acceptably validated.

The applicant has confirmed that methods AM027115MF1 and AM027115MF2 are different versions of the same method and that the later version was created to "correct typing errors" relating to the chromatographic conditions. They have confirmed that "all testing (including accelerated and cold storage stability) have been performed according to the validated parameters in AM027115MF2".

The differences between the validated method and the method used in this study appear to be minor and limited to the rinsing flow rate of the HPLC system and the amount of test item analysed. The only significant impact of these changes is in relation to the final extract concentration, which, for this analysis now falls outside of the validated linear range. Linearity data were not included in this report as concentrations were measured against a single point recovery determination (thus assuming linearity between the measured concentrations). While this is not ideal, it is noted that the proposed endpoint is a greater-than value therefore this data can be used as an indicator of spray concentration.

The proposed endpoint is an ER_{50} value of > 75.0 g a.s./ha as no adverse effects on survival, shoot height or dry shoot weight reached or exceeded the 50 % effect level in any species of plant tested. The RMS considers this value acceptable for use in the risk assessment.

B.9.11.2. Extended laboratory studies on non-target plants

No extended laboratory studies on non-target plants were submitted.

B.9.11.3. Semi-field and field tests on non-target plants

No semi-field or field tests on non-target plants were submitted.

B.9.12. RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev2 final, 2002). It is restricted to off-field situations, as non-target plants are defined as non-crop plants located outside the treated area. Spray drift from the treated areas may lead to deposition of the applied product on plants in adjacent off-crop areas, or else onto ground where non-target plants will shortly germinate and emerge.

The vegetative vigour and seedling emergence studies following exposure to 'Isoflucypram EC 50' at the GAP rate of 75 g a.s./ha are summarised in B.9.11 and the endpoints are summarised in table B.9.12-1:

Table B.9.12-1: Effect values relevant for the risk assessment for non-target terrestrial plants for the product Isoflucypram EC 50

Test organism	Study type	Max. effects	Most sensitive species	References
Maximum application rate: 75 g a.s./ha (equivalent to 1.5 L product/ha)				
Terrestrial non-target plants; 10 species	Vegetative vigour; Tier 2 dose response 21 days	No effects ≥ 50 % at a rate of 75 g a.s./ha	Corn (*Zea mays*)	Koehler, P.; 2017; M-589028-01-1 KCP 10.6.2/01
Maximum application rate: 75 g a.s./ha (equivalent to 1.5 L product/ha)				
Terrestrial non-target plants; 10 species	Seedling emergence; Tier 1 single dose 21 days	No effects ≥ 50 % at a rate of 75 g a.s./ha	Onion (*Allium cepa*)	Koehler, P.; 2017; M-596298-01-1 KCP 10.6.2/02
Terrestrial non-target plants; 4 species	Seedling emergence; Tier 2 dose response 21 days	No effects ≥ 50 % at a rate of 75 g a.s./ha	Soy bean (*Glycine max*)	Köhler, P.; 2017; M-607264-01-1 KCP 10.6.2/03

Seedling emergence tier 1 data (single dose of 75.0 g a.s./ha) on 10 species showed that there were no effects ≥ 50 % at a rate of 75 g a.s./ha, showing an acceptable risk. Despite this, the applicant provided a dose-response seedling emergence study on 4 species (TER conducted on this data below). The applicant did not provide reasoning for this further test, but it is noted that the 4 species test were the only ones that showed a significant inhibition on either shoot dry weight or shoot length in the Tier 1 test.

There was no screening data for vegetative vigour. Instead, a dose-response study was conducted on 10 species and this is detailed in Table B.9.12-2 below, alongside a tier II deterministic risk assessment for both pre- and post-emergence exposure to non-target plants.

Table B.9.12-2: Dose-response data and risk assessment for non-target terrestrial plants treated with 75.0 g a.s./ha 'Isoflucypram EC 50'

Species	Test substance	ER_{50} (g a.s./ha) vegetative vigour	ER_{50} (g a.s./ha) emergence	Exposure[1] (g a.s./ha)	TER	Trigger
Beta vulgaris	'Isoflucypram EC 50'	> 75.0	> 75.0	2.0775	>36.1	5
Brassica napus						
Cucumis sativus[2]						
Glycine max						
Helianthus annuus[2]						
Solanum lycopersicum[2]						
Allium cepa						
Avena sativa[2]						
Lolium perenne[2]						
Zea mays[2]						
Extended laboratory studies : None submitted. Semi-field and field test: None submitted.						

[1] exposure has been estimated based on maximum application rate x drift factor of 0.0277 (based on Ganzelmeier *et* al, 1995 drift data)

[2] This species was not included in the seedling emergence dose-response study.

Conclusion

The maximum application rate from the GAP was tested on more than 6 species of terrestrial plants, encompassing both dicotyledonous and monocotyledonous species for both seedling emergence and vegetative vigour effects and no effects ≥ 50% were observed, meaning endpoints defined for risk assessment were pre- and post-emergence ER_{50} > 75 g a.s./ha. These endpoints were considered in a tier II deterministic risk assessment and resultant TER values were greater than 5, indicating acceptable risk to non-target terrestrial plants. No further refinements are necessary.

B.9.13. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No further data submitted.

B.9.14. RISK ASSESSMENT FOR OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No assessment required.

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

A study was submitted which measured the respiration rate of activated sludge exposed to Isoflucypram (active substance).

The study summary is in section B.9.8 of Volume 3 – B.9 (AS). The following table (Table B.9.16-1) lists the respiration rate endpoint for activated sludge exposed to Isoflucypram (as a.s.) for use in the risk assessment.

Table B.9.16-1 : Endpoint for activated sludge exposed to Isoflucypram (as a.s.)

Test item	Test system	Endpoint (µg a.s./L)		Reference
Isoflucypram	Activated sludge respiration rate	EC50 based on respiration rate	1 000 000	Neuhalm (2018)

Conclusion:

The max PEC_{SW} at FOCUS step 1 (as confirmed in the dossier for Environmental Fate and Behaviour B8 for 'Isoflucypram EC 50') is 9.63 µg a.s./L. As the EC_{50} is much greater than the PEC_{SW} value, no adverse effects are expected with regard to activated sewage sludge and the risk does not require further consideration.

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**	**Previous evaluation**
KCP 10.2.1 / 01	[redacted]	2017	BCS-CN88460 EC 50 G acute toxicity to rainbow trout (Oncorhynchus mykiss) under static conditions - Final report Bayer Report No.: E 203 05016-7 Edition Number: M-595274-01-1 Date: 2017-07-07 GLP/GEP: Yes, unpublished	Yes	Yes	New data for a new active substance	Bayer	No
KCP 10.2.1 / 02	Kuhl, K.	2017	Acute toxicity of BCS-CN88460 EC 50 G to the waterflea Daphnia magna in a static	No	Yes	New data for a new active substance	Bayer	No

			laboratory test system Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EBLNN499 Edition Number: M-607779-01-1 Date: 2017-11-10 GLP/GEP: Yes, unpublished					
KCP 10.2.1 / 03	Kuhl, K.	2017	Pseudokirchneriella subcapitata growth inhibition test - BCS-CN88460 EC50 G Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EBLNN500 Edition Number: M-600970-01-1 Date: 2017-09-15 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCP 10.3.1.1.1 / 01	Ehmke, A.	2016	BCS-CN88460 EC 50 (50.0 g/L): Effects (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory - Final report - IBACON GmbH, Rossdorf, Germany Bayer Report No.: 113451035	No	Yes	New data for a new active substance	Bayer	No

			Edition Number: M-571280-01-1 Date: 2016-11-16 GLP/GEP: Yes, unpublished					
KCP 10.3.1.5 / 01	Peters, B.; Rohland, A.	2016	Study on the effect of BCS-CN88460 EC 50 G (50 g/L) on honey bees (Apis mellifera L.) under semi-field conditions tier3 solutions GmbH, Leverkusen, Germany Bayer Report No.: P15019 Edition Number: M-549363-01-1 Date: 2016-03-02 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCP 10.3.1.5 / 02	Hein, R.	2017	Assessment of side effects of BCS-CN88460 EC 50 on the honeybee (Apis mellifera L.) in the semi-field after one application on Phacelia tanacetifolia in Germany 2016 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Niefern-Oeschelbronn, Germany Bayer Report No.: S16-02869	No	Yes	New data for a new active substance	Bayer	No

			Edition Number: M-581949-01-1 Date: 2017-03-01 GLP/GEP: Yes, unpublished					
KCP 10.3.1.5 / 03	Schmitzer, S.	2017	Isoflucypram EC 50 G: Toxicity testing on honey bees (Apis mellifera L.) under semi-field conditions in Germany - Tunnel test IBACON GmbH, Rossdorf, Germany Bayer Report No.: 122701037 Edition Number: M-606834-01-1 Date: 2017-11-14 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCP 10.3.1.5 / 04	Vallon, A.	2017	Assessment of side-effects of isoflucypram EC 50 G on the honeybee (Apis mellifera L.) in a semi-field study after application in flowering Phacelia tanacetifolia in Spain 2017 Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer Report No.: EBLN0008 Edition Number: M-607771-01-1	No	Yes	New data for a new active substance	Bayer	No

			Date: 2017-11-24 GLP/GEP: Yes, unpublished					
KCP 10.3.2.1 / 01	Waibel, J.	2017	Toxicity to the parasitoid wasp Aphidius rhopalosiphi (Hymenoptera: Braconidae) using a laboratory test BCS-CN88460 EC 50 g/L Bayer AG, Crop Science Division, Frankfurt am Main, Germany Bayer Report No.: CW16/036 Edition Number: M-593743-01-1 Date: 2017-06-23 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCP 10.3.2.1 / 02	Waibel, J.	2017	Toxicity to the predatory mite Typhlodromus pyri (Acari: Phytoseiidae) using a laboratory test BCS-CN88460 EC 50 g/L Bayer AG, Crop Science Division, Frankfurt am Main, Germany Bayer Report No.: CW16/035 Edition Number: M-593747-01-1 Date: 2017-06-23 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCP 10.3.2.2 / 01	Waibel, J.	2017	Toxicity to the parasitoid wasp Aphidius	No	Yes	New data for a new active substance	Bayer	No

			rhopalosiphi (Hymenoptera: Braconidae) using an extended laboratory test on barley BCS-CN88460 EC 50 g/L Bayer AG, Crop Science Division, Frankfurt am Main, Germany Bayer Report No.: CW16/038 Edition Number: M-583441-01-1 Date: 2017-03-17 GLP/GEP: Yes, unpublished					
KCP 10.3.2.2 / 02	Waibel, J.	2017	Toxicity to the predatory mite Typhlodromus pyri (Acari: Phytoseiidae) using a laboratory test on bean BCS-CN88460 EC 50 g/L Bayer AG, Crop Science Division, Frankfurt am Main, Germany Bayer Report No.: CW16/037 Edition Number: M-608958-01-1 Date: 2017-12-04 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCP 10.3.2.2 / 03	Waibel, J.	2017	Toxicity to the green lacewing Chrysoperla carnea (Neuroptera: Chrysopidae) using an extended	No	Yes	New data for a new active substance	Bayer	No

			laboratory test on bean - BCS-CN88460 EC 50 g/L Bayer AG, Crop Science Division, Frankfurt am Main, Germany Bayer Report No.: CW16/039 Edition Number: M-601137-01-1 Date: 2017-09-18 GLP/GEP: Yes, unpublished					
KCP 10.3.2.2 / 04	Müller, R. U.	2017	Toxicity to the ladybird beetle Coccinella septempunctata (Coleoptera: Coccinellidae) using an extended laboratory test on bean BCS-CN88460 EC 50 g/L Bayer AG, Crop Science Division, Frankfurt am Main, Germany Bayer Report No.: CW17/010 Edition Number: M-608806-01-1 Date: 2017-11-28 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCP 10.3.2.2 / 05	Jans, D.	2017	Toxicity to the parasitoid wasp Aphidius rhopalosiphi in an extended laboratory test with aged residues on maize soflucypram EC 50 g/L	No	Yes	New data for a new active substance	Bayer	No

			Bayer AG, Crop Science Division, Frankfurt am Main, Germany Bayer Report No.: CW17/014 Edition Number: M-600692-01-1 Date: 2017-09-13 GLP/GEP: Yes, unpublished					
KCP 10.4.1.1 / 01	Frommholz, U.	2016	BCS-CN88460 EC 50 G: Effects on survival, growth and reproduction of the earthworm Eisenia fetida tested in artificial soil Bayer Report No.: E 312 04951-5 Edition Number: M-574897-01-1 Date: 2016-12-13 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCP 10.4.2.1 / 01	Larnaudie Lopez, M. I.	2017	BCS-CN88460 EC 50 G: Influence on mortality and reproduction of the collembolan species Folsomia candida tested in artificial soil Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: E 314 05007-0 Edition Number: M-591834-01-1	No	Yes	New data for a new active substance	Bayer	No

			Date: 2017-06-08 GLP/GEP: Yes, unpublished					
KCP 10.4.2.1 / 02	Larnaudie Lopez, M. I.	2017	BCS-CN88460 EC 50 G: Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: E 428 05008-7 Edition Number: M-592571-01-1 Date: 2017-06-09 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCP 10.5 / 01	Schulz, L.	2017	Amendment no. 1 to the final report - BCS-CN88460 EC 50 G: Effects on the activity of soil microflora (Nitrogen transformation test) - Final report BioChem agrar GmbH, Gerichshain, Germany Bayer Report No.: 16 10 48 062 N Edition Number: M-574633-02-1 Date: 2016-12-05 ... amended: 2017-11-13	No	Yes	New data for a new active substance	Bayer	No

			GLP/GEP: Yes, unpublished					
KCP 10.6.2 / 01	Koehler, P.	2017	BCS-CN88460 EC 50 g/L - Effects on the vegetative vigor of ten species of non-target terrestrial plants (Tier 2) Bayer AG, Crop Science Division, Frankfurt am Main, Germany Bayer Report No.: VV17/001 Edition Number: M-589028-01-1 Date: 2017-05-22 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCP 10.6.2 / 02	Koehler, P.	2017	BCS-CN88460 EC 50 g/L - Effects on the seedling emergence and growth of ten species of non-target terrestrial plants (tier 2) - Final report - Bayer AG, Crop Science Division, Frankfurt am Main, Germany Bayer Report No.: SE17/008 Edition Number: M-596298-01-1 Date: 2017-07-10 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	No
KCP 10.6.2 / 03	Köhler, P.	2017	Effects on the seedling emergence and growth of four species of non-target terrestrial	No	Yes	New data for a new active substance	Bayer	No

			plants (Tier 2) Isoflucypram EC 50 g/L Bayer AG, Crop Science Division, Frankfurt am Main, Germany Bayer Report No.: SE17/056 Edition Number: M-607264-01-1 Date: 2017-11-15 GLP/GEP: Yes, unpublished					