Document Title

Summary of the Ecotoxicological studies Isoflucypram EC 50 (50 g/L) (Code: BCS-CN88460 EC 50)

Data Requirement(s)

Regulation (EC) No 1107/2009 & Regulation (EU) No 284/2013

Document MCP

Section 10: Ecotoxicological studies

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

Date

2018-04-17

Author(s)

[redacted]

[redacted].

Bayer AG

Crop Science Division

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Version history

Date [yyyy-mm-dd]	Data points containing amendments or additions[1] and brief description	Document identifier and version number
2018-02-06	Original Document MCP Section 10	M-613610-01-1
2018-04-17	Dossier update - CP 10.2.1: additional information added	M-613610-02-1

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

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CP 10 ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

INTRODUCTION

The purpose of this MCP-Dossier Section 10 is to support the approval process of the new active substance isoflucypram in the territory of Europe under (EC) No. 1107/2009.

Isoflucypram EC 50 as the representative formulation is an emulsifiable concentrate (EC) containing 50 g/L isoflucypram for use in cereal crops.

Isoflucypram is a novel broad spectrum fungicide of the chemical class of N-cyclopropyl-N-benzyl-pyrazole-carboxamides with an outstanding efficacy against the major economically important fungal diseases of cereal crops (wheat, triticale, rye, barley and oats) and excellent crop safety.
Since isoflucypram is an SDH inhibitor and thus assigned to the FRAC resistance Group 7 the application scope of isoflucypram-containing products on cereals with only one foliar spray at a maximum of 75 g a.s./ha supports an effective anti-resistance management strategy.
Tailor-made and broad spectrum isoflucypram combinations show highly beneficial properties in terms of plant physiology beside the long-lasting and certain curative efficacy to control fungal diseases and to maximize the full yield potential of the cereal crops.

This document summarises all ecotoxicological data, risk assessments and classification proposal, which are relevant for the approval of isoflucypram and the proposed intended uses, including the representative uses, under Regulation (EC) No 1107/2009 in accordance with the requirements laid down in the Commission Regulation (EU) No 283/2013 and under Classification Regulation (EC) No 1272/2008.

Details of the literature search undertaken for isoflucypram, its metabolites and products have been summarized in the Document MCA Section 9.

Throughout the development of the formulation Isoflucypram EC 50 the following synonyms may have been used and referred to in individual study reports: Bayer Code: BCS-CN88460 EC 50 and the Bayer-internal abbreviation short Code: ISY EC 50. All products described by either of these codes refer to the same formulation with identical composition.

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

Use pattern considered in this risk assessment

Table 10- 1: Intended application pattern

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

Definition of the residue for risk assessment

Table10- 2: Definition of the residue for risk assessment

Compartment	Residue definition for risk assessment
Soil	Isoflucypram and BCS-CN88460-carboxylic acid *(M12)*
Groundwater	Isoflucypram and BCS-CN88460-carboxylic acid *(M12)*
Surface water	Isoflucypram and BCS-CN88460-carboxylic acid *(M12)*
Sediment	Isoflucypram and BCS-CN88460-carboxylic acid *(M12)*
Air	Isoflucypram

CP 10.1 Effects on birds and other terrestrial vertebrates

The risk assessment has been performed according to "European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA" (EFSA Journal 2009; 7(12):1438. doi:10.2903/j.efsa.2009.1438), referred to in the following as "EFSA GD 2009".

CP 10.1.1 Effects on birds

Table 10.1.1- 1: Endpoints used in risk assessment

Test substance	Risk assessment	Species	Endpoint	Reference
Isoflucypram	Acute	Bobwhite quail, Wild canary	LD_{50} **> 2000 mg as/kg bw**	[redacted]; [redacted]; 2015; M-535551-01-1 KCA 8.1.1.1/01 [redacted], M.; 2016; M-547051-01-1 KCA 8.1.1.1/02
	Long-term	Mallard duck	NOEC 1000 ppm **NOEL 60 mg a.s./kg bw/d**	[redacted]; [redacted]; 2017; M-597500-01-1 KCA 8.1.1.3/01

Endpoints in **bold** considered relevant for risk assessment

Metabolites of isoflucypram

The metabolite BCS-CN88460-propanol-Glyc-MA (M21) is a plant metabolite, which was found in wheat hay at an amount of 10.3% TRR in a metabolism study with wheat plants (see MCA Summary Section 6, Point 6.2). Therefore it may be ingested by herbivorous birds or mammals. This metabolite is assumed to be cleaved under acidic conditions in the stomach of birds and mammals to the metabolite BCS-CN88460-propanol (M01). The metabolite M01 was found in the metabolic pathway in rat and hen (see MCA Summary Section 5, Point 5.1.1 and MCA Summary Section 6, Point 6.2). Therefore the metabolite M21 is covered by the risk assessment with the active substance isoflucypram.

Table 10.1.1- 2: Relevant generic avian focal species for Tier 1 risk assessment

Crop	Scenario	Generic focal species	Representative species	Shortcut value (SV)	
				Long-term RA based on RUD_m	Acute RA based on RUD_{90}
Cereals	BBCH 30-39	Small omnivorous bird “lark”	Woodlark (*Lullula arborea*)	5.4	12.0
	BBCH ≥ 40	Small omnivorous bird “lark”	Woodlark (*Lullula arborea*)	3.3	7.2

ACUTE DIETARY RISK ASSESSMENT

Table 10.1.1- 3: Tier 1 acute risk assessment for birds

Crop scenario	Generic focal species	DDD			DDD	LD_{50} [mg a.s./kg bw]	TER_A	Trigger
		Appl. rate [kg a.s./ha]	SV_{90}	MAF_{90}				
BBCH 30-39	Small omnivorous bird “lark”	0.075	12.0	1.0	0.90	> 2000	> 2222	10
BBCH ≥ 40	Small omnivorous bird “lark”		7.2		0.54		> 3704	

The TER_A values calculated in the acute risk assessment on Tier 1 level exceed the a-priori-acceptability trigger of 10 for all evaluated scenarios. Thus, the acute risk to birds can be considered as low and acceptable without need for further, more realistic risk assessment.

Acute risk assessment for birds drinking contaminated water from pools in leaf whorls

For the fungicidal use in crops under assessment in this evaluation (cereals) the leaf scenario is not considered relevant according to the EFSA Guidance Document for risk assessment for bird and mammals (2009).

Acute risk assessment for birds drinking contaminated water from puddles

Table 10.1.1- 4: Evaluation of potential concern for exposure of birds from drinking water (escape clause)

Crop	Koc [L/kg]	AR_{eff} (Application rate × MAF) [g a.s./ha]	LD_{50} [mg as/kg	Ratio AR_{eff} / LD_{50}	“Escape clause” No concern if ratio	Conclusion
Cereals	1579.6	75	> 2000	< 0.04	≤ 3000	No concern

According to the EFSA Guidance document for risk assessment for bird and mammals (2009) “*no specific calculations of exposure and TER are necessary when the ratio of effective application rate*

(in g/ha) to relevant endpoint (in mg/kg bw) does not exceed 3000 in the case of more sorptive substances (Koc > 500 L/kg).” This is the case for isoflucypram and therefore the acute risk for birds from drinking water that may contain residues from isoflucypram is acceptable.

LONG-TERM REPRODUCTIVE ASSESSMENT

Table 10.1.1- 5: Tier 1 reproductive risk assessment for birds

Crop	Generic focal species	DDD				DDD	NOAEL [mg a.s./ kg bw/d]	TER_{LT}	Trigger
		Appl. rate [kg a.s./ha]	SV_m	MAF_m	f_{TWA}				
BBCH 30-39	Small omnivorous bird “lark”	0.075	5.4	1.0	0.53	0.21	60	286	5
BBCH ≥ 40	Small omnivorous bird “lark”	0.075	3.3	1.0	0.53	0.13	60	462	5

The TER_{LT} values calculated in the chronic risk assessment on Tier 1 level exceed the a-priori-acceptability trigger of 5 for all evaluated scenarios. Thus, the long-term risk to birds can be considered as low and acceptable without need for further, more realistic risk assessment.

Long-term risk assessment for birds drinking contaminated water from puddles

Table 10.1.1- 6: Evaluation of potential concern for exposure of birds from drinking water (escape clause)

Crop	K_{oc} [L/kg]	AR_{eff} (Application rate × MAF) [g a.s./ha]	NO(A)EL [mg a.s./ kg bw/d]	Ratio (Application rate × MAF) / NO(A)EL)	“Escape clause” No concern if ratio	Conclusion
Cereals	1579.6	75	60	1.25	≤ 3000	No concern

According to the EFSA Guidance Document for risk assessment for bird and mammals (2009) “no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 3000 in the case of more sorptive substances (Koc > 500 L/kg).” This is the case for isoflucypram and therefore, the long-term risk for birds from drinking water that may contain residues from isoflucypram is acceptable.

RISK ASSESSMENT OF SECONDARY POISONING

Table 10.1.1- 7: Log Pow values

Substance	log Pow	Reference
Isoflucypram	4.0 (pH 4-9)	KCA 2.7/01 [redacted]; [redacted]; 2014; M-484656-01-1
BCS-CN88460-carboxylic acid (M12)	2.11 (pH 5) 0.22 (pH 7) -1.1 (pH 9)	KCA 2.7/02 [redacted]; [redacted]; 2015; M-519996-01-1

Effects on secondary poisoning have been assessed for isoflucypram, due to a log P_{OW} value above 3. The metabolite BCS-CN88460-carboxylic acid (M12) is assessed in the aquatic risk assessment and in the risk assessment for soil organisms. The Log P_{OW} values of this metabolite are below 3 at ecologically relevant pH values. Therefore there is no risk to birds from BCS-CN88460-carboxylic acid (M12) through secondary poisoning.

Table 10.1.1- 8: Avian generic focal species for the Tier 1 risk assessment of secondary poisoning

Generic avian indicator species	Body weight [g]	Example	FIR/bw
Earthworm eater	100	Thrush	1.05
Fish eater	1000	Heron	0.159

Long-term DDD and TER calculation for earthworm-eating birds

Table 10.1.1- 9: Tier 1 long-term DDD and TER calculation for earthworm-eating birds

	Isoflucypram
K_{OW}	10000
K_{OC} [mL/g]	1580[A]
f_{OC}	0.02
BCF_{worm}	3.82
PEC_{soil} (twa, 21 d) [mg/kg]	0.03[B]
PEC_{worm} [mg/kg]	0.11
FIR/bw	1.05
DDD [mg/kg bw/d]	0.12
NO(A)EL [mg/kg bw/d]	60
TER_{LT}	500
Trigger	5

[A] K_{OC} value given in MCP 9.2.4.1 (Table 9.2.4-1)
[B] $PEC_{soil, accu}$ value used for risk assessment (MCP 9.1.3; Table 9.1.3-3)

Long-term DDD and TER calculation for fish-eating birds

Table 10.1.1- 10: Tier 1 long-term DDD and TER calculation for fish-eating birds

Substance	Isoflucypram
BCF_{fish}	370 (whole fish)
FOCUS Step 2 PEC_{sw}* (twa, 21 d)[mg/L]	0.00272
PEC_{fish} [mg/kg]	1.0064
FIR/bw	0.159
DDD [mg/kg bw/d]	0.160
NO(A)EL [mg/kg bw/d]	60
TER_{LT}	375
Trigger	5

* 21 twa PEC_{sw} value given in MCP 9.2.5 (Table 9.2.4.3-6)

The TER values for isoflucypram are above the trigger of concern of 5, indicating no risk from secondary poisoning for earthworm- and fish-eating birds.

CP 10.1.1.1 Acute oral toxicity

For animal welfare reasons, no acute oral toxicity study with the preparation was performed. Such a study is not deemed necessary, given the fact that the active substance is not acutely toxic to birds.

CP 10.1.1.2 Higher tier data on birds

In view of the results presented above, no further studies were necessary.

CP 10.1.2 Effects on terrestrial vertebrates other than birds

Table 10.1.2- 1: Endpoints used in risk assessment

Test substance	Risk assessment	Species	Endpoint		Reference
Isoflucypram	Acute	Rat	LD_{50}	**> 2000 mg /kg bw**	[redacted], V.; 2014; M-485872-01-1 KCA 5.2.1/01
	Long-term	Rat	$NOAEL_{reproduction}$	**92.9 mg/kg bw/d**	[redacted], R.; 2016; M-612750-01-1 KCA 5.6.1/01

Endpoints in **bold** considered relevant for risk assessment

Metabolites of isoflucypram

The metabolite BCS-CN88460-propanol-Glyc-MA (M21) is a plant metabolite, which was found in wheat hay at an amount of 10.3% TRR in a metabolism study with wheat plants (see MCA Summary Section 6, Point 6.2). Therefore it may be ingested by herbivorous birds or mammals. This metabolite is assumed to be cleaved under acidic conditions in the stomach of birds and mammals to the metabolite BCS-CN88460-propanol (M01). The metabolite M01 was found in the metabolic pathway in rat and hen (see MCA Summary Section 5, Point 5.1.1 and MCA Summary Section 6, Point 6.2). Therefore the metabolite M21 is covered by the risk assessment with the active substance isoflucypram.

Table 10.1.2- 2: Relevant generic focal species for Tier 1 risk assessment

Crop	Scenario	Generic focal species	Representative species	Shortcut value (SV)	
				Long-term RA based on RUD_{mean}	Acute RA based on RUD_{90}
Cereals	BBCH ≥ 20	Small insectivorous mammal "shrew"	Common shrew (*Sorex araneus*)	1.9	5.4
	BBCH ≥ 40	Small herbivorous mammal "vole"	Common vole (*Microtus arvalis*)	21.7	40.9
	BBCH 30-39	Small omnivorous mammal "mouse"	Wood mouse (*Apodemus sylvaticus*)	3.9	8.6
	BBCH ≥ 40	Small omnivorous mammal "mouse"	Wood mouse (*Apodemus sylvaticus*)	2.3	5.2

ACUTE DIETARY RISK ASSESSMENT

Table 10.1.2- 3: Tier 1 acute risk assessment for wild mammals

Crop	Generic focal species	DDD			DDD	LD_{50} [mg a.s./kg bw]	TER_A	Trigger
		Appl. rate [kg a.s./ha]	SV_{90}	MAF_{90}				
BBCH ≥ 20	Small insectivorous mammal "shrew"	0.075	5.4	1.0	0.405	> 2000	> 4938	10
BBCH ≥ 40	Small herbivorous mammal "vole"		40.9		3.068		> 652	
BBCH 30-39	Small omnivorous mammal "mouse"		8.6		0.645		> 3101	
BBCH ≥ 40	Small omnivorous mammal "mouse"		5.2		0.390		> 5128	

The TER_A values calculated in the acute risk assessment on Tier 1 level for wild mammals exceed the a-priori-acceptability trigger of 10 for all evaluated scenarios. Thus, the acute risk to wild mammals can be considered as low and acceptable without need for further, more realistic risk assessment.

Acute risk assessment for mammals drinking contaminated water

The puddle scenario is relevant for the acute risk assessment.

Table 10.1.2- 4: Evaluation of potential concern for exposure of mammals drinking water

Crop	K_{oc} [L/kg]	AR_{eff} (Application rate × MAF)	LD_{50} [mg a.s./ kg bw/d]	Ratio (AR_{eff}) / LD_{50}	"Escape clause" No concern if ratio	Conclusion
Cereals	1579.6	75	> 2000	< 0.04	≤ 3000	No concern

According to the EFSA Guidance document for risk assessment for bird and mammals (2009) "*no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 3000 in the case of more sorptive substances (Koc > 500 L/kg).*" This is the case for isoflucypram and therefore, the long-term risk for birds from drinking water that may contain residues from isoflucypram is acceptable.

LONG-TERM REPRODUCTIVE ASSESSMENT

Table 10.1.2- 5: Tier 1 reproductive risk assessment for wild mammals

Crop	Generic focal species	DDD				DDD	NOAEL [mg a.s./ kg bw/d]	TER_{LT}	Trigger
		Appl. rate [kg a.s./ha]	SV_m	MAF_m	f_{TWA}				
BBCH ≥ 20	Small insectivorous mammal "shrew"	0.075	1.9	1.0	0.53	0.076	92.9	1222	5
BBCH ≥ 40	Small herbivorous mammal "vole"		21.7			0.863		108	
BBCH 30-39	Small omnivorous mammal "mouse"		3.9			0.155		599	
BBCH ≥ 40	Small omnivorous mammal "mouse"		2.3			0.091		1021	

The TER_{LT} values calculated in the reproductive risk assessment on Tier 1 level for wild mammals exceed the a-priori-acceptability trigger of 5 for all evaluated scenarios. Thus, the long-term risk to wild mammals can be considered as low and acceptable without need for further, more realistic risk assessment.

Long-term risk assessment for mammals drinking contaminated water

Table 10.1.2- 6: Evaluation of potential concern for exposure of mammals drinking water (escape clause)

Crop	K_{oc} [L/kg]	AR_{eff} (Application rate × MAF) [g a.s./ha]	NO(A)EL [mg a.s./ kg bw/d]	Ratio (Application rate * MAF) / NO(A)EL)	"Escape clause" No concern if ratio	Conclusion
Cereals	1579.6	75	92.9	0.81	**≤ 3000**	No concern

According to the EFSA Guidance Document for risk assessment for bird and mammals (2009) *"no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 3000 in the case of more sorptive substances (Koc > 500 L/kg).*" This is the case for isoflucypram and therefore, the long-term risk for mammals from drinking water that may contain residues from isoflucypram is acceptable.

RISK ASSESSMENT OF SECONDARY POISONING

Effects on secondary poisoning have been assessed for isoflucypram, due to a log P_{OW} value above 3. The metabolite BCS-CN88460-carboxylic acid (M12) is assessed in the aquatic risk assessment and in the risk assessment for soil organisms. The Log P_{OW} values of this metabolite are below 3 at ecologically relevant pH values. Therefore there is no risk to birds from BCS-CN88460-carboxylic acid (M12) through secondary poisoning.

Table 10.1.2- 7: Mammalian generic focal species for the Tier 1 risk assessment of secondary poisoning

Generic avian indicator species	Body weight [g]	Example	FIR/bw
Earthworm eater	100	Common shrew	1.28
Fish eater	1000	Otter	0.142

Long-term DDD and TER calculation for earthworm-eating mammals

Table 10.1.2- 8: Tier 1 long-term ETE and TER calculation for earthworm eating mammals

	Isoflucypram
PEC_{worm} [mg/kg]	0.11*
FIR/bw	1.28
DDD [mg/kg bw/d]	0.14
NO(A)EL [mg/kg bw/d]	92.9
TER_{LT}	664
Trigger	5

*See calculation presented in Table 10.1.1- 9.

Long-term toxicity exposure ratio for fish-eating mammals

Table 10.1.2- 9: Tier 1 long-term ETE and TER calculation for fish eating mammals

Substance	Isoflucypram
PEC_{fish} [mg/kg]	1.0064*
FIR/bw	0.142
DDD [mg/kg bw/d]	0.14
NO(A)EL [mg/kg bw/d]	92.9
TER_{LT}	663
Trigger	5

*See calculation presented in Table 10.1.1- 10.

The TER values for isoflucypram are above the trigger of concern of 5, indicating no risk from secondary poisoning for earthworm- and fish-eating mammals.

CP 10.1.2.1 Acute oral toxicity to mammals

According to the Regulation (EC) No 1272/2008 Annex 3.1.3.6.1, the classification of a mixture such as a formulated plant protection product may be estimated with a calculation method.
The representative formulation Isoflucypram EC 50 contains no ingredients relevant for calculation of an oral ATEmix. Therefore, ISY EC 50 should not be classified for oral toxicity. For details, please refer to the CONFIDENTIAL Document JCP, Point 7.4 and Point 12.3.

Conclusion

As the formulation is not classified for acute oral toxicity the formulation is not considered to be more toxic than the active substance.

CP 10.1.2.2 Higher tier data on mammals

In view of the results presented above, no further studies were necessary.

CP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

Information on effects of isoflucypram on reptiles or amphibians is not available. No guidelines for studies with terrestrial amphibian life stages and reptiles are available and no risk assessments schemes are established so far. Therefore no further studies can be suggested for these groups of organisms.

CP 10.2 Effects on 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.

Table 10.2- 1: Endpoints used in risk assessment and studies for isoflucypram

Test substance	Test species	Endpoint		Reference
Isoflucypram EC 50	Fish, acute *Oncorhynchus mykiss*	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*	96 h LC_{50}	**0.153 mg a.s./L**[B]	Geometric mean acc. to new aquatic Guidance Document (EFSA Journal 2013;11(7):3290)
	Invertebrate, acute *Daphnia magna*	48 h EC_{50}	2.22 mg/L (nom) (**~0.117 mg a.s./L**)[A]	[redacted]; 2017; M-607779-01-1 KCP 10.2.1/02
	Invertebrate, acute *Daphnia magna Americamysis bahia*	EC_{50}	**0.203 mg a.s./L**[B]	Geometric mean acc. to new aquatic Guidance Document (EFSA Journal 2013;11 (7))
	Algae *Pseudokirchneriella subcapitata*	72h E_rC_{50}	3.39 mg/L (nom) (**~0.179 mg a.s./L**)[A]	[redacted]; 2017; M-600930-01-1 KCP 10.2.1/03
Isoflucypram	Fish, acute *Pimephales promelas*	96 h LC_{50}	**0.081 mg a.s./L** (nom)[C]	[redacted]; 2018; M-542897-02-1 KCA 8.2.1/01
	Fish, acute *Pimephales promelas, Oncorhynchus mykiss, Cyprinodon variegatus*	96 h LC_{50}	**0.1628 mg a.s./L**[D]	Geometric mean acc. to new aquatic Guidance Document (EFSA Journal 2013;11(7):3290)
	Fish, chronic (ELS) *Pimephales promelas*	33 d NOEC	**0.0156 mg a.s./L** (nom)	[redacted]; [redacted]; 2017; M-580247-01-1 KCA 8.2.2.1/01
	Fish, BCF flow-through *Lepomis macrochirus*	BCF	**370** (kinetic BCF lipid normalized and growth corrected)	[redacted]; [redacted], R.; 2017; M-610008-01-1 KCA 8.2.2.3/01
	Invertebrate, acute *Daphnia magna*	48 h EC_{50}	**0.201 mg a.s./L** (gmm)	[redacted]; 2016; M-574184-01-1 KCA 8.2.4.1/01
	Invertebrate, acute *Daphnia magna, Americamysis bahia*	EC_{50}	**0.233 mg a.s./L**[D]	Geometric mean acc. to new aquatic Guidance Document (EFSA Journal 2013;11(7):3290)
	Invertebrate, chronic *Daphnia magna*	21 d EC_{10}	**0.0661 mg a.s./L** (mm)	[redacted]; 2017; M-593961-01-1 KCA 8.2.5.1/01
	Sediment dweller *Chironomus dilutus*	61 d NOEC 61 d LOEC	**85 mg a.s./kg** (mm) > 85 mg a.s./kg (mm)	[redacted]; 2017; M-596883-01-1 KCA 8.2.5.4/01
	Freshwater diatom, *Navicula pelliculosa*	72h-E_rC_{50} 72 h-E_yC_{50}	**> 2.0 mg a.s./L** (gmm) **> 2.0 mg a.s./L** (gmm)	[redacted], J. R.; [redacted], A. I.; [redacted], J. R.; [redacted], K. H.; 2017; M-604809-01-1 KCA 8.2.6.2/03
	Aquatic macrophyte, *Lemna gibba*	7d-E_rC_{50}	**> 3.02 mg a.s./L** (gmm)	[redacted]; 2017; M-593965-01-1 KCA 8.2.7/01

Test substance	Test species	Endpoint		Reference
BCS-CN88460-carboxylic acid (M12)	Fish, acute *Oncorhynchus mykiss*	96 h LC_{50}	**> 33.5 mg p.m./L** (gmm)	[redacted]; 2017; M-587655-01-1 KCA 8.2.1/04
	Invertebrate, acute *Daphnia magna*	48 h EC_{50}	**> 24 mg p.m./L** (nom)	[redacted]; 2016; M-573296-01-1 KCA 8.2.4.1/02
	Green algae, *Pseudokirchneriella subcapitata*	72h-E_rC_{50}	**> 35.1 mg p.m./L** (gmm)	[redacted]; 2017; M-587659-01-1 KCA 8.2.6.1/02

Bold: endpoints used in risk assessment
Nom = nominal concentrations, mm = mean measured concentration, gmm = geometric mean measured concentration
[A] 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 product risk assessments'
[C] Endpoint corrected for purity. In study report uncorrected values are cited.
[D] 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 under MCA 8.2: 'Selection of endpoints for Tier 2 risk assessments with the active substance'

Selection of endpoints for Tier 2 product risk assessments

The effect data for the product Isoflucypram EC 50 observed for aquatic organisms are very similar to the effect data derived for the active substance.
In the table 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 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 formulation is not increasing the toxicity of the active substance.

Table 10.2- 2: 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 for the active substance more species have been investigated compared to the product we propose to consider this information as well for the product based risk assessment. Otherwise the environmental risk assessment would not make full use out of the existing information. Therefore we propose to use the Tier 2A as well for the product risk assessment. To consider the fact that rainbow trout and *Daphnia magna* data exist twice, the notifier proposes to use the geomean for data resulting from active substance testing and from product testing, before using the data in a Tier 2A approach.

Fish acute

If more species are tested as required according to the data requirements laid down in Regulation (EU) No 283/2013 it might be appropriate to use Tier 2A. In case of isoflucypram the data requirements were exceeded for acute testing of fish. Data for the different fish species: The trout (*Oncorhynchus mykiss*), the fathead minnow (*Pimephales promelas*) and the sheepshead minnow (*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 out of the respective two endpoints is used.

The following LC_{50} values (based on the real content of the active ingredient) for the three tested species are then available:

Table 10.2- 3: Endpoints for fish acute

Species	Species (Scientific name)	96h LC_{50} (mg a.s. /L)
Rainbow trout	*Oncorhynchus mykiss*	0.082 (Geometric mean of a.s. and product study)
Fathead minnow	*Pimephales promelas*	0.081
Sheepshead minnow	*Cyprinodon variegtaus*	0.544
Geometric mean:	-	**0.153**

For the taxonomic group of fish the data requirements (one species) were exceeded. Data for three species are available. Therefore the geomean 96 h LC_{50} for these three species was calculated.
Before this value can be used it has to be checked whether the geometric mean approach has been biased by introducing insensitive species. According to the Guidance on tiered risk assessment for edge of field surface waters (EFSA, 2013) an assessment of this has to be made when the difference in sensitivity exceeds 1 or 2 orders of magnitude. In case of fish a factor of 100 should not be exceeded.
The highest and the lowest 96 h LC_{50} derived for fish and isoflucypram differ by a factor of 6.7. Therefore the use of the geomean approach for the fish acute risk assessment is appropriate.
In addition the chronic fish data can be used to check the appropriateness of the Tier 2A related regulatory acceptable concentration (RAC) for acute fish of 0.00153 mg a.s./L. This RAC is a factor of 10.2 below the lowest observed chronic NOEC for fish, resulting from a Fish Early Life Stage (FELS) test with fathead minnow. The acute RAC based on Tier 2A for fish is even a factor of 12.9 below the Tier 2A chronic fish value based on the two existing FELS studies for fathead minnow and sheepshead minnow.
The above presented information demonstrates that the Tier 2A fish acute 96 h LC_{50} of 0.153 mg a.s./L (product) and the resulting Tier 2A RAC of 0.00153 mg/L is protective and can therefore be used within the aquatic risk assessment of the product Isoflucypram EC50.

Crustacean acute

If more species are tested as required according to the data requirements laid down in Regulation (EU) No 283/2013 than it might be appropriate to use Tier 2a. In case of isoflucypram the data requirements were exceeded for acute testing of invertebrates, especially crustaceans. Data for two different crustacean species, the waterflea (*Daphnia magna*) and the mysid shrimp (*Americamysis bahia*) are provided. The available data are not sufficient to use 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 out of the two endpoints is used.
The following EC_{50} values (based on the real content of the active ingredient) for the two tested species are then available:

Table 10.2- 4: Endpoints for crustacean acute

Species	Species (Scientific name)	EC_{50} [mg a.s. /L]
Water flea	*Daphnia magna*	0.153 (48h, geometric mean of a.s. and product study)
Mysid shrimp	*Americamysis bahia*	0.270 (96h)
Geometric mean:	-	**0.203**

The resulting Tier 2A EC_{50}, based on information for the waterflea *Daphnia magna* and the mysid shrimp *Americamysis bahia* is 0.203 mg/L.
The notifier proposes to use this value for the product risk assessment according to Tier 2A (geomean approach).

Selection of algae and macrophytes endpoints for risk assessment

Processes in ecosystems are dominantly rate driven and therefore, the unit development per time (growth rate) is more suitable to measure effects in algae and macrophytes. Also, growth rates and their inhibition can easily be compared between species, test durations and test conditions, which is not the case for yield or biomass based endpoints. Following current state of science, the test guidelines OECD TG 201 and 221, the EU-Method C3, the Regulation for Classification and Labelling (Regulation (EC) No 1272/2008), the PPR Opinion (EFSA Journal 461, 1-44; 2007), the EFSA supporting publication 2015 (EN-924 published 22 December 2015) and also the EFSA Aquatic Guidance Document (AGD, 2013, noted by SCFCAH on July 10-11th, 2014), list growth rate as the relevant endpoint of the algae and the *Lemna* growth inhibition test. Therefore the risk assessment is based on the E_rC_{50}, when available.

Metabolites

In the risk assessment the isoflucypram metabolite BCS-CN88460-carboxylic acid (M12) has to be addressed:
The EFSA AGD (2013) stepwise approach was used for all metabolites to be addressed in the risk assessment:

- 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 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.

Predicted environmental concentrations used in the risk assessment

Predicted environmental concentrations of isoflucypram and its metabolites in surface water were calculated according to FOCUS Steps 1-3 for the use in cereals.

Table 10.2- 5: Initial max PEC_{sw} values – FOCUS Steps 1 and 2

Compound	FOCUS Scenario	Cereals* 1 × 75 g a.s./ha, BBCH 30-69
		$PEC_{sw,max}$ [µg/L]
Isoflucypram	STEP 1	8.7383
	STEP 2 – North	1.5622
	STEP 2 - South	**2.8386**
BCS-CN88460-carboxylic acid (M12)	STEP 1	4.1971
	STEP 2 – North	0.6781
	STEP 2 – South	**1.3090**

*Worst case PEC_{sw} values considering all scenarios relevant for use in winter and spring cereals
Bold values were considered in risk assessment

Table 10.2- 6: Initial max PEC_{sw} values – FOCUS Steps 3

Compound	FOCUS Scenario	1 × 75 g a.s./ha, BBCH 30-69	
		Winter cereals	Spring cereals
		$PEC_{sw,max}$ [µg/L]	$PEC_{sw,max}$ [µg/L]
Isoflucypram	D1 ditch	1.2430	0.9975
	D1 stream	0.7788	0.6253
	D2 ditch	1.1690	-
	D2 stream	0.7315	-
	D3 ditch	0.4754	0.4749
	D4 pond	0.0785	0.0874
	D4 stream	0.4102	0.4089
	D5 pond	0.0821	0.0766
	D5 stream	0.4425	0.4140
	D6 ditch	0.6339	-
	R1 pond	0.0493	-
	R1 stream	0.3133	-
	R3 stream	0.4414	-
	R4 stream	0.3930	0.3893

Risk assessment for aquatic organisms

According to the new Aquatic Guidance Document (EFSA PPR Panel Guidance, 2013), the risk to aquatic organisms is evaluated based on the derivation of Regulatory Acceptable Concentrations (RACs) as follows:

Acute risk assessment:

$RAC_{sw, ac} = LC_{50}$ or EC_{50} / 100

The risk is considered acceptable, if the $RAC_{sw, ac} \geq PEC_{sw, max}$.

Chronic risk assessment:

$RAC_{sw, ch} = NOEC$ or EC_{10} / 10

$RAC_{sw, ch} = E_rC_{50}$ / 10

The risk is considered acceptable, if the $RAC_{sw, ch} \geq PEC_{sw, max}$.

To summarise, these abbreviations are used in subscript following the term PEC or RAC: ac: acute, ch: chronic, sw: surface water, max: maximum.

ACUTE RISK ASSESSMENT FOR AQUATIC ORGANISMS

Table 10.2- 7: Acute risk assessment based on FOCUS Step 2

Compound	Species	Endpoint [µg/L]	RAC# [µg/L]	$PEC_{sw,max}$ [µg/L]	RAC# ≥ PEC_{sw}
Isoflucypram EC 50	Fish, acute *Oncorhynchus mykiss*	LC_{50} >68	0.68	2.8386	**No**
	Invertebrate, acute *Daphnia magna*	EC_{50} 117	1.17		**No**
Isoflucypram	Fish, acute *Pimephales promelas*	LC_{50} 81	0.810		**No**
	Invertebrate, acute *Daphnia magna*	EC_{50} 201	2.01		**No**
BCS-CN88460-carboxylic acid (M12)	Fish, acute *Pimephales promelas*	LC_{50} > 33500	> 335	1.3090	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} > 24000	> 240		Yes

For isoflucypram the acute trigger was not met for fish and invertebrates. For the isoflucypram metabolite BCS-CN88460-carboxylic acid (M12) acceptable acute risk could be proven for fish and invertebrates. A risk assessment for isoflucypram under consideration of more realistic FOCUS Step 3 water concentrations is presented below.

Winter cereals

Table 10.2- 8: Acute risk assessment based on FOCUS Step 3 for winter cereals

Compound	Species	Endpoint [µg/L]	RAC# [µg/L]	FOCUS Scenario	$PEC_{sw,max}$ [µg/L]	RAC#/PEC_{sw}
Isoflucypram EC 50	Fish, acute *Oncorhynchus mykiss*	LC_{50} 68	0.680	D1 ditch	1.2430	**No**
				D1 stream	0.7788	**No**
				D2 ditch	1.1690	**No**
				D2 stream	0.7315	**No**
				D3 ditch	0.4754	Yes
				D4 pond	0.0785	Yes
				D4 stream	0.4102	Yes
				D5 pond	0.0821	Yes
				D5 stream	0.4425	Yes
				D6 ditch	0.6339	Yes
				R1 pond	0.0493	Yes
				R1 stream	0.3133	Yes
				R3 stream	0.4414	Yes
				R4 stream	0.3930	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} 117	1.17	D1 ditch	1.2430	**No**
				D1 stream	0.7788	Yes
				D2 ditch	1.1690	Yes
				D2 stream	0.7315	Yes
				D3 ditch	0.4754	Yes
				D4 pond	0.0785	Yes
				D4 stream	0.4102	Yes
				D5 pond	0.0821	Yes
				D5 stream	0.4425	Yes
				D6 ditch	0.6339	Yes
				R1 pond	0.0493	Yes
				R1 stream	0.3133	Yes
				R3 stream	0.4414	Yes
				R4 stream	0.3930	Yes

Table 10.2- 8 (continued): Acute risk assessment based on FOCUS Step 3 for winter cereals

Isoflucypram	Fish, acute *Pimephales promelas*	LC_{50}	81	0.810	D1 ditch	1.2430	**No**
					D1 stream	0.7788	Yes
					D2 ditch	1.1690	**No**
					D2 stream	0.7315	Yes
					D3 ditch	0.4754	Yes
					D4 pond	0.0785	Yes
					D4 stream	0.4102	Yes
					D5 pond	0.0821	Yes
					D5 stream	0.4425	Yes
					D6 ditch	0.6339	Yes
					R1 pond	0.0493	Yes
					R1 stream	0.3133	Yes
					R3 stream	0.4414	Yes
					R4 stream	0.3930	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50}	201	2.01	D1 ditch	1.2430	Yes
					D1 stream	0.7788	Yes
					D2 ditch	1.1690	Yes
					D2 stream	0.7315	Yes
					D3 ditch	0.4754	Yes
					D4 pond	0.0785	Yes
					D4 stream	0.4102	Yes
					D5 pond	0.0821	Yes
					D5 stream	0.4425	Yes
					D6 ditch	0.6339	Yes
					R1 pond	0.0493	Yes
					R1 stream	0.3133	Yes
					R3 stream	0.4414	Yes
					R4 stream	0.3930	Yes

Spring cereals

Table 10.2- 9: Acute risk assessment based on FOCUS Step 3 for spring cereals

Compound	Species	Endpoint [µg/L]	RAC# [µg/L]	FOCUS Scenario	$PEC_{sw,max}$ [µg/L]	RAC#/PEC_{sw}
Isoflucypram EC 50	Fish, acute *Oncorhynchus mykiss*	LC_{50} 68	0.680	D1 ditch	0.9975	**No**
				D1 stream	0.6255	Yes
				D3 ditch	0.4749	Yes
				D4 pond	0.0874	Yes
				D4 stream	0.4089	Yes
				D5 pond	0.0766	Yes
				D5 stream	0.4140	Yes
				R4 stream	0.3893	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} 117	1.17	D1 ditch	0.9975	Yes
				D1 stream	0.6255	Yes
				D3 ditch	0.4749	Yes
				D4 pond	0.0874	Yes
				D4 stream	0.4089	Yes
				D5 pond	0.0766	Yes
				D5 stream	0.4140	Yes
				R4 stream	0.3893	Yes
Isoflucypram	Fish, acute *Pimephales promelas*	LC_{50} 81	0.810	D1 ditch	0.9975	**No**
				D1 stream	0.6255	Yes
				D3 ditch	0.4749	Yes
				D4 pond	0.0874	**No**
				D4 stream	0.4089	Yes
				D5 pond	0.0766	Yes
				D5 stream	0.4140	Yes
				R4 stream	0.3893	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} 201	2.01	D1 ditch	0.9975	Yes
				D1 stream	0.6255	Yes
				D3 ditch	0.4749	Yes
				D4 pond	0.0874	Yes
				D4 stream	0.4089	Yes
				D5 pond	0.0766	Yes
				D5 stream	0.4140	Yes
				R4 stream	0.3893	Yes

For use of isoflucypram in winter cereals acceptable acute risk to fish could be proven for most FOCUS Step 3 scenarios. For invertebrates the risk assessment at FOCUS Step 3 leads to acceptable risk for almost all scenarios. For use of isoflucypram in spring cereals the risk assessment on the basis of FOCUS Step 3 PEC_{sw} values is passed almost for all scenarios whereas for invertebrates acceptable risk could be proven for all FOCUS Step 3 scenarios.
A refined risk assessment for the use in winter and spring cereals for the acute risk to fish is presented below. Those scenarios are presented which do not pass the risk assessment at Tier 1.

Refined risk assessment (Tier 2a)

Winter cereals

Table 10.2- 10: Acute risk assessment based on FOCUS Step 3 for winter cereals

Compound	Species	Endpoint [µg/L]	RAC# [µg/L]	FOCUS Scenario	$PEC_{sw,max}$ [µg/L]	RAC# ≥ PEC_{sw}
Isoflucypram EC 50	Fish, acute *Pimephales promelas*, *Oncorhynchus mykiss*, *Cyprinodon variegatus*	LC_{50} 153	1.53	D1 ditch	1.2430	Yes
				D1 stream	0.7788	Yes
				D2 ditch	1.1690	Yes
				D2 stream	0.7315	Yes
	Invertebrate, acute *Daphnia magna* *Americamysis bahia*	EC_{50} 203	2.03	D1 ditch	1.2430	Yes
Isoflucypram	Fish, acute *Pimephales promelas*, *Oncorhynchus mykiss*, *Cyprinodon variegatus*	LC_{50} 162.8	1.628	D1 ditch	1.2430	Yes
				D2 ditch	1.1690	Yes

Spring cereals

Table 10.2- 11: Acute risk assessment based on FOCUS Step 3 for spring cereals

Compound	Species	Endpoint [µg/L]	RAC# [µg/L]	FOCUS Scenario	$PEC_{sw,max}$ [µg/L]	RAC# ≥ PEC_{sw}
Isoflucypram EC 50	Fish, acute *Pimephales promelas*, *Oncorhynchus mykiss*, *Cyprinodon variegatus*	LC_{50} 153	1.53	D1 ditch	0.9975	Yes
Isoflucypram	Fish, acute *Pimephales promelas*, *Oncorhynchus mykiss*, *Cyprinodon variegatus*	LC_{50} 162.8	1.628	D1 ditch	0.9975	Yes
				D4 pond	0.0874	Yes

For the use of Isoflucypram EC 50 in cereals, calculated PEC/RAC ratios for the formulation and the active substance isoflucypram indicate acceptable chronic risk for all aquatic organisms.

CHRONIC RISK ASSESSMENT FOR AQUATIC ORGANISMS

Table 10.2- 12: Chronic risk assessment based on FOCUS Step 2

Compound	Species	Endpoint [µg/L]		RAC [µg/L]	$PEC_{sw,max}$ [µg/L]	RAC ≥ PEC_{sw}
Isoflucypram EC 50	Algae, chronic *Pseudokirchneriella subcapitata*	E_rC_{50}	179	17.9	2.8386	Yes
Isoflucypram	Fish, chronic *Oncorhynchus mykiss*	NOEC	15.6	1.56		**No**
	Invertebrate, chronic *Daphnia magna*	NOEC	66.1	6.61		Yes
	Sediment dweller, chronic *Chironomus riparius*	NOEC	85000	8500		Yes
	Algae, chronic *Navicula pelliculosa*	E_rC_{50}	> 2000	> 200		Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50}	> 3020	> 302		Yes
BCS-CN88460-carboxylic acid (M12)	Fish, chronic # *Oncorhynchus mykiss*	NOEC	15.6	1.56	1.3090	Yes
	Invertebrate, chronic # *Daphnia magna*	NOEC	66.1	6.61		Yes
	Algae, chronic *Pseudokirchneriella subcapitata*	E_rC_{50}	> 35100	> 3510		Yes

As the metabolite is at least acutely 10 times less toxic than the parent (on a molar basis) the chronic parent endpoints for all Tier 1 taxonomic groups can be taken as surrogates.

For isoflucypram the chronic trigger was not met for fish. For the isoflucypram metabolite BCS-CN88460-carboxylic acid (M12) acceptable chronic risk could be proven for fish, invertebrates and algae. The consideration of the more realistic FOCUS Step 3 water concentrations is presented below.

Winter cereals

Table 10.2- 13: Chronic risk assessment based on FOCUS Step 3 for winter cereals

Compound	Species	Endpoint [µg/L]		RAC# [µg/L]	FOCUS Scenario	$PEC_{sw,max}$ [µg/L]	RAC# ≥ PEC_{sw}
Isoflucypram	Fish, chronic *Oncorhynchus mykiss*	NOEC	15.6	1.56	D1 ditch	1.2430	Yes
					D1 stream	0.7788	Yes
					D2 ditch	1.1690	Yes
					D2 stream	0.7315	Yes
					D3 ditch	0.4754	Yes
					D4 pond	0.0785	Yes
					D4 stream	0.4102	Yes
					D5 pond	0.0821	Yes
					D5 stream	0.4425	Yes
					D6 ditch	0.6339	Yes
					R1 pond	0.0493	Yes
					R1 stream	0.3133	Yes
					R3 stream	0.4414	Yes
					R4 stream	0.3930	Yes

Spring cereals

Table 10.2- 14: Chronic risk assessment based on FOCUS Step 3 for spring cereals

Compound	Species	Endpoint [µg/L]		RAC# [µg/L]	FOCUS Scenario	$PEC_{sw,max}$ [µg/L]	RAC# ≥ PEC_{sw}
Isoflucypram	Fish, chronic *Oncorhynchus mykiss*	NOEC	15.6	1.56	D1 ditch	0.9975	Yes
					D1 stream	0.6255	Yes
					D3 ditch	0.4749	Yes
					D4 pond	0.0874	Yes
					D4 stream	0.4089	Yes
					D5 pond	0.0766	Yes
					D5 stream	0.4140	Yes
					R4 stream	0.3893	Yes

For the use of isoflucypram in cereals, calculated PEC/RAC ratios for the active substance isoflucypram indicate acceptable chronic risk for all aquatic organisms.

CP 10.2.1 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. These studies need to cover (among others) as well additional studies with fish. As these data exist they have to be part of the submitted dossier and therefore have been submitted by the notifier for regulatory review.

Report: KCP 10.2.1/01; [redacted]; 2017; M-595274-01-1
Title: BCS-CN88460 EC 50 G acute toxicity to rainbow trout (Oncorhynchus mykiss) under static conditions - Final report
Report No.: E 203 05016-7
Document No.: M-595274-01-1
Guideline(s): EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985)
OCSPP 850.1075 (Public Draft, 1996)
OECD No. 203 (rev.1992)
JMAFF, 12 Nousan No. 8147 (2000)
Guideline deviation(s): none
GLP/GEP: yes

Material and methods

Test material	BCS-CN88460 EC 50 G lot/batch 2016-001002 Specification 102000031262 analyzed content of active substance: 51.45 g/L (5.28% w/w)
Guideline(s) adaptation	None specified
Test species	Rainbow trout (*Oncorhynchus mykiss*)
Acclimation	At least 14 days, fed daily with commercial trout food Health during acclimation: less than 5% mortality

Organism age/size at study initiation	Mean length: 4.4 ± 0.9 cm Mean body weight: 0.9 ± 0.4 g
Test solutions	Nominal concentrations: 0.178, 0.355, 0.710, 1.42 and 2.84 mg form./L 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 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
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	Required	Obtained
Mortality within the 48-h settling-in period	≤ 5%	0%
Mortality in control during test	≤ 10%	0%
Dissolved oxygen saturation	≥ 60%	≥ 94%

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 the toxicity has to be attributed to the tested formulation as a whole, all results were related to nominal test concentrations of the formulated product.

Nominal conc. form. [mg/L]	Nominal conc. BCS-CN88460 [mg/L]	Measured concentration * of BCS-CN88460 [%]			
		Day 0	Day 1	Day 2	Day 4
0.178	0.00938	124	-	97.6	85.3
0.355	0.0188	128	-	102	88.6
0.710	0.0375	124	-	103	89.4
1.42	0.0750	123	-	102	105
2.84	0.1500	122	114	-*	-*

* No measurement (all fish dead)

Biological results:

Observations
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.

Cumulative mortality over the test period

Exposure time (hours)	24	48	96
Test conc. form. [mg/L]	No of dead (%)	No of dead (%)	No of dead (%)
Control	0	0	0
0.178	0	0	0
0.355	0	0	0
0.710	0	0	0
1.42	10	60	90
2.84	100	100	100

Conclusion

The study meets the validity criteria and the 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)**
LOEC: lowest concentration with an effect	0.710 mg form./L
NOEC: highest concentration without adverse effects	0.355 mg form./L
LC_0: highest concentration without mortality	0.710 mg form./L
LC_{100}: lowest concentration with 100% mortality	1.84 mg form./L

Report: KCP 10.2.1/02; [redacted]; 2017; M-607779-01-1
Title: Acute toxicity of BCS-CN88460 EC 50 G to the waterflea Daphnia magna in a static laboratory test system
Report No.: EBLNN499
Document No.: M-607779-01-1
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
Guideline(s) adaptation	None specified
Test species	Water flea (*Daphnia magna*)
Organism age/size at study initiation	First instar neonates, less than 24 hours old
Test solutions	Nominal concentrations: 0.194, 0.427, 0.939, 2.07, 4.55 and 10.0 mg form./L Control: water control
Replication	No. of vessels per concentration (replicates): 6 No. of vessels per control (replicates): 6
Organisms per replicate	No. of organisms per vessel: 5
Exposure	Static; total exposure duration: 48 hours

Feeding during test	None
Test conditions	Temperature: 20.2 - 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.
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

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

Analytical results:

The accompanying chemical analysis of BCS-CN88460 in the freshly prepared test solutions at test initiation revealed measured contents between 105% and 114% of the aspired nominal concentrations. 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.

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:

Observations
An immobilisation of 3.3% as observed for untreated control animals ranged well below the 10% value which is regarded to represent the limit for natural mortality.

Immobility

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
0.939	30	0	0	1	3.3
2.07	30	5	16.7	7	23.3
4.55	30	16	53.3	30	100
10.0	30	30	100	30	100

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)

Report:	KCP 10.2.1/03; [redacted]; 2017; M-600970-01-1
Title:	Pseudokirchneriella subcapitata growth inhibition test - BCS-CN88460 EC50 G
Report No.:	EBLNN500
Document No.:	M-600970-01-1
Guideline(s):	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; U.S. EPA Pesticide Assessment Guidelines, Subdivision J, §122-2, 123-2; OCSPP Guideline 850.4500 (January 2012)
Guideline deviation(s):	none
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
Guideline(s) adaptation	None specified.
Test species	Freshwater green algae (*Pseudokirchneriella subcapitata*) Strain SAG 61.81
Culturing conditions	400 µL of a 7-10 days old stock culture was transferred into a 300 mL cotton plugged Erlenmeyer flask containing about 100 mL of nutrient medium every 7-10 days. 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[1] algae culture. Pre-cultures were prepared from stock cultures 3 days before the start of the test using OECD medium.
Organism age/size at study initiation	Pre cultures were prepared from stock cultures 3 days before the start of the test using OECD medium.
Test solutions	Nominal concentrations: 0.0954, 0.305, 0.977, 3.13 and 10.0 mg/L Controls: Water control Evidence of undissolved material: No precipitations observed.
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4
Exposure	Static Total exposure duration: 96 hours
Initial cells density	10^4 cells/mL in each test group

[1] 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)
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 examiniation 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 and the given results are expressed as 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 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%	12.9%
3) The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%.	< 7%	1.2%

Analytical results:

Measured concentrations of the samples ranged from approximately 91 to 112% 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).

Nominal Concentration (mg/L)	0-hour % Nominal*	72-hour % Nominal*	96-hour % Nominal*
0.0954	95	91	87
0.305	103	101	112
0.977	111	99	97
3.13	108	107	110
10.0	111	112	123

* Mean of two determinations

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

72 hours

Nominal concentrations (mg/L)	Mean cell number after 72 h (cells/mL)	Inhibition of average specific growth rate (%)
Water Control	714000	-
0.0954	712000	0.1
0.305	664000	1.1
0.977	588000	4.5
3.13	145000	45.2
10.0	8000	122.9

Nominal concentrations (mg/L)	Yield (cells × 1000/mL)	Inhibition of yield (%)
Water Control	70.4	-
0.0954	70.1	0.4
0.305	65.4	7.1
0.977	57.8	17.9
3.13	10.5	85.1
10.0	-0.2	100.3

Nominal concentrations (mg/L)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Water Control	1277.3	0.0
0.0954	1237.1	3.1
0.305	1194.8	6.5
0.977	1038.6	18.7
3.13	211.9	83.4
10.0	13.9	98.9

96 hours

Nominal concentrations (mg/L)	Mean cell number after 96 h (cells/mL)	Inhibition of average specific growth rate (%)*
Water Control	2133000	-
0.0954	2121000	0.1
0.305	2006000	1.1
0.977	1864000	2.5
3.13	490000	28.9
10.0	20000	87.3

* % Inhibition: Increase in growth relative to the pooled control

Nominal concentrations (mg/L)	Yield (cells × 1000/mL)	Inhibition of yield (%)
Water Control	70.4	-
0.0954	70.1	0.4
0.305	65.4	7.1
0.977	57.8	17.9
3.13	10.5	85.1
10.0	-0.2	100.3

Nominal concentrations (mg/L)	Area under the growth curve (biomass integral)	Inhibition of Biomass integral (%)
Water Control	1277.3	0.0
0.0954	1237.1	3.1
0.305	1194.8	6.5
0.977	1038.6	18.7
3.13	211.9	83.4
10.0	13.9	98.9

Conclusion

The study meets the validity criteria and 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)
LOE_rC 72 hours: Lowest concentration with an effect	3.13 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)
LOE_rC 72 hours: Lowest concentration with an effect	0.305 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)
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)
LOE_yC 96 hours: Lowest concentration with an effect	0.977 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)
LOE_bC 96 hours: Lowest concentration with an effect	0.977 mg/L
NOE_bC 96 hours: highest concentration without adverse effects	0.305 mg/ L

CP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

No additional studies were necessary based on the current data requirements. Please refer to Document MCA Section 8, Point 8.2.

CP 10.2.3 Further testing on aquatic organisms

No studies were necessary based on the current data requirements. Please refer to Document MCA, Section 8, Point 8.2.

CP 10.3 Effects on arthropods

CP 10.3.1 Effects on 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 under MCA Section 8, Point 8.3.1.
Further data on honeybees was generated under semi-field conditions with the representative formulation Isoflucypram EC 50, which is described in detail in this MCP Section 10:

- Semi-field brood studies following OECD Guidance Document No. 75 using a more realistic spray scenario onto flowering *Phacelia* covering effects on brood (eggs) and their development and colony parameters.
- Semi-field brood studies following EPPO 170 (4) using a more realistic spray scenario onto flowering *Phacelia* covering effects on mortality, foraging activity as well as general colony development.

These tunnel tests with the representative formulation Isoflucypram EC 50 according to OECD GD 75 and EPPO 170 are presented in the MCP-Summary, Section 10 under Point 10.3.1.5.

Table 10.3.1- 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 LD_{50} – contact	> 106.3 µg a.s./bee > 100 µg a.s./bee	[redacted]; 2014; M-503824-01-1 KCA 8.3.1.1.1/01 KCA 8.3.1.1.2/01
	Honeybee, 48 h	LD_{50} – oral LD_{50} – contact	> 109.5 µg a.s./bee > 100 µg a.s./bee	[redacted]; [redacted]; 2013; M-472468-01-1 KCA 8.3.1.1.1/02 KCA 8.3.1.1.2/02
	Honeybee larva, 22 days	NOEC ≥ 406 mg a.s./kg NOED ≥ 62.5 µg a.s./larva		[redacted]; 2017; M-587515-01-1 KCA 8.3.1.3/01
	Bumble bee, 48 h	LD_{50} – oral	> 200.2 µg a.s./bumble bee	[redacted]; 2015; M-542774-01-1 KCA 8.3.1.1.1/03
	Bumble bee, 48 h	LD_{50} – contact	> 100 µg a.s./bumble bee	[redacted]; 2015; M-509048-01-1 KCA 8.3.1.1.2/03

Test substance	Test species/ study type	Endpoint	References
Isoflucypram SC 200	Honeybee, 10 day chronic adult feeding study	LDD_{50} > 89.7 µg a.s./bee/day NOEDD ≥ 89.7 µg a.s./bee/day	[redacted], A.; 2015; M-540173-01-1 KCA 8.3.1.2/01
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	[redacted]; 2016; M-571280-01-1 KCP 10.3.1.1/01
	Honeybee Brood - Semi-Field (OECD GD 75)	Overall, no adverse effects on brood development (brood termination rate, brood index and compensation index), mortality (adult and pupae), foraging activity, behaviour, colony condition and strength after application of 75 g a.s./ha onto flowering *Phacelia tanacetifolia*.	[redacted]; [redacted]; 2016; M-549363-01-1 KCP 10.3.1.5/01
	Honeybee Brood - Semi-Field (OECD GD 75)	Overall, no adverse effects on brood development (brood termination rate, brood index and compensation index), mortality (adult and pupae), foraging activity, behaviour, colony condition and strength after application of 75 g a.s./ha onto flowering *Phacelia tanacetifolia*.	[redacted]; 2017; M-581949-01-1 KCP 10.3.1.5/02
	Honeybee Colony Development - Semi-Field (EPPO 170)	Overall, no adverse effects on mortality (adult and pupae), foraging activity, behaviour, colony development and condition and colony strength after application of 75 g a.s./ha onto flowering *Phacelia tanacetifolia*.	[redacted]; 2017; M-606834-01-1 KCP 10.3.1.5/03
	Honeybee Colony Development - Semi-Field (EPPO 170)	Overall, no adverse effects on mortality (adult and pupae), foraging activity, behaviour, colony development and condition and colony strength after application of 75 g a.s./ha onto flowering *Phacelia tanacetifolia*.	[redacted], A.; 2017; M-607771-01-1 KCP 10.3.1.5/04

Risk assessment for bees

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 10.3.1- 2: Hazard quotients for bees – oral exposure

Compound	Oral LD_{50} [µg a.s./bee]	Max. application rate [g/ha]	Hazard quotient Q_{HO}	Trigger	*A-priori* acceptable risk for adult bees
Isoflucypram tech.	> 109.5	75	< 0.69	50	yes
Isoflucypram EC 50	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 10.3.1- 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	*A-priori* acceptable risk for adult bees
Isoflucypram tech.	> 100.0	75	< 0.75	50	yes
Isoflucypram EC 50	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. $Q_{HC} < 50$).

Further considerations for the risk assessment

The active substance isoflucypram (tech.) is non- toxic to bees with acute LD_{50} values for adult honeybees in excess of 100 µg a.s./bee for oral and contact routes of administration. The formulated product Isoflucypram EC 50 is slightly toxic to honeybees with an acute oral LD_{50} value of 69.1 µg a.s./bee and 14.1 µg a.s./bee for contact toxicity. All calculated HQ values based on these toxicity endpoints are considerably lower than the levels regarded to indicate a risk to bees.
Acute laboratory toxicity tests on adult bumble bees resulted in LD_{50} values which are in excess of 200 and 100 µg a.s./bumble bee for oral and contact exposure, respectively, indicating that bumble bees are not more sensitive than honeybees to isoflucypram.

Isoflucypram was further subjected to chronic laboratory testing with adult honeybees (KCA 8.3.1.2; [redacted], A.; 2015; M-540173-01-1).
This chronic study was designed as a dose-response test by exposing adult honeybees for 10 consecutive days to nominal concentrations of 208, 417, 833, 1667, 3333 mg isoflucypram/kg feeding solution. The actual test was conducted by using the formulated product Isoflucypram SC 200 (202.3 g/L) to overcome the limitations of solubility that a technical active ingredient may have. After exposing honeybees for ten consecutive days exclusively to feeding solution containing the a.s. at the respective treatment levels, the 10 day LC_{50} (Lethal Concentration) was determined to be > 3333 mg isoflucypram/kg, which corresponds to a LDD_{50} (Lethal Dietary Dose) of >89.7 µg a.s./bee/day. The respective NOEC (No Observed Effect Concentration) for mortality was determined to be >3333 mg isoflucypram/kg, which corresponds to the NOEDD (No Observed Effect Dietary Dose) of ≥ 89.7 µg a.s./bee/day. The findings from this study indicate that isoflucypram is of equally low toxicity over a 10-day period compared to acute exposure.

Additional laboratory testing was performed with Isoflucypram by feeding of honeybee larvae (KCA 8.3.1.3; [redacted]; 2017; M-587515-01-1).

This repeated feeding study was designed as a dose-response test by exposing young honeybee larvae at three feeding events to nominal concentrations of 10.4, 26.0, 65.0, 162 and 406 mg a.s./kg diet, equivalent to cumulative doses of 1.60, 4.00, 10.0, 24.9 and 62.5 µg a.s./larva per developmental period. After exposure mortality was assessed during the larval and pupal phase with a final assessment of adult emergence on day 22. As study endpoint the 22-day NOEC (No Observed Effect Concentration) for emergence was determined to be >406 mg isoflucypram/kg, which corresponds to the NOED (No Observed Effect Dose) of ≥ 62.5 µg a.s./larva.

In order to clarify whether isoflucypram would pose a risk to honeybee brood in particular and to the survival of adult honeybees and colony development in general under more realistic worst-case conditions, two higher tier semi-field honeybee brood studies were conducted according to the provisions of the OECD Guidance Document 75. Under these forced/confined exposure conditions the representative formulation Isoflucypram EC 50 was applied at 75 g a.s./ha in tunnels to the full flowering and highly bee attractive surrogate crop *Phacelia tanacetifolia*. In the two semi-field (tunnel) tests conducted by [redacted]; [redacted]; 2016; M-549363-01-1 and [redacted]; 2017; M-581949-01-1 the honeybee colonies were exposed to spray residues by adult bees foraging on flowers (nectar and pollen) from the treated plants. The bee colonies were kept within the tunnels for approx. 1 week (confined exposure phase) and were then relocated out of the tunnels and transferred to a monitoring site without flowering crops and intensive agricultural activity for further monitoring for approx. 2.5 weeks. Daily, throughout the confined exposure phase, mortality of worker bees, larvae and pupae was assessed along with assessments of foraging activity and behaviour. Mortality assessments were continued along with behaviour around the hive during the post-exposure observation period. Colony assessments (colony strength, brood area, food stores) were made before and after the confinement period and at the end of the study. Detailed brood assessments included investigation of the fate of more than 200 individually marked cells in each replicate of each treatment group in both studies and were performed on several occasions throughout the studies, covering an entire brood cycle of honeybees.

Differences between test item and control were only observed in one out of the two studies. In [redacted]; 2017; M-581949-01-1 a slight but statistically significant increase was observed in adult mortality on day 14 after application and a slight but statistically significant decrease in flight intensity was observed on the application day and two days after application, and for the mean over the entire observation period. These defects were either transient or so minor in nature that they are not seen as biologically relevant. When summarized, in both studies it was found that the application of Isoflucypram EC 50 at the rate of 75 g a.s./ha under tunnel conditions to the full flowering and highly bee attractive surrogate crop *Phacelia tanacetifolia* generally did not cause any adverse effects on individual brood development (brood termination rate, brood index, compensation index), adult and larval/pupal survival, foraging activity as well as on general colony development (brood status, colony strength and condition) in the test item treatment groups when compared to the respective parallel running controls.

To further investigate exposure of honeybees and honeybee colonies under more realistic worst-case conditions two higher tier semi-field honeybee studies were conducted following the guideline EPPO 170 (4) with focus on mortality, foraging activity and general colony development. Under these forced/confined exposure conditions the representative formulation Isoflucypram EC 50 was applied at 75 g a.s./ha in tunnels to the full flowering and highly bee attractive surrogate crop *Phacelia tanacetifolia*. In the two semi-field (tunnel) tests conducted by [redacted]; 2017; M-606834-01-1 and [redacted], A.; 2017; M-607771-01-1 the honeybee colonies were exposed to spray residues by adult bees foraging on flowers (nectar and pollen) from the treated plants. The bee colonies were kept within the tunnels for approx. 1 week (confined exposure phase) and were then transferred to a monitoring site without flowering crops and intensive agriculture for further monitoring for approx. 6 weeks, covering the period of 2 brood cycles. Daily, throughout the confined exposure phase, mortality of worker bees, larvae and pupae was assessed along with assessments of foraging activity and

behaviour. Mortality assessments were continued along with behaviour around the hive during the post-exposure observation period. Colony assessments (colony strength, brood area, food stores) were made before and after the confinement period and at the end of the study.
Few differences between test item and control were detected in these two studies. In the study by [redacted]; 2017; M-606834-01-1 a slight but statistically significant increase was observed in pairwise comparisons in adult mortality on 3 individual days. In the study by [redacted], A.; 2017; M-607771-01-1 a slight but statistically significant increase was observed in pairwise comparisons in adult mortality on the day of application. Both detects were transient and so minor in nature that they are not seen as biologically relevant. In sum in both studies it was found that the application of Isoflucypram EC 50 at the rate of 75 g a.s./ha under tunnel conditions to the full flowering and highly bee attractive surrogate crop *Phacelia tanacetifolia* did not cause any adverse effects on adult and larval/pupal survival, foraging activity as well as on general colony development (brood status, colony strength and condition) in the test item treatment groups when compared to the respective parallel running controls.

Conclusion

Overall, it can be concluded that Isoflucypram EC 50, when applied at the maximum application rate of 1.5 L product/ha (75 g a.s./ha) even during the flowering period of a bee-attractive crop, does not pose an unacceptable risk to honeybees and honeybee colonies so that it can be safely assumed that an application in cereals will result in a safe use of the product when applied as recommended.

CP 10.3.1.1 Acute toxicity to bees

CP 10.3.1.1.1 Acute oral toxicity to bees

Report:	KCP 10.3.1.1.1/01; [redacted]; 2016; M-571280-01-1
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
Document No.:	M-571280-01-1
Guideline(s):	OECD 213 and 214 (1998); US EPA OCSPP 850.3020, 850.supp.
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of this study was to determine the acute contact and oral toxicity of BCS-CN88460 EC 50 G to the honeybee (*A. mellifera* L.) in the laboratory. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Material and methods:

Test item: BCS-CN88460 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.
Test organism: female worker honeybees (*Apis mellifera*), obtained from a healthy and queen-right colony, bred by IBACON.
Under laboratory conditions *Apis mellifera* 30 worker bees per treatment level were exposed for 96 hours to doses of 80.0, 36.4, 16.5, 7.5, 3.4 and 1.6 µg a.s. per bee by topical application (contact dose response test) and 30 worker bees per treatment level were exposed for 72 hours to doses of 90.7, 81.1, 39.8, 19.9 and 9.1 µg a.s. per bee by feeding (oral dose response test, value based on the actual

intake of the test item). Due to increasing mortality between 24 and 48 hours both contact and oral test were prolonged for further 24 hours up to 72 hours. Additionally, the contact test was prolonged up to 96 hours because of increasing mortality between 48 and 72 hours.
Furthermore, each test consisted of a control and a reference item group. In the contact test, tap water containing 0.5% Adhaesit was used as control. In the oral test a 50 % w/v sucrose solution was used as control. In both tests Perfekthion EC (active ingredient 420.3 g/L dimethoate, Batch no.: FRE-001226) was used as toxic standard. Each treatment group consisted out of 3 replicates (test units) with 10 bees per replicate. Test units were stainless steel cages with 8 cm × 6 cm × 4 cm (length × height × width). The tests were conducted in darkness, temperature was 23.8 – 25.4°C and relative humidity was between 59.2 and 64.1%. Biological observations, including mortality and behavioural changes were recorded 4, 24, 48, 72 and 96 h after application in the contact test and 4, 24, 48 and 72 h after application in the oral test.
The software used to perform the statistical analysis was ToxRat Professional.

Results:

Biological findings:

Test Item	**BCS-CN88460 EC 50 (50.0 g/L)**	
Test object	***Apis mellifera L.***	
Exposure	**contact (solution in Adhaesit (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	24 hours: 36.8 48 hours: 25.7 72 hours: 17.6 96 hours: 14.1	24 hours: 74.8 48 hours: 69.1 72 hours: 69.1
LD_{20} µg a.s./bee	24 hours: 16.7 48 hours: 7.7 72 hours: 5.3 96 hours: 5.9	24 hours: 44.6 48 hours: 50.4 72 hours: 50.4
LD_{10} µg a.s./bee	24 hours: 11.0 48 hours: 4.1 72 hours: 2.9 96 hours: 3.7	24 hours: 34.1 48 hours: 42.7 72 hours: 42.7
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, α = 0.05).

Observations:

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 mortality of 100.0, 80.0, 56.7, 30.0, 10.0 and 13.3 % at test termination (96 hours), respectively. 6.7 % mortality occurred in the control group (water + 0.5 % Adhaesit).

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	50.0	50.0	80.0	3.3
16.5	0	80.0	26.7	30.0	46.7	6.7	53.3	3.3	56.7	0
7.5	0	40.0	3.3	10.0	20.0	3.3	30.0	3.3	30.0	0
3.4	0	3.3	3.3	3.3	10.0	0	10.0	0	10.0	0
1.6	0	0	0	0	3.3	0	13.3	0	13.3	0
Water	0	0	0	0	0	0	3.3	0	6.7	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

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 mortality of 100.0, 40.0, 13.3, 3.3 and 3.3 % 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).

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	40	0	40	0
39.8	0	20	13.3	0	13.3	0	13.3	0
19.9	0	0	3.3	0	3.3	0	3.3	0
9.1	0	0	0	0	3.3	0	3.3	0
Water	0	0	0	0	3.3	0	3.3	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	73.3	0	76.7	0
0.08	0	16.7	3.3	0	10	0	16.7	0
0.06	0	6.7	0	0	0	0	3.3	0

Results are average from three replicates (ten bees each) per dosage/control
Behav. abnorm. = Behavioural abnormalities

Validity criteria:

All validity criteria of the test were met.

Validity criteria according to OECD 213 and 214	Obtained in this study
Control mortality should not exceed 10% at test end	Contact test Control: 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

Conclusion:

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.

CP 10.3.1.1.2 Acute contact toxicity to bees

Please refer to CP 10.3.1.1.1.

CP 10.3.1.2 Chronic toxicity to bees

A 10-day chronic oral toxicity study was conducted with formulated isoflucypram (SC 200). The corresponding summary is filed in MCA Section 8, Point 8.3.1.2.

CP 10.3.1.3 Effects on honey bee development and other honey bee life stages

A repeated honeybee larvae feeding study was conducted with isoflucypram tech. The corresponding summary is filed under MCA Section 8, Point 8.3.1.3.

CP 10.3.1.4 Sub-lethal effects

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

CP 10.3.1.5 Cage and tunnel tests

Honeybee semi-field studies with focus on brood development (OECD GD 75) have been conducted with Isoflucypram EC 50 and are summarised below.

Report:	KCP 10.3.1.5/01; [redacted]; [redacted]; 2016; M-549363-01-1
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
Document No.:	M-549363-01-1
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
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of the study was to investigate potential side effects of a spray application of BCS-CN88460 EC 50 on the honeybee (*Apis mellifera* L.) under semi-field conditions by following the OECD Guidance Document No. 75 (2007), with methodological improvements by the AG Bienenschutz (PISTORIUS *et al.*, 2012).

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.

Test species were honeybees (*Apis mellifera L.*, Hymenoptera, Insecta); 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.

The test was conducted in tunnel tents in order to assess potential side effects of the product BCS-CN88460 EC 50 G (50 g/L) on honeybee colonies under semi-field conditions. A plot of *Phacelia tanacetifolia* with an effective crop area of ca. 85 m² (2 × 42.5 m^2) was prepared for each tunnel (21 m long, 5.5 m wide and 2.5 m high) and each plot constituted one replicate. For each treatment group (control, test item and reference item), 4 tunnels/replicates were set up, resulting in 12 tunnels in total. Per tunnel one honeybee colony was used. The bee colonies were placed into the tunnels with *Phacelia* (BBCH 60) six days before application. After the exposure period all bee colonies including dead-bee traps were removed from the study field and placed at a monitoring location.

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). Control tunnels were sprayed with water (400 L/ha), test item tunnels received 75 g a.s. in 400 L tap water/ha (corresponding to 1.512 mL product/ha) and reference item tunnels received 300 g a.s. in 400 L tap water/ha (corresponding to nominal 1200 g Insegar/ha).

The following endpoints were assessed:

- Mortality assessment: Dead worker bees, larvae, pupae and drones were assessed in dead-bee traps and on non-woven sheets.
- Flight activity: Numbers of bees that were both foraging on flowering plants and flying around the crop were recorded.
- Behavioural abnormalities of the bees were recorded.

- Colony conditions (estimation of bees, brood, pollen and nectar) assessments were conducted.
- The development of bee brood (ontogenesis of eggs) was evaluated for appropriate amount of eggs (> 200) from each colony.

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.

Findings:

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 ($p < 0.001$). This was consistent with the expected effect of the reference substance "Insegar" and demonstrated that the foraging activities were sufficient to instigate a high level of exposure to the treatments in the bees and their brood.

	Phase	Control		Test item			Reference item		
		Mean	± SD	Mean	± SD	stat.	Mean	± SD	stat.
Workers in dead –bee traps	pre-exposure (DAT-5 to 0/0)	3.8	1.8	2.8	2.2	>0.05	3.8	1.5	> 0.05
	exposure (DAT 0/1 to 8	3.3	2.1	4.1	3.3		3.0	2.4	
	post-exposure (DAT 9 to 28)	13.8	16.8	14.4	16.4		15.1	6.7	
Workers on non-woven sheets	pre-exposure (DAT-5 to 0/0)	6.7	5.9	8.5	7.1	> 0.05	9.8	10.0	> 0.05
	exposure (DAT 0/1 to 8	4.2	2.7	5.9	3.4		5.4	3.5	
Pupae and larvae in dead bee traps	pre-exposure (DAT-5 to 0/0)	0.3	0.4	0.3	0.3	> 0.05	0.4	0.3	< 0.001
	exposure (DAT 0/1 to 8	2.1	2.8	1.0	1.3		3.6	5.3	
	post-exposure (DAT 9 to 28)	0.3	0.5	0.2	0.2		27.6	26.8	

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 ($p > 0.05$) while a reduction ($p < 0.05$) was observed when comparing control and toxic reference item.

Phase	Control		Test item			Reference item		
	Mean	± SD	Mean	± SD	stat.	Mean	± SD	stat.
pre-exposure (DAT-2 to 0/0)	18.3	4.6	16.7	6.5	>0.05	15.0	2.9	< 0.05
exposure (DAT-2 to 0/0)	14.8	7.1	11.5	7.1		10.1	4.5	

Behaviour

No acute symptoms of poisoning (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

In general, development of the bees was normal for the seasonal period. 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 normal range. 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.

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).

	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

Conclusion:

In order to assess potential effect 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.

Report:	KCP 10.3.1.5/02; [redacted]; 2017; M-581949-01-1
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
Document No.:	M-581949-01-1
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)
Guideline deviation(s):	no major deviations
GLP/GEP:	**yes**

Objective:

The purpose of the study was to investigate potential side effects of a spray application of BCS-CN88460 EC 50 on the honeybee (*Apis mellifera* L.) under semi-field conditions by following the OECD Guidance Document No. 75 (2007), with methodological improvements by the AG Bienenschutz (PISTORIUS *et al.*, 2012).

Material and methods:

Test item: BCS-CN88460 EC 50 G (50 g/L). BCS-CN88460: 5.18 % w/w (analytical); batch ID: 2016-001002; Sample Description: TOX20246-00; Specification No.: 102000031262; density: 0.974 g/mL.

Test species: Honeybees (*Apis mellifera L.*; Hymenoptera, Insecta). The hives contained only 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 study design comprised one treatment group T, treated with the test item, one treatment group R, treated with the reference item and one treatment group C, treated with tap water, each with four replicates. Applications were made at full-flowering (BBCH 65) while honeybees were actively foraging on the crop.

The test item BCS-CN88460 EC 50 was applied at a mean rate of 78.9 g a.s./ha. The envisaged target rate was 75 g a.s./ha. Tap water was applied in the treatment group C. Insegar was applied at a target rate of 1200 g product/ha in the reference item group (corresponding to 300 g fenoxycarb/ha). The spray volume was 400 L/ha in all treatment groups.

The initial mean colony sizes per treatment group were in the range of 4501 to 6023 bees. The honeybees remained in the tunnels for 12 days and colonies were assessed twice during the confined period and seven times afterwards.

The following endpoints were assessed:
- Total and mean number of dead bees on the linen sheets in tunnels, in the dead bee traps and in the dead bee bottoms before as well as after the start of exposure in T and the application in C and R, respectively.
- Flight intensity (mean number of forager bees/m² *Phacelia tanacetifolia*) before as well as after the start of exposure in T and the application in C and R, respectively.
- Behaviour of the bees in the crop and around the hive.
- Condition of the colonies (colony strength and area of the different brood stages and food storage per colony and assessment date).
- Development of the bee brood assessed in individual brood cells. For this particular assessment, between 229 and 266 individually marked cells per colony were selected.

Findings:

Mortality

Throughout the study (before and following exposure) and especially on the application day, no increases of the adult honeybee mortality were found in the treatment groups T or R when tested against C, indicating no effect of the test item. A statistically significant difference between C and T was found on 14DAA but this was only minor in nature and not related to the treatment.

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).

As increased pupal mortality, starting at about 10DAA, is a typical and well known effect of the reference item, the increased pupal mortality rates refer to a strong toxic effect of the reference item. The observation of pupae with malformations (white eyes) on 8, 9, 11, 13, 14, 15, 17, 18, 22 and 25DAA confirm this observation of a strong toxic reference item effect.

Treatment group		Control (C)	Test item(T)	Reference Item (R)
Daily mean mortality (dead worker bees/colony) ± STD	4DBA to 0DBA	42.5 ± 14.4	48.1 ± 15.9	59.5 ± 34.3
	0DAA	86.0 ± 20.8	80.5 ± 36.5	109.3 ± 22.9
	0DAA to 7DAA	60.3 ± 16.7	55.5 ± 16.8	72.6 ± 29.8
	8DAA to 26DAA	17.1 ± 5.4	13.7 ± 3.2	20.7 ± 11.4
	0DAA to 26DAA	29.9 ± 8.1	26.1 ± 6.5	36.1 ± 7.3
Daily mean mortality (dead larvae + pupae/ colony) ± STD	4DBA to 0DBA	1.2 ± 2.3	0.6 ± 0.7	0.5 ± 0.4
	0DAA	0.0 ± 0.0	0.7 ± 1.2	0.3 ± 0.5
	0DAA to 7DAA	1.5 ± 1.9	0.7 ± 0.8	0.4 ± 0.4
	8 DAA to 26DAA	1.3 ± 1.7	0.4 ± 0.6	16.4 * ± 21.7
	0DAA to 26DAA	1.3 ± 1.4	0.4 ± 0.7	11.7 * ± 15.2

* = statistically significant higher than control group

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, α = 0.05). On the following day (1DAA) there was already no statistically significant difference between the flight activity in T and C (T: 20.4, C: 20.5 forager bees/m²). A small but 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 in T and C was statistically significant (T: 18.2, C: 20.7 forager bees/m²). The difference was only 2.5 bees/m² which is minor in nature and therefore biologically negligible. Therefore, no adverse effect of the test item treatment on honeybee flight activity could be discerned.

Treatment group		Control (C)	Test item (T)	Reference Item (R)
Daily mean flight intensity (bees/m²) ± STD	4DBA to 0DBA	14.1 ± 2.8	15.1 ± 2.3	14.6 ± 2.0
	0DAA	21.7 ± 6.0	14.7* ± 1.7	18.3 ± 1.6
	0DAA to 7DAA	20.7 ± 2.3	18.2* ± 0.7	20.4 ± 0.8

DAA = days after application; DBA = days before application; STD = standard deviation
* = statistically significant lower than control group

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

Strength of the colonies
The overall development of colony strength of all treatment groups showed fluctuations in a typical and normal range. The colony strength values of the test item group were on approximately the same level during the entire study compared the corresponding values of the control group. Therefore, no test-item related adverse effects on colony strength were observed.

Development of the brood area
The mean amount of brood in the colonies (sum of cells containing eggs, larvae and pupae) was assessed. Overall, on the level of whole colonies, honeybee brood development in the test item treatment group T as not affected, 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. All colonies were well provided during the course of the study and there was no lack of pollen or nectar in any colony at any assessment date. Except in treatment group R, where a decline in mean colony strength and food storage area at the end of the first brood cycle (21DAA) was recorded. 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.

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

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, flight intensity and behaviour were observed over the entire test period.
The effect of the toxicity of the reference item was clearly 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.

Honeybee semi-field studies with focus on colony development have been conducted with Isoflucypram EC 50 and are summarised in the following.

Report:	KCP 10.3.1.5/03; [redacted]; 2017; M-606834-01-1
Title:	Isoflucypram EC 50 G: Toxicity testing on honey bees (Apis mellifera L.) under semi-field conditions in Germany - Tunnel test
Report No.:	122701037
Document No.:	M-606834-01-1
Guideline(s):	OEPP/EPPO No. 170 (4)(2010) Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP Not Applicable
Guideline deviation(s):	None
GLP/GEP:	**yes**

Objective:

The purpose of the study was to investigate potential side effects of a spray application of Isoflucypram EC 50 on the honeybee (*Apis mellifera* L.) under semi-field conditions in Germany by following OEPP/EPPO Guideline No. 170(4).

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.
Test species: Honeybees (*Apis mellifera* L.; Hymenoptera, Insecta). Colonies with 11 combs (4-7 brood combs) and one queen were used. Three days before application the colonies consisted of a mean of 4770 to 6233 honey bees/colony. The colonies were produced at the same time with sister queens in order to guarantee uniform bee material in all treatments.
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.

The following endpoints were assessed:
- Mortality: 3 days before to 42 days after application
- Foraging activity of the bees: 3 days before to 7 days after application
- Behavioural abnormalities: 3 days before to 42 days after application
- Colony assessments (including assessments of brood status (food stores, colony strength and hive populations): once before application on day – 3 and on days 7, 14, 21, 28, 34, 42.
- Results of residue analysis

Findings:

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 low with a mean of 10.9 dead bees per day and colony, which was not statistical significant 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 statistical significant

different to the control. 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.
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 statistical significant 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) so that these statistical detections are seen to be of no biological relevance.

Treatment group		Control	Test item	Reference Item
Daily mean mortality (dead worker bees/colony) ± STD	3DBA to 1DBA	43.2 ± 14.7	31.2 ± 9.7	42.6 ± 15.4
	0DBA	35.3 ± 12.2	32.0 ± 6.9	22.0 ± 10.8
	0DAA	17.8 ± 6.4	23.3 ± 3.3	1287.5 ± 234.6
	0DAA to 7DAA	42.6 ± 23.9	36.8 ± 17.5	246.1 ± 430.5
	8DAA to 21DAA	14.6 ± 9.7	10.9 ± 7.9	5.5 ± 5.0
	22DAA to 42DAA	5.8 ± 2.6	6.6 ± 1.6	4.6 ± 1.5
	0DAA to 42DAA	15.3 ± 6.6	13.5 ± 2.1	48.8 ± 4.9

DBA = Days before application, DAA = Days after application

<u>Foraging activity</u>
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.

Treatment group		Control (C)	Test item (T)	Reference Item (R)
Daily mean foraging activity (bees/m²) ± STD	3DBA to 1DBA	13.0 ± 8.4	13.3 ± 10.5	13.8 ± 11.0
	0DBA	12.8 ± 1.9	12.2 ± 1.1	13.7 ± 0.9
	0DAA	18.4 ± 3.2	17.0 ± 1.4	0.1 ± 0.1
	0DAA to 7DAA	16.8 ± 7.8	16.3 ± 7.7	0.1 ± 0.1*

DAA = days after application, DBA = days before application
* = Statistically significant lower than control group

<u>Behavioural abnormalities</u>
No behavioural abnormalities occurred in the test item treated group at any assessment day.

<u>Strength of the colonies</u>
The mean number of honey bees per colony in all treatment groups was similar three days before application and did not differ statistically significantly (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 have been observed throughout the study.

<u>Development of the brood area</u>
At the beginning of the trial all colonies to be used for the test were similar according to the season. 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.

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. 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 and the day after 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.

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

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.
No 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.

Report:	KCP 10.3.1.5/04; [redacted], A.; 2017; M-607771-01-1
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
Document No.:	M-607771-01-1
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
Guideline deviation(s):	None
GLP/GEP:	**yes**

Objective:

The purpose of the study was to investigate potential side effects of a spray application of Isoflucypram EC 50 on the honeybee (*Apis mellifera* L.) under semi-field conditions in Spain by following OEPP/EPPO Guideline No. 170(4).

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.: 102000003162; density: 0.975 g/mL.
Test species: 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.
The study design comprised one of three treatment groups in total, one being treated with the test item (T), one treated with the reference item dimethoate (R) and one treated with tap water, each with four replicates (tunnels). Applications were made at full flowering (BBCH 65) and during daily bee flight.
The test item Isoflucypram 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 400 g a.s./ha in 400 L water/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 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.

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

Findings:

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. The mean daily mortality values were approximately on the same level in the treatment groups C and T throughout this period 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. However, this statistical significance was not considered to be of any biological relevance since the mean mortality rates for the post-application period in C and T were on the same level. This conclusion is supported by the fact that no statistically significantly higher values compared to the control group were observed when calculated for single observation days during the post-application period. 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.

The application of the reference item in treatment R had a clear effect on honey bee mortality. During the post-application period (0DAA to 7DAA), mortality was in the range from 51.0 to 890.5 dead honey bees/colony/day (mean: 307.2 dead honey bees/day) 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.

Treatment group		Control	Test item	Reference Item
Daily mean mortality (dead worker bees, larvae and pupae/colony) ± STD	3DBA to 0DBA	17.0 ± 7.0	38.5 ± 29.2	33.9 ± 7.7
	0DBA to 7DAA	14.8 ± 7.4	24.1 ± 19.8	307.2* ± 36.5
	0DAA to 43 DAA	3.7 ± 1.8	5.2 ± 3.6	62.0* ± 14.2
	8DAA to 43 DAA	1.2 ± 0.7	1.1 ± 0.4	7.6 ± 13.0

DBA = Days before application, DAA = Days after application
* Statistically significantly higher compared to the control

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. A statistically significantly different value in T compared to the control group was observed directly before the application on 0DBA. However, this statistical significance was not considered to be of any biological relevance since the mean flight intensities for the pre- and post-application period in C and T were on the same level (for detailed values see further below). This conclusion is supported by the fact that no statistically significantly lower values compared to the control group were observed when calculated for single observation days during the post-application period.

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.
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) and a clear impact was observed in the reference item treatment R after the application.

Treatment group		**Control (C)**	**Test item (T)**	**Reference Item (R)**
Daily mean flight intensity (bees/m²) ± STD	3DBA to 0DBA	21.5 ± 1.2	18.1 ± 2.3	16.3* ± 3.4
	0DBA	28.5 ± 4.5	26.1 ± 2.3	3.3** ± 1.6
	1DAA	23.3 ± 1.4	24.6 ± 7.2	0.0** ± 0.0
	0DAA to 7DAA	28.4 ± 1.9	27.5 ± 4.6	0.5** ± 0.2

DBA = Days before application; DAA = Days after application
* = Statistically significantly different compared to the control
** = Statistically significantly lower compared to the control

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

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). 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.

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.
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.

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.

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: 128 - 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 biologically relevant 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.

CP 10.3.1.6 Field tests with honeybees

Not necessary when considering the outcome of the risk assessment and the results of the lower-tiered studies.

CP 10.3.2 Effects on 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[2]).

Table 10.3.2- 1: Ecotoxicological endpoints for non-target arthropods

Test species, Dossier-file-No. Reference	Tested Formulation, study type, exposure	Ecotoxicological Endpoint
Aphidius rhopalosiphi [redacted]; 2017; M-593743-01-1 Rep.No: CW16/036 KCP 10.3.2.1/01	Isoflucypram EC 50 Laboratory, glass plates	LR_{50} 14.13 g a.s./ha Corr. Mortality [%]
	7.5 g a.s./ha	10.2
	13.3 g a.s./ha	32.2
	23.7 g a.s./ha	100.0
	42.2 g a.s./ha	100.0
	75.0 g a.s./ha	100.0
Typhlodromus pyri [redacted]; 2017; M-593747-01-1 Rep.No: CW16/035 KCP 10.3.2.1/02	Isoflucypram EC 50 Laboratory, glass plates	LR_{50} 30.6 g a.s./ha Corr. Mortality [%]
	7.5 g a.s./ha	2.3
	13.3 g a.s./ha	26.9[A]
	23.7 g a.s./ha	27.6
	42.2 g a.s./ha	80.5
	75.0 g a.s./ha	96.6
Aphidius rhopalosiphi [redacted]; 2017; M-583441-01-1 Rep.No: CW16/038 KCP 10.3.2.2/01	Isoflucypram EC 50 Extended Lab., exposure on barley seedlings	LR_{50} > 75 g a.s./ha; ER_{50} 17.8 g a.s./ha Corr. Mortality [%] Effect on Reproduction [%] Wasps on plants [%]
	7.5 g a.s./ha	0 45.8 34.7
	13.3 g a.s./ha	0 53.8 21.5
	23.7 g a.s./ha	6.7 77.0 19.3
	42.2 g a.s./ha	6.7 62.8 25.2
	75 g a.s./ha	3.3 60.6 18.2
Typhlodromus pyri [redacted]; 2017; M-608958-01-1 Rep.No: CW16/037 KCP 10.3.2.2/02	Isoflucypram EC 50 Extended Lab., exposure on bean leaves	LR_{50} > 75 g a.s./ha; ER_{50} > 42.2 g a.s./ha Corr. Mortality [%] Effect on Reproduction [%]
	7.5 g a.s./ha	6.7 44.4
	13.3 g a.s./ha	9.0 31.3
	23.7 g a.s./ha	4.5 19.2
	42.2 g a.s./ha	19.9 21.2
	75 g a.s./ha	10.1 64.0

[2] 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		
Chrysoperla carnea	Isoflucypram EC 50	LR_{50} > 75 g a.s./ha; no effects on reproduction		
[redacted]; 2017;	Extended Lab., exposure on detached bean leaves	Corr. Mortality [%]	Eggs/Female/Day	Hatching [%]
M-601137-01-1	7.5 g a.s./ha	-5.3[A]	18.3	75.7
Rep.No: CW16/039	13.3 g a.s./ha	13.2	22.0	79.6
KCP 10.3.2.2/03	23.7 g a.s./ha	0.0	23.0	83.2
	42.2 g a.s./ha	13.2	21.7	79.7
	75 g a.s./ha	10.5	20.0	82.7
Coccinella septempunctata	Isoflucypram EC 50	LR_{50} > 75 g a.s./ha; no effects on reproduction		
[redacted], R. U.; 2017;	Extended Lab., exposure on detached bean leaves	Corr. Mortality [%]	Eggs/Female/Day	Hatching [%]
M-608806-01-1	7.5 g a.s./ha	-5.3[A]	8.1	93.4
Rep.No: CW17/010	13.3 g a.s./ha	-2.2[A]	8.8	97.8
KCP 10.3.2.2/04	23.7 g a.s./ha	-11.8[A]	7.0	96.5
	42.2 g a.s./ha	2.9	6.9	93.5
	75 g a.s./ha	-2.9[A]	7.6	95.9
Aphidius rhopalosiphi	Isoflucypram EC 50			
[redacted], D.; 2017; M-600692-01-1 Rep.No: CW17/014 KCP 10.3.2.2/05	aged residues spray deposits on maize plants, 1 appl. of 75 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 exposure scenario is based on the use pattern as given in Table 10- 1. 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

Application rates: 1.5 L product/ha (= 75 g a.s./ha)
MAF (multiple application factor) = 1.0 (1 application)

Table 10.3.2- 2: Exposure calculation for in-field assessment

Crop / no. of applications	Appl. rate [g a.s./ha]	MAF	in-field PEC_{max} [g a.s./ha]
Cereals / 1	75	1.0	75

Table 10.3.2- 3: Exposure calculation for the off-field scenario

Crop / no. of applications	Appl. rate [g a.s./ha]	MAF	Drift [%]	VDF	Correction factor	Off-field PEC_{max} [g a.s./ha]
Cereals / 1	75	1.0	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[3]).

Tier 1 in-field risk assessment for non-target arthropods

Table 10.3.2- 4: Tier 1 in-field risk assessment for non-target arthropods

Crop	Species	In-field $PEC_{max.}$ [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. Therefore, a Tier 2 risk assessment is presented with the two standard species and the two additional species *Chrysoperla carnea* and *Coccinella septempunctata.*

Table 10.3.2- 5: Tier 2 in-field risk assessment for non-target arthropods

Crop	Species	In-field $PEC_{max.}$ [g a.s./ha]	ER_{50} [g a.s./ha]	Risk acceptable if:	Refined assessment required?
Cereals	*A. rhopalosiphi*	75	17.8	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.

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

Refined in-field risk assessment for *Aphidius rhopalosiphi*

An extended aged residue laboratory study was performed with the most sensitive species *Aphidius rhopalosiphi.* 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). After exposure to aged residues (14DAT1) a reduction in reproduction of 13.8% was observed which was not statistically significantly different to the control. Since effects on mortality and reproduction dropped below 14% after aging of the residues for 14 days it can be concluded that the potential for recovery is given within two weeks after the application. Therefore, no unacceptable effects on non-target arthropods in the in-field area are expected from the intended use of isoflucypram EC 50.

Tier 1 off-field risk assessment for non-target arthropods

Table 10.3.2- 6: Tier 1 off-field risk assessment for non-target arthropods

Crop	Species	Off-field PEC_{max} [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.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

Report: KCP 10.3.2.1/01; [redacted]; 2017; M-593743-01-1
Title: Toxicity to the parasitoid wasp Aphidius rhopalosiphi (Hymenoptera: Braconidae) using a laboratory test BCS-CN88460 EC 50 g/L
Report No.: CW16/036
Document No.: M-593743-01-1
Guideline(s): EU Directive 91/414/EEC
Regulation (EC) No. 1107/2009
US EPA OCSPP Not Applicable
MEAD-BRIGGS ET AL. (2000)
CANDOLFI ET AL. (2001)
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:

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

Material and methods:

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

The test item was applied on glass plates at rates of 7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha in 200 L deionised water/ha using a calibrated laboratory track sprayer (mean measured application rate: 198 L/ha). The effects of the test item on the parasitoid wasp *Aphidius rhopalosiphi* were compared to those of a deionised water treated control. A reference item (active substance: dimethoate) applied at 0.04 g a.s./ha in 200 L deionised water/ha was included.
Mortality of 60 adult wasps, not older than 48 h at study start (4 replicates with 15 wasps per test group), was assessed 2, 24 and 48 h after exposure (food = feeding solution which consisted of 3 parts of water + 1 part of honey).

The climatic test conditions during the study were 19.5 - 20.5 °C temperature and 65 - 83% relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 930 - 1250 Lux.

The LR_{50} value (lethal rate causing 50% mortality) was calculated by Spearman-Karber method.
The computer program SAS (Version 9.4) was used to perform the statistical analyses.

Findings:

In this laboratory test the effects of BCS-CN88460 EC 50 g/L residues on the survival of *Aphidius rhopalosiphi* were determined at 7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha, applied to glass plates.
After 48 h of the study 1.7% of the wasps were found dead in the control group. In all test item rates a statistically significant mortality was found (Fisher`s Exact test (one-sided, $\alpha = 0.05$).
In the test item rates of 7.5 and 13.3 g a.s./ha, a corrected mortality of 10.2% and 32.2% occurred, respectively. In all higher test item rates of 23.7, 42.2 and 75.0 g a.s./ha, the corrected mortality was 100%.

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

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

	Validity criteria	**Finding**
Mortality in water control	≤ 13%	**1.7%**
Corrected mortality reference substance	≥ 50%	**100%**

Conclusion:

The LR_{50} was calculated to be 14.13 g a.s./ha. The NOER for mortality was < 7.5 g a.s./ha.
The figures obtained fulfil the validity criteria of the laboratory method using glass plates (MEAD-BRIGGS ET AL., 2000).

Report:	KCP 10.3.2.1/02; [redacted]; 2017; M-593747-01-1
Title:	Toxicity to the predatory mite Typhlodromus pyri (Acari: Phytoseiidae) using a laboratory test BCS-CN88460 EC 50 g/L
Report No.:	CW16/035
Document No.:	M-593747-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable BLÜMEL ET AL. (2000) CANDOLFI ET AL. (2001)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

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

Material and Methods:

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

The test item was applied onto glass plates at rates of 7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha in 200 L deionised water/ha using a calibrated laboratory track sprayer (mean measured application rate: 189 L/ha). 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 5.0 g a.s./ha in 200 L deionised water/ha was included.
Mortality of 100 predatory mites, protonymphs at study start (5 replicates of 20 individuals per test group), was assessed 4 and 7 days after exposure by counting the number of living and dead mites (food = pollen mixture, one part birch ; one part pine). The number of escaped mites was calculated as the difference from the total number exposed.
The climatic test conditions during the study were 24.0 - 25.5 °C temperature and 60 - 72% relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 119 - 540 Lux.
The LR_{50} value (lethal rate causing 50% mortality) was calculated by Probit analysis.
The computer program SAS (Version 9.4) was used to perform the statistical analyses.

Findings:

In this laboratory test the effects of BCS-CN88460 EC 50 g/L residues on the survival of the predatory mite *Typhlodromus pyri* were determined at the rates of 7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha applied to glass cover slides.
The mortality / escaping rate in the control exposure units up to day 7 after treatment was 13.0%.
In the higher test item rates of 23.7, 42.2 and 75.0 g a.s./ha, a statistically significantly different mortality compared to the control was found (Fisher`s Exact test, one-sided). At the lower test item rates of 7.5 and 13.3. g a.s/ha, a corrected mortality of 2.3% and -6.9% has been observed, respectively. At the test item rates of 23.7, 42.2 and 75.0 g a.s./ha, the corrected mortality was 27.6%, 80.5% and 96.6%, respectively.
A summary of the effects observed in this study is given below.

Test item		**BCS-CN88460 EC 50 g/L**		
Test organism		***Typhlodromus pyri***		
Exposure on		**Glass plates**		
		Mortality after 7 days [%]		
Treatment	**g a.s./ha**	**Uncorrected**	**Corrected (*)**	**P-Value (**)**
Control	0.0	13.0		
Test item	7.5	15.0	2.3	0.839 not significant
Test item	13.3	7.0	-6.9	0.951 not significant
Test item	23.7	37.0	27.6	<0.001 significant
Test item	42.2	83.0	80.5	<0.001 significant
Test item	75.0	97.0	96.6	<0.001 significant
Reference item	5.0	95.0	94.3	

LR_{50}: 30.6 g a.s./ha; 95 % Confidence Interval: 27.0 - 34.3 (calculated with Probit analysis)
* Corrected mortality according to SCHNEIDER-ORELLI (1947)
** Fisher`s Exact test (one-sided), p-values are adjusted according to Bonferroni-Holm

	Validity criteria	**Finding**
MortEsc.-rate in the control group on day 7	≤ 20%	**13.0%**
Average corr. mortality in the reference item	≥ 50%	**94.3%**

Conclusion:

The LR_{50} was calculated to be 30.6 g a.s./ha. The NOER for mortality was ≥ 13.3 g a.s./ha.
The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates (BLÜMEL ET AL., 2000).

CP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

Report: KCP 10.3.2.2/01; [redacted], 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; US EPA OCSPP not applicable
Guideline deviation(s): none
GLP/GEP: **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).

The test item was applied on barley seedlings (*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 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.

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.
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.

Findings:

In this extended laboratory test the effects of BCS-CN88460 EC 50 residues on the survival of *Aphidius rhopalosiphi* were determined at 7.5, 13.3, 23.7, 42.2 and 75.0 g a.s./ha, applied to barley seedlings (*Hordeum vulgare*).
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.
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. No further repellent effects were observed after 24 h.
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 the following table.

Test item		**BCS-CN88460 EC 50**							
Test organism		***Aphidius rhopalosiphi***							
Exposure on		**Barley seedlings**							
		Mortality after 48 h [%]			**Reproduction**			**Repellency (first 24 h)**	
Treat-ment	**g a.s./ha**	**un-corr.**	**corr.**	**P-Value (*)**	**Rate (mummies per female)**	**Reduc-tion 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	0.000 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)
* 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

	Validity criteria	**Finding in study**
Mortality in water control	≤ 10%	0%
Corrected mortality reference item	≥ 50%	87%
Mean reproduction per female in water control	≥ 5	31
Number of wasps in the water control producing zero values for reproduction	≤ 2	0

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 17.8 g a.s./ha. 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).

Report:	KCP 10.3.2.2/02; [redacted]; 2017; M-608958-01-1
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/GEP:	**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).

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.

Mortality of 100 predatory mites, protonymphs at study start (5 replicates with 20 individuals per test group), was assessed on day 4 and 7 after exposure by counting the number of living and dead mites (food = pollen mixture, one part birch : one part pine). The number of escaped mites was calculated as the difference from the total number exposed.
The reproduction rate of surviving mites in the control and test item group was then evaluated on day 7, 10, 12 and 14 after application by counting the total number of offspring (eggs and larvae) produced.
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.

Findings:

In this extended laboratory test the effects of BCS-CN88460 EC 50 g/L residues on the survival of the predatory mite *Typhlodromus pyri* 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*).
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.
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, α = 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, α = 0.05).

Test item		**BCS-CN88460 EC 50**					
Test organism		***Typlodromus pyri***					
Exposure on		**Detached bean leaves**					
		Mortality after 7 days [%]			**Reproduction**		
Treat-ment	**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, α = 0.05)
n.a. = not assessed, n. sign = not significant, sign. = significant.

	Validity criteria	**Finding in study**
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**

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).

Report:	KCP 10.3.2.2/03; [redacted]; 2017; M-601137-01-1
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/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 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).

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

Findings:

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 (Fisher`s Exact test, one-sided) in all test item rates with a corrected mortality below 14%.
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).

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 %]
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
Test item	13.3	17.5	13.2	0.386 n.sign.	22.0	79.6
Test item	23.7	5.0	0.0	1.000 n.sign.	23.0	83.2
Test item	42.2	17.5	13.2	0.386 n.sign.	21.7	79.7
Test item	75.0	15.0	10.5	0.395 n.sign.	20.0	82.7
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

	Validity criteria	Finding
Mortality in water control	≤ 20%	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%

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 reproductive performance was not 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).

Report: KCP 10.3.2.2/04; [redacted], R. U.; 2017; M-608806-01-1
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).

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.
The preimaginal mortality of 40 larvae, 4 days old at study start (per test group), was assessed till the hatch of the imagines up to 15 days (food = *Acyrthosiphon pisum*). 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.
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. The computer program SAS (Version 9.4) was used to perform the statistical analyses.

Findings:

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*).
All test item rates of BCS-CN88460 EC 50 g/L had no or only slight influence on the preimaginal mortality.
Reproduction was assessed for all rates of BCS-CN88460 EC 50 g/L. There were no adverse effects of the test item rates of 7.5, 13.3, 23.7, 42.2 and 75 g a.s./ha on the reproductive performance. The mean number of fertile eggs per female and day was above the lower limit given as validity criterion. Since the reproductive performance was within the range of the historical data base for control beetles (≥ 2 fertile eggs per female and day; SCHMUCK ET AL. 2000) this parameter is considered as not affected at all test item rates.

Test item		BCS-CN88460 EC 50 g/L				
Test organism		*Coccinella septempunctata*				
Exposure on		Detached bean leaves				
		Preimaginal mortality [%]			Reproduction	
Treat-ment	g a.s./ha	uncorrected	corrected (*)	P-Value (**)	Fertile eggs per female and day	
Control	0.0	15.0			10.0	
Test item	7.5	10.5	-5.3	1.000 n.sign.	8.1	
Test item	13.3	13.2	-2.2	1.000 n.sign.	8.8	
Test item	23.7	5.0	-11.8	1.000 n.sign.	7.0	
Test item	42.2	17.5	2.9	1.000 n.sign.	6.9	
Test item	75.0	12.5	-2.9	1.000 n.sign.	7.6	
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, α=0.05), p-values are adjusted according to Bonferroni-Holm
n.a. not assessed n.sign. not significant

	Validity criteria	Finding
Preimaginal mortality in water control	≤ 30%	15.0%
Preimaginal mortality reference item	≥ 40%	97.5%
Mean number of fertile eggs per female and day in water control	≥ 2	10.0

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 is not considered to be impacted by the test item.
The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates (SCHMUCK ET AL. 2000).

Report:	KCP 10.3.2.2/05; [redacted], D.; 2017; M-600692-01-1
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)
Guideline deviation(s):	none
GLP/GEP:	**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).

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. 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).

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 (0DAT = 0 days after treatment) and the last two weeks later (2DAT1).

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 infested with *Rhopalosiphum padi* for a period of 24 h. 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).

Findings:

In this extended laboratory test the effects of Isoflucypram EC 50 g/L residues (fresh and aged under semi-field conditions, with rain protection during the whole study) on the parasitoid wasp *Aphidius rhopalosiphi* were determined after an application of 75 g a.s./ha onto maize plants (*Zea mays*).

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).

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)

	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

Conclusion:

Both bioassays (started on 0DAT1 and 14DAT1) resulted in a corrected mortality of < 50% as well a reduction of reproduction of < 50%. The figures obtained fulfil the validity criteria of the extended laboratory method (MEAD-BRIGGS ET AL., 2010).

CP 10.3.2.3 Semi-field studies with non-target arthropods

In view of the results presented above, no semi-field studies were deemed necessary.

CP 10.3.2.4 Field studies with non-target arthropods

In view of the results presented above, no semi-field studies were deemed necessary.

CP 10.3.2.5 Other routes of exposure for non-target arthropods

No relevant exposure of non-target arthropods is expected by other routes of exposure.

CP 10.4 Effects on non-target soil meso- and macrofauna

The risk assessment procedure follows the requirements as given in the Council Directive 91/414/EEC (Annex III), Council Directive 97/57/EC (Annex VI) and the Guidance Document on Terrestrial Ecotoxicology.

Predicted environmental concentrations used in risk assessment

For details of PEC_{soil} calculations refer to MCP Summary Section 9, Point 9.1.3.

Table 10.4- 1: PEC_{soil} values for isoflucypram, its metabolite BCS-CN88460-carboxylic acid (M12) and the product Isoflucypram EC 50 (for details see MCP Section 9, Point 9.1.3)

Compound	Cereals (1 × 75 g a.s./ha)		
	$PEC_{soil,\ initial}$ [mg/kg]	$PEC_{soil,\ plateau,\ 20\ cm}$ [mg/kg]	$PEC_{soil,\ accu}$*
Isoflucypram	0.020	0.010	0.030
BCS-CN88460-carboxylic acid (M12)	0.002	< 0.001	0.002
Isoflucypram EC 50	0.390 [1)]	-	-

* $PEC_{soil,\ accu}$ means the sum of $PEC_{soil,\ initial}$ and $PEC_{soil,\ plateau}$

[1)] The PEC_{soil} value for the product Isoflucypram EC 50 is calculated based on the initial rate of the product (1.5 L/ha) in a single application, the portion reaching soil (BBCH 30-69, interception of 80%), the standard soil density (1.5 g/cm^3), the standard soil depth (5 cm) and the density of the formulation (0.974 g/mL).

The Tier 1 risk assessments are based on worst case PEC_{soil} values for the application in cereals.

CP 10.4.1 Earthworms

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.

Table 10.4.1- 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 ≥ 280 mg prod./kg dws* ≥ 14.5 mg a.s./kg dws*# EC_{10} not calculable[1)]	[redacted]; 2016; M-574897-01-1 KCP 10.4.1.1/01
Isoflucypram	*Eisenia fetida* reproduction 56 d, mixed	NOEC ≥ 163 mg a.s./kg dws* EC_{10} not calculable[1)]	[redacted]; 2016; M-548749-01-1 KCA 8.4.1/01
BCS-CN88460-carboxylic acid (M12)	*Eisenia fetida* reproduction 56 d, mixed	NOEC 50 mg p.m./kg dws* EC_{10} not calculable[1)]	[redacted]; 2017; M-579263-01-1 KCA 8.4.1/02

dws = dry weight soil; a.s. = active substance; p.m. = pure metabolite.
*Endpoint corrected due to lipophilic substance (log P_{ow} > 2)
[#] Endpoint calculated on the basis of analysed isoflucypram content in the formulation (5.18% w/w; as given in study report)
1) for details see study summaries

Risk assessment for earthworms

Table 10.4.1- 2: TER calculation for earthworms for the product Isoflucypram EC 50

Compound	Species, study type	Endpoint [mg prod./kg]	PEC_{soil} [mg prod./kg]	TER_{LT}	Trigger
Isoflucypram EC 50	Earthworm, reproduction	NOEC ≥ 280	0.390	≥ 718	5

Table 10.4.1- 3: TER calculations for earthworms for isoflucypram and its metabolite BCS-CN88460-carboxylic acid (M12)

Compound	Species, study type	Endpoint [mg/kg]	$PEC_{soil,max}$ [mg/kg]	TER_{LT}	Trigger
Isoflucypram	Earthworm, reproduction	NOEC ≥ 163	0.030	≥ 5433	5
BCS-CN88460-carboxylic acid (M12)	Earthworm, reproduction	NOEC 50	0.002	25000	5

The TER values clearly 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.

CP 10.4.1.1 Earthworms sub-lethal effects

Report:	KCP 10.4.1.1/01; [redacted]; 2016; M-574897-01-1
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
Document No.:	M-574897-01-1
Guideline(s):	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP Not Applicable
Guideline deviation(s):	minor deviations
GLP/GEP:	**yes**

Objective:

The purpose of this study was to assess the effect of BCS-CN88460 EC 50 G (Isoflucypram EC 50) on survival and reproduction of the earthworm *Eisenia fetida* in artificial soil. The test was performed according to the International Standard ISO 11268-2 (1998) and OECD 222 (April 13, 2004).

Material and methods:

Test item: BCS-CN88460 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.

Adult *Eisenia fetida*, approx. 7 months old, 8 x 10 earthworms for the control group and 4 x 10 animals per test concentration of the treatment groups, were exposed to control and treatment. Non-reusable plastic boxes (length x width x height ca. 16.5 cm × 12 cm × 6 cm, area approximately 200 cm^2) were used as test vessels. Nominal test concentrations of 10, 18, 32, 56, 100, 180, 320 and 560 mg test item/kg dry weight artificial soil were mixed into the artificial soil. During the study they were fed with animal manure. A temperature of 20 ± 2 °C and a light regime of 400 – 800 lux, 16 h light and 8 h dark during the conduct of the study were applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 70% fine quartz sand, 10% Sphagnum peat, air dried and finely ground, 20% Kaolin clay. Each test vessel contained an amount of approximately 500 g artificial soil (dry weight) to obtain a depth of approximately 5 cm soil in the test vessels. After 28 days the number of surviving adult earthworms and their weight alteration was determined. Therefore they were removed from the artificial soil. After further 28 days, the number of offspring was determined.

Findings:

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 (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.

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	132.5	151.8	127.8	124.5
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	25.4	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		
LOEC [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 Binomial Test, one-sided greater, α = 0.05), + significant, - not significant
** (William's t-test, two-sided, α = 0.05), + = significant, - = not significant
*** (William's t-test, one-sided smaller, α = 0.05), + significant, - not significant
n.d. – could not be determined, see observations and conclusions.

Experimental conditions:
The pH values measured in the control and the treatments ranged from 5.87 to 6.14 at test start and from 6.03 to 6.14 at test end. The water content during the whole study was between 49.14 to 60.82 of WHC_{max}.

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 most recent toxic standard reference test, with the reference test item mixed into the artificial soil, was performed from August 25 to November 19, 2015 (Report No. kra-Rg-R-Ref 26/15; NON-GLP). Effects on mortality and growth of the adults after an exposure period of 28 days and the number of offspring after 56 days were determined.
No mortality of the adult earthworms was observed 28 days after application. The change of body weight of the adult earthworms of the test concentration of 5.0 mg a.s./kg dry weight soil was statistically significant reduced in comparison to the control (results of a Williams multiple sequential t-test, two-sided, α = 0.05). 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, α = 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.

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 test item/kg dry weight soil. Thus, the overall LOEC is determined to be > 560 mg test item/kg dry weight soil. Due to the lack of a clear concentration-response relationship no reliable $EC_{10/20}$ calculation was possible.

CP 10.4.1.2 Earthworms field studies

In view of the results presented above, no field studies were necessary.

CP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

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. In case EC_{10} values were lower than the NOEC and the calculation was reliable they were used for the calculations of TER values.

Table 10.4.2- 1: 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 EC_{10}	50 mg prod./kg dws* 2.59 mg a.s./kg dws*# 49 mg prod./kg dws*	[redacted]; 2017; M-591834-01-1 KCP 10.4.2.1/01
Isoflucypram	*Folsomia candida* reproduction 28 d, mixed	NOEC EC_{10}	49.5 mg a.s./kg dws* not calculable[1)]	[redacted]; 2015; M-522863-01-1 KCA 8.4.2.1/01
BCS-CN88460-carboxylic acid (M12)	*Folsomia candida* reproduction 28 d, mixed	NOEC EC_{10}	9 mg p.m./kg dws* 6.7 mg p.m./kg dws*	[redacted]; 2017; M-587760-01-1 KCA 8.4.2.1/02
Soil mites, reproduction				
Isoflucypram EC 50	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC EC_{10}	158 mg prod./kg dws* 8.18 mg a.s./kg dws*# 181 mg prod./kg dws*	[redacted] 2017; M-592571-01-1 KCP 10.4.2.1/02
Isoflucypram	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC EC_{10}	≥ 495 mg a.s./kg dws* not calculable[1)]	[redacted]; 2015; M-528194-01-1 KCA 8.4.2.1/03
BCS-CN88460-carboxylic acid (M12)	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC EC_{10}	≥ 495 mg a.s./kg dws* not calculable[1)]	[redacted]; 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)
1) for details see study summaries

Risk assessment for non-target soil meso- and macrofauna (other than earthworms)

Table 10.4.2- 2: TER calculations for the product Isoflucypram EC 50 for other non-target soil meso- and macrofauna

Compound	Species	Endpoint [mg prod./kg]		PEC_{soil} [mg prod./kg]	TER	Trigger
Isoflucypram EC 50	*Folsomia candida*	EC_{10}	49	0.390	126	5
Isoflucypram EC 50	*Hypoaspis aculeifer*	NOEC	158	0.390	405	5

Table 10.4.2- 3: TER calculations for Isoflucypram and its metabolite BCS-CN88460-carboxylic acid (M12) for other non-target soil meso- and macrofauna

Compound	Species	Endpoint [mg/kg]		$PEC_{soil,max}$ [mg/kg]	TER_{LT}	Trigger
Isoflucypram, a.s.	*Folsomia candida*	NOEC	49.5	0.030	1650	5
BCS-CN88460-carboxylic acid (M12)	*Folsomia candida*	EC_{10}	6.7	0.002	3350	5
Isoflucypram, a.s.	*Hypoaspis aculeifer*	NOEC	≥ 495	0.030	≥ 16500	5
BCS-CN88460-carboxylic acid (M12)	*Hypoaspis aculeifer*	NOEC	≥ 495	0.002	≥ 247500	5

Endpoint calculated on the basis of analysed isoflucypram content in the formulation (5.18% w/w; as given in study report)

All TER values clearly 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.

CP 10.4.2.1 Species level testing

Report: KCP 10.4.2.1/01; [redacted]; 2017; M-591834-01-1
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
Document No.: M-591834-01-1
Guideline(s): EU Directive 91/414/EEC
Regulation (EC) No. 1107/2009
US EPA OCSPP Not Applicable
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:

The purpose of this study was to assess the effect of BCS-CN88460 EC 50 (Isoflucypram EC 50) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Material and methods:

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)
10 collembolans (10-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatment. Concentrations of 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil were mixed into the artificial soil. During the study, they were fed with granulated dry yeast. A temperature of 20 ± 2 °C and a light regime of 400 – 800 lux, 16 h light : 8 h darkness during the conduct of the study was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75% fine quartz sand, 5% Sphagnum peat, air dried and finely ground, 20% Kaolin clay. Mortality and reproduction were determined after 28 days.

Findings:

Biological results:

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
LOEC (mg test item/kg dry weight artificial soil)				562	178
				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, α = 0.05, + = significant, - = not significant)
(**) = (Bonferroni-Welsh-t-test; t-test, one-sided smaller, α = 0.05, + = significant, - = not significant)
1) Mortality = Weibull analysis; reproduction = Probit analysis
2) = Evaluation with 3 replicates

Observations:

Mortality

In the control group 8.8% of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20% 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 Lowest-Observed-Effect-Concentration (LOEC) for mortality is 562 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, α = 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 Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 178 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.

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

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

<u>Validity criteria:</u>

All validity criteria were met in this study.

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%

<u>Toxic reference test:</u>
The most recent non-GLP-test (LAR-Coll-Ref-28/16, Maria Ivonne [redacted], November 16, 2016) with the reference item Boric acid was performed at test concentrations 44, 67, 100, 150, 225 mg Boric acid/kg dry weight artificial soil. 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).
The $NOEC_{reproduction}$ was calculated to be < 44 mg Boric acid/kg dry weight artificial soil and accordingly the $LOEC_{reproduction}$ is 44 mg Boric acid/kg dry weight artificial soil according Welsh-t-test after Bonferroni-Holm, α = 0.05, one-sided smaller. This shows that test organisms are sufficiently sensitive.

Conclusions:

$NOEC_{mortality}$:	316 mg test item/kg dry weight artificial soil
$LOEC_{mortality}$:	562 mg test item/kg dry weight artificial soil
$NOEC_{reproduction}$:	100 mg test item/kg dry weight artificial soil
$LOEC_{reproduction}$:	178 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

Report:	KCP 10.4.2.1/02; [redacted]; 2017; M-592571-01-1
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
Document No.:	M-592571-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP: Not Applicable
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of this study was to assess the effect of BCS-CN88460 EC 50 G (Isoflucypram EC 50) on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil by comparing control and treatment.

Material and methods:

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)).
Ten adult, fertilized female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatments. Concentrations of 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil were mixed into the artificial soil. During the test, the *Hypoaspis aculeifer* were fed with nematodes bred on watered oat flakes. During the study a temperature of 20 ± 2 °C and a light regime of 400 – 800 Lux, 16 h light : 8 h dark were applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75% fine quartz sand, 5% Sphagnum peat, air dried and finely ground, 20% Kaolin clay.
After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20% ethylene glycol, 80% deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Findings:

Biological results**:**

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	-	185.5 ± 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
LOEC (mg test item/kg dry weight artificial soil)	>1000	562
EC_{10} mg test item/kg dry weight artificial soil1) (95% confidence limits)		362 (349 – 375)
EC_{20} mg test item/kg dry weight artificial soil1) (95% confidence limits)		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

Observations:

Mortality

In the control group 3.8% of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20% 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. The Lowest-Observed-Effect-Concentration (LOEC) 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 Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 562 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.

Experimental conditions:
All values were within the range recommended by the guideline.

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

Validity criteria:

All validity criteria were met in this study.

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%

Toxic reference test:
The most recent non-GLP-test (Maria Ivonne [redacted], LAR HR-O-24/16, August 09, 2016) with the reference item dimethoate was performed at test concentrations of 1.0, 1.8, 3.2, 5.6 and 10 mg dimethoate/kg dry weight artificial soil.
Dimethoate showed a LC_{50} of 1.8 mg a.s./kg for mortality of the adult mites according Probit analysis using maximum likelihood regression (confidence limits from 1.6 mg a.s./kg to 2.0 mg a.s./kg).
The reproduction of the soil mites was not significantly reduced in comparison to the control up to and including 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg dry weight artificial soil and accordingly the LOEC is 5.6 mg a.s./kg dry weight artificial soil. Since variances of the data were homogenous, Williams t-test, $\alpha = 0.05$, one-sided smaller was used. 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.

Conclusion:
$NOEC_{adult\ mortality}$: ≥1000 mg test item/kg dry weight artificial soil
$LOEC_{adult\ mortality}$: >1000 mg test item/kg dry weight artificial soil

$NOEC_{reproduction}$: 316 mg test item/kg dry weight artificial soil
$LOEC_{reproduction}$: 562 mg test item/kg dry weight artificial soil

EC_{10}-$_{reproduction}$: 362 mg test item/kg dry weight artificial soil
EC_{20}-$_{reproduction}$: 422 mg test item/kg dry weight artificial soil

CP 10.4.2.2 Higher tier testing

In view of the results presented in Section CP 10.4.2, no further testing is necessary.

CP 10.5 Effects on soil nitrogen transformation

Table 8.5 - 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)	[redacted], 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)	[redacted]; 2015; M-532055-01-1 KCA 8.5/01
BCS-CN88460-carboxylic acid (M12)	Study duration, 28 days	no unacceptable effects at an application rate of 0.54 mg a.s./kg soil (403 g p.m./ha)	[redacted], 2015; M-538059-01-1 KCA 8.5/02

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

Risk assessment for Soil Nitrogen Transformation

Table 8.5 - 2: Risk Assessment for the product Isoflucypram EC 50 for soil micro-organisms

Compound	Species	Endpoint [mg prod./kg]	$PEC_{soil,max}$ [mg prod./kg]	Refinement required
Isoflucypram EC 50	Soil micro-organisms	9.74	0.390	No

Table 8.5 - 3: Risk Assessment for Isoflucypram and its metabolite BCS-CN88460-carboxylic acid for soil micro-organisms

Compound	Species	Endpoint [mg/kg]	$PEC_{soil,max}$ [mg/kg]	Refinement required
Isoflucypram	Soil micro-organisms	0.5	0.030	No
BCS-CN88460-carboxylic acid (M12)	Soil micro-organisms	0.54	0.002	No

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.

Report:	KCP 10.5/01; [redacted]; 2017; M-574633-02-1
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
Document No.:	M-574633-02-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 (2009) US EPA OCSPP Not Applicable
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The aim of this study was to determine the effects of the test item on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 (2000) by measuring the nitrogen turnover.

Material and methods:

Test item: BCS-CN88460 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.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.1 mg a.s./kg soil dry weight, 0.5 mg a.s./kg soil dry weight and a control. Each treatment consisted of 3 replicates. Application rates were equivalent to 75 g a.s./ha (1.5 L product/ha) and 375 g a.s./ha (7.5 L product/ha) corresponding to test concentrations of 0.1 mg a.s./kg soil dry weight (1.95 mg prod./kg soil) and 0.5 mg a.s/kg soil dry weight (9.74 mg prod./kg soil). The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5%). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless Dinoterb is tested routinely as reference item in a separate study to verify the sensitivity of the test system.

Findings:

Experimental conditions:
The test conditions were: 19.7-21.0 °C in a climatic and dark room, 43.29 to 45.24% of WHC_{max} and pH values of 6.2 to 6.3 in the soil. The water content of the soil in each test vessel was determined at test start (after application) and adjusted once a week to the required range of 40-50% of WHC_{max}. The pH values in the soil used in the test were measured at test start (after application) and at the final sampling on day 28.
A statistical evaluation of the test results was performed by means of a 2-sided Student-t-test (for homogeneous variances at 5% significance level).

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).

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, $p \leq 0.05$)

*s Statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

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).

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%
Effect of toxic standard ≥ 25%	- 37.0% (separate study)

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).

CP 10.6 Effects on terrestrial non-target higher plants

For the product Isoflucypram EC 50 a dose response study on Terrestrial Plant Vegetative Vigour and a single dose study as well as a dose response study on Terrestrial Plant Seedling Emergence were conducted to determine possible effects on seedling emergence and plant growth. The vegetative vigour and seedling emergence tests were carried out according to the OECD 227 and OECD 208 guidelines for the testing of chemicals, respectively.

Table 10.6- 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*)	[redacted]; 2017; M-589028-01-1
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*)	[redacted]; 2017; M-596298-01-1
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*)	[redacted], P.; 2017; M-607264-01-1 KCP 10.6.2/03

In the case of Isoflucypram EC 50, the Tier 2 vegetative vigour study and the Tier 1 and Tier 2 seedling emergence study showed no phytotoxic effects ≥50 % at the tested rate of 75 g a.s./ha (equivalent to 1.5 L product/ha).

Risk assessment for Terrestrial Non-Target Higher Plants

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev. 2 final, 2002). It is restricted to off-field situations, as non-target plants are defined as non-crop plants located outside the treated area. Effects on non-target plants are of concern in the off-field environment, where non-target plants may be exposed to spray drift.

As it is clearly indicated that the maximum single application rate of 75 g a.s./ha (corresponding to 1.5 L product/ha) does not results in effects > 50 %, according to the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev. 2 final, 2002), no risk for non-target terrestrial plants is expected. Thus, no further risk assessment is required and no risk mitigation measures are regarded necessary.

Conclusion:

From the data presented above, it is concluded that unacceptable effects of Isoflucypram EC 50 on non-target terrestrial plants are not to be expected when the product is used as recommended.

CP 10.6.1 Summary of screening data

Not necessary as guideline GLP studies for terrestrial non-target plants are available (see Point 10.6.2 in this MCP Summary).

CP 10.6.2 Testing on non-target plants

Report:	KCP 10.6.2/01; [redacted]; 2017; M-589028-01-1
Title:	BCS-CN88460 EC 50 g/L - Effects on the vegetative vigor 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 vigor
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: Brown light turbid liquid. A total of 10 species, 6 dicotyledonous and 4 monocotyledonous species were tested in this vegetative vigour test representing 8 plant families. The plants were grown in a greenhouse in 15 cm pots (filled with approx. 1.2 L soil). The used soil was a silt loam. Planting density included 2 or 4 plants per pot with 16 or 8 replicate pots, respectively, for a total of 32 plants per treatment level.

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. Details of the range of test item rates per species are summarized in the following table:

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
Cucumis sativus	CUMSA	Cucumber	X	X	X	X	X
Glycine max	GLXMA	Soybean	X	X	X	X	X
Helianthus annuus	HELAN	Sunflower	X	X	X	X	X
Solanum lycopersicum	LYPES	Tomato	X	X	X	X	X
Allium cepa	ALLCE	Onion	X	X	X	X	X
Avena sativa	AVESA	Oat	X	X	X	X	X
Lolium perenne	LOLPE	Ryegrass	X	X	X	X	X
Zea mays	ZEAMA	Corn	X	X	X	X	X

X: Plant species tested with test item rate.

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.
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 %. 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.
Statistical analyses of the data were performed to obtain NOER (No Observed Effect Rate), LOER (Lowest Observed Effect Rate), ER_{25}/ER_{50} (Effect Rate producing 25 %/50 % effect) for survival, IR_{25}/IR_{50} (Inhibition Rate producing 25 %/ 50% effect) for shoot length and shoot dry weight, using ToxRat statistical software.

Findings:

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 in equal manner with the same soil.
The analysis of BCS-CN88460 content in the initial test item stock solution revealed measured concentrations of 114 % of nominal.
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.
The NOER, LOER, ER_{25}/ER_{50} for survival, IR_{25}/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 following tables for the final assessment (on day 21 after application).

Survival

Plant Species	ER_{25} (g a.s./ha)	95% Confidence Limits		ER_{50} (g a.s./ha)	95% Confidence Limits		LOER (g a.s./ha)	NOER (g a.s./ha)
		lower	upper		lower	upper		
Beta vulgaris	>75 [a]	n.d.	n.d.	>75 [a]	n.d.	n.d.	>75	75
Brassica napus	>75 [a]	n.d.	n.d.	>75 [a]	n.d.	n.d.	>75	75
Cucumis sativus	>75 [a]	n.d.	n.d.	>75 [a]	n.d.	n.d.	>75	75
Glycine max	>75 [a]	n.d.	n.d.	>75 [a]	n.d.	n.d.	>75	75
Helianthus annuus	>75 [a]	n.d.	n.d.	>75 [a]	n.d.	n.d.	>75	75
Solanum lycopersicum	>75 [a]	n.d.	n.d.	>75 [a]	n.d.	n.d.	>75	75
Allium cepa	>75 [a]	n.d.	n.d.	>75 [a]	n.d.	n.d.	>75	75
Avena sativa	>75 [a]	n.d.	n.d.	>75 [a]	n.d.	n.d.	>75	75
Lolium perenne	>75 [a]	n.d.	n.d.	>75 [a]	n.d.	n.d.	>75	75
Zea mays	>75 [a]	n.d.	n.d.	>75 [a]	n.d.	n.d.	>75	75

n.d.: Confidence limits not determined (outside the range tested)
[a]: No effects were observed up to the highest concentration tested.

Shoot Length

Plant Species	IR_{25} (g a.s./ha)	95% Confidence Limits		IR_{50}* (g a.s./ha)	95% Confidence Limits		LOER (g a.s./ha)	NOER (g a.s./ha)
		lower	upper		lower	upper		
Beta	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Brassica	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Cucumis	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Glycine max	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Helianthus	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Solanum	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Allium cepa	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Avena sativa	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Lolium	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Zea mays	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	75	37.5

*: 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

Plant Species	IR_{25} (g a.s./ha)	95% Confidence Limits		IR_{50}* (g a.s./ha)	95% Confidence Limits		LOER (g a.s./ha)	NOER (g a.s./ha)
		lower	upper		lower	upper		
Beta vulgaris	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	75	37.5
Brassica napus	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	75	37.5
Cucumis sativus	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Glycine max	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	75	37.5
Helianthus annuus	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	75	37.5
Solanum lycopersic	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	75	37.5
Allium cepa	>75 [c]	n.d.	n.d.	>75 [c]	n.d.	n.d.	>75	75
Avena sativa	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Lolium perenne	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Zea mays	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	18.8	9.4

*: 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).

Shoot Length

Plant Species	IR_{25} (g a.s./ha)	95% Confidence Limits		IR_{50}* (g a.s./ha)	95% Confidence Limits		LOER (g a.s./ha)	NOER (g a.s./ha)
		lower	upper		lower	upper		
Beta vulgaris	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Brassica napus	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Cucumis sativus	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Glycine max	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Helianthus annuus	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Solanum lycopersicum	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Allium cepa	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Avena sativa	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Lolium perenne	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	>75	75
Zea mays	>75 [b]	n.d.	n.d.	>75 [b]	n.d.	n.d.	75	37.5

*: IR_{50} corresponds to ER_{50}.
n.d.: Confidence limits not determined (outside the range tested)
[b]: Not calculated (outside the range tested).

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 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 lycopersicum	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.

For survival of all species no effects were observed up to the highest test item rate tested, there was 100 % survival of all species for all rates. Therefore the LOER was outside the range tested (>75 g a.s./ha) and the NOER reported as the highest rate tested (75 g a.s./ha) and the ER_{25} and ER_{50} values could not be calculated and are reported as >75 g a.s./ha for all species.

For shoot length the IR_{25} and IR_{50} values could not be calculated and are reported as >75 g a.s./ha for all species tested. The LOER was outside the range tested (>75 g a.s./ha) and the NOER reported as the highest rate tested (75 g a.s./ha) except for *Zea mays*. For *Zea mays*, the LOER was calculated to be 75 g a.s./ha and the NOER 37.5 g a.s./ha.

For shoot dry weight the IR_{25} and IR_{50} values could not be calculated and are reported as >75 g a.s./ha for all species tested. The LOER was outside the range tested (>75 g a.s./ha) and the NOER reported as the highest rate tested (75 g a.s./ha) for the following species *Cucumis sativus*, *Allium cepa*, *Avena sativa* and *Lolium perenne*. For *Beta vulgaris*, *Brassica napus*, *Glycine max*, *Helianthus annuus* and *Solanum lycopersicum* the LOER was calculated to be 75 g a.s./ha and the NOER 37.5 g a.s./ha. For *Zea mays*, the LOER was calculated to be 18.8 g a.s./ha and the NOER 9.4 g a.s./ha. The most sensitive species for this parameter was *Helianthus anuus*, showing 16.0 % inhibition at the test rate of 75 g a.s./ha.

Report:	KCP 10.6.2/02; [redacted]; 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 2) - 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: Brown light turbid liquid. A total of 10 species, 6 dicotyledonous and 4 monocotyledonous species were tested in this seedling emergence test representing 8 plant families. 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.
Planting density included 2 or 4 seeds per pot, with 20 or 10 replicate pot 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.

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.

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.
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 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. 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.

Findings:

The germination rate of the seeds used in this study was ≥ 70 %.
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 in equal manner with the same soil.
The analysis of BCS-CN88460 content in the initial test item stock solution revealed measured concentrations of 113 % of nominal.
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.
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).
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)*.
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.
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.
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.

The following table summarises per cent 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.

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 (%) inhibition)*	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 [c]	30-31	30-31
Cucumis sativus	0.0	-2.6	-4.4	3.7	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.

Report:	KCP 10.6.2/03; [redacted], P.; 2017; M-607264-01-1
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. Four crop species, 3 dicotyledonous and 1 monocotyledonous species were tested in this seedling emergence test representing 4 different plant species. 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 used soil was a silt loam.
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 the following table.

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	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 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 were performed to obtain NOER (No Observed Effect Rate), LOER (Lowest Observed Effect Rate), ER_{25}/ER_{50} (Effect Rate) for emergence, survival, IR_{25}/IR_{50} (Inhibition Rate) for shoot length and shoot dry weight, using ToxRat statistical software.

Findings:

The germination rate of the seeds used in this study was $\geq$ 70%.
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 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 each species. The pots used for all species of this study were filled in equal manner with the same soil.
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 NOER, LOER, ER_{25}/ER_{50} for emergence and survival, IR_{25}/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 following tables for the final assessment (21 days after 50% emergence of the control seedlings).

Emergence								
Plant species	**ER_{25} (g a.s./ha)**	**95% Confidence limits**		**ER_{50} (g a.s./ha)**	**95% Confidence limits**		**LOER (g a.s./ha)**	**NOER (g a.s./ha)**
		lower	**upper**		**lower**	**upper**		
Beta vulgaris	**>75[a]**	n.d.	n.d.	**>75[a]**	n.d.	n.d.	>75	75
Brassica napus	**>75[a]**	n.d.	n.d.	**>75[a]**	n.d.	n.d.	>75	75
Glycine max	**>75[a]**	n.d.	n.d.	**>75[a]**	n.d.	n.d.	>75	75
Allium cepa	**>75[a]**	n.d.	n.d.	**>75[a]**	n.d.	n.d.	>75	75

n.d.: Confidence limits not determined (outside the range tested)
[a]: Not calculated (outside the range tested).

Survival								
Plant species	**ER_{25} (g a.s./ha)**	**95% Confidence**		**ER_{50} (g a.s./ha)**	**95% Confidence limits**		**LOER (g a.s./ha)**	**NOER (g a.s./ha)**
		lower	**upper**		**lower**	**upper**		
Beta vulgaris	**>75[b]**	n.d.	n.d.	**>75[b]**	n.d.	n.d.	>75	75
Brassica napus	**>75[b]**	n.d.	n.d.	**>75[b]**	n.d.	n.d.	>75	75
Glycine max	**>75[a]**	n.d.	n.d.	**>75[a]**	n.d.	n.d.	>75	75
Allium cepa	**>75[a]**	n.d.	n.d.	**>75[a]**	n.d.	n.d.	>75	75

n.d.: Confidence limits not determined (outside the range tested)
[a]: Not calculated (outside the range tested).
[b]: Not calculated (no effect observed).

Shoot Length								
Plant species	**IR_{25}* (g a.s./ha)**	**95% Confidence limits**		**IR_{50}* (g a.s./ha)**	**95% Confidence limits**		**LOER (g a.s./ha)**	**NOER (g a.s./ha)**
		lower	**upper**		**lower**	**upper**		
Beta vulgaris	**>75[a]**	n.d.	n.d.	**>75[a]**	n.d.	n.d.	**>75**	**75**
Brassica napus	**>75[a]**	n.d.	n.d.	**>75[a]**	n.d.	n.d.	**>75**	**75**
Glycine max	**>75[a]**	n.d.	n.d.	**>75[a]**	n.d.	n.d.	**>75**	**75**
Allium cepa	**>75[a]**	n.d.	n.d.	**>75[a]**	n.d.	n.d.	**>75**	**75**

*: IR corresponds to ER.
n.d.: Confidence limits not determined (outside the range tested)
[a]: Not calculated (outside the range tested).

Shoot Dry Weight								
Plant species	**IR_{25}* (g a.s./ha)**	**95% Confidence limits**		**IR_{50}* (g a.s./ha)**	**95% Confidence limits**		**LOER (g a.s./ha)**	**NOER (g a.s./ha)**
		lower	**upper**		**lower**	**upper**		
Beta vulgaris	>75[a]	n.d.	n.d.	>75[a]	n.d.	n.d.	>75	75
Brassica napus	>75[a]	n.d.	n.d.	>75[a]	n.d.	n.d.	>75	75
Glycine max	>75[a]	n.d.	n.d.	>75[a]	n.d.	n.d.	>75	75
Allium cepa	>75[a]	n.d.	n.d.	>75[a]	n.d.	n.d.	>75	75

*: IR corresponds to ER.
n.d.: Confidence limits not determined (outside the range tested)
[a]: Not calculated (outside the range tested).

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

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.

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.
No statistically significant differences were found for emergence, survival, shoot length or shoot dry weight between treated plants and plants in the control of any species tested. For all measurements and species, the LOER was outside the range tested and the NOER reported as the highest test item rate of 75 g a.s./ha. The ER_{25}/IR_{25} and ER_{50}/IR_{50} values for all species tested were higher than the highest test item rate and could not be calculated and are therefore reported as > 75 g a.s./ha.

CP 10.6.3 Extended laboratory studies on non-target plants

In view of the results presented under Point CP 10.6.2 above, no further studies are deemed necessary.

CP 10.6.4 Semi-field and field tests on non-target plants

In view of the results presented under Point CP 10.6.2 above, no further studies are deemed necessary.

CP 10.7 Effects on other terrestrial organisms (flora and fauna)

No further tests on other terrestrial organism deemed to be necessary due to the low to moderate acute and chronic ecotoxicity of Isoflucypram EC 50 as presented under the Points CP 10.1 to CP 10.6 in this MCP Summary.

CP 10.8 Monitoring data

No monitoring data has been collected by the applicant nor have they been reported in any of the public literature references as evaluated in Document MCA, Section 9. Due to the low to moderate acute and chronic ecotoxicity of Isoflucypram EC 50 as presented under the Points CP 10.1 to CP 10.7, no monitoring of non-target organism is deemed to be necessary.