Bayer CropScience

Document Title

Summary of the ecotoxicological studies

Isoxaflutole + Cyprosulfamide SC 480 (240+240) g/L

Data Requirements

EU Regulation 1107/2009 & EU Regulation 284/2013

Document MCP

Section 10: Ecotoxicological studies

According to the guidance document, SANCO 10181/2013, for preparing dossiers for the approval of a chemical active substance

Date

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Version history

Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number

[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

Use pattern considered in this risk assessment

Table10- 1: Intended application pattern

Crop	Timing of application (range)	Number of applications	Application interval [days]	Maximum label rate (range) [L/ha]	Maximum application rate, individual treatment [g/ha] isoxaflutole
Maize, Pre-emergence	BBCH 00 - 13	1		0.417	100

Definition of the residue for risk assessment

Justification for the residue definition for risk assessment is provided in MCA Section 7, Point 7.4.1 and MCA Section 6, Point 6.7.1.

Table10- 2: Definition of the residue for risk assessment

Compartment	Compound / Code
Soil	RPA 202248 RPA 203328
Groundwater	RPA 202248 RPA 203328
Surface water	RPA 202248 RPA 203328 RPA 205834

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).

CP 10.1.1 Effects on birds

Table 10.1.1- 1: Endpoints used in risk assessment

Test substance	Exposure	Species/origin	Endpoint	Reference
Isoxaflutole	Acute risk assessment	Mallard duck	LD_{50} > 2150 mg a.s./kg bw	[redacted] 1994b M-166863-01-1 KCA 8.1.1.1/02 (EU-evaluated)
RPA 202248 (Isoxaflutole-diketonitrile DKN)	Long-term risk assessment	Bobwhite quail	NOEL ≥ 43.6 mg p.m./kg bw/d	[redacted] et al. 1999 M-238510-01-1 KCA 8.1.1.3/01

Table 10.1.1- 2: Relevant avian indicator species for risk assessment on screening level

Crop	Indicator species	Shortcut value	
		For long-term RA based on RUD_m	For acute RA based on RUD_{90}
Bare soil	Small granivorous bird	11.4	24.7
Maize	Small omnivorous bird	64.8	158.8

Risk assessment for birds

ACUTE DIETARY RISK ASSESSMENT

Table 10.1.1- 3: Acute DDD and TER calculation for birds on screening level

Compound / Crop	Indicator species	DDD			DDD	LD_{50} [mg a.s./kg bw]	TER_A	Trigger
		Appl. rate [kg a.s./ha]	SV_{90}	MAF_{90}				
Isoxaflutole								
Bare soil	Small granivorous bird	0.1	24.7	1	2.5	2150	870	10
Maize	Small omnivorous bird		158.8		15.9		135	10

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

As the IFT + CSA SC 480 is applied pre-emergence to bare soil (BBCH 00-09) or on not fully developed maize plants (pre-emergence to 3 leave stage), no pools in leaf axils where an acute exposure possibly might occur are to be expected.

LONG-TERM REPRODUCTIVE RISK ASSESSMENT

Table 10.1.1- 4: Long-term DDD and TER calculation for birds on screening level

Compound / Crop	Generic focal species	DDD				DDD	NO(A)EL [mg a.s. kg/bw/d]	TER_{LT}	Trigger
		Appl. rate [kg a.s./ha]	SV_m	MAF_m	f_{twa}				
RPA 202248									
Bare soil	Small granivorous bird	0.1	11.4	1	0.53	0.6	43.6	72	5
Maize	Small omnivorous bird		64.8			3.4		12.7	5

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

Table 10.1.1- 5: Evaluation of potential concern for exposure of birds drinking water (escape clause)

Crop	Koc [L/kg]	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
RPA 202248 [a]						
Bare soil/ maize	108	1 × 100	43.6	2.3	≤ 50	No concern

[a] BCS considers that the metabolite RPA 202248 covers the environmental fate properties better than the parent isoxaflutole. For details see the text above. 100% conversion from the parent to the metabolite RPA 202248 will be assumed and no mass correction is needed.

Overall, there is no unacceptable risk for birds from the acute or chronic exposure to isoxaflutole.

RISK ASSESSMENT OF SECONDARY POISONING

Table 10.1.1- 6: Log P_{ow} values

Substance	log P_{ow}	Reference
Isoxaflutole	2.34	MCA Sec.2, Point 2.7 [redacted], 1995, M-162438-03-1 (KCA 2.7 /01)
RPA 202248	-0.37 (pH 4.4)	[redacted], 1994, M-202428-01-1 (KCA 8.2.2.3/02)
RPA 205834	1.67 (pH 4.4)	
RPA 203328	-1.0 (pH 4.4)	

As the log P_{ow} values for isoxaflutole and its metabolites are below the trigger value of 3, no potential for bioaccumulation is assumed and effects on secondary poisoning are not assessed.

CP 10.1.1.1 Acute oral toxicity

For studies already evaluated during the first EU review of isoxaflutole, please refer to corresponding section in the Baseline Dossier (KCP: D-009257-01-1) provided by Bayer CropScience and in the Monograph.

No further studies are required.

CP 10.1.1.2 Higher tier data on birds

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

CP 10.1.2 Effects on terrestrial vertebrates other than birds

Table 10.1.2- 1: Endpoints used in risk assessment

Test substance	Exposure	Species/origin	Endpoint		Reference
Isoxaflutole	Acute risk assessment	Rat	LD_{50}	> 5000 mg a.s./kg bw	[redacted] 1993, M-158376-01-1, KCA 5.2.1/01, (EU evaluated)
	Long-term risk assessment	Rat	NOAEL	20 mg a.s./kg bw/d	[redacted] 1995, M-213063-01-1, KCA 5.8.1/03, (EU evaluated)

Table 10.1.2- 2: Relevant mammal indicator species for risk assessment on screening level

Crop	Indicator species	Shortcut value	
		For long-term RA based on RUD_m	For acute RA based on RUD_{90}
Bare soil	Small granivorous mammal	6.6	14.4
Maize	Small herbivorous mammal	72.3	136.4

Risk assessment for other terrestrial vertebrates

ACUTE DIETARY RISK ASSESSMENT

Table 10.1.2- 3: Acute DDD and TER calculation for mammals on screening level

Compound / Crop	Indicator species	DDD			DDD	LD_{50} [mg a.s. /kg bw]	TER_A	Trigger
		Appl. rate [kg a.s. /ha]	SV_{90}	MAF_{90}				
Isoxaflutole								
Bare soil	Small granivorous mammal	0.1	14.4	1	1.4	≥ 5000	≥ 3472	10
Maize	Small herbivorous mammal		136.4		13.6		≥ 367	10

LONG-TERM REPRODUCTIVE RISK ASSESSMENT

Table 10.1.2- 4: Long-term DDD and TER calculation for mammals on screening level

Compound / Crop	Generic focal species	DDD				DDD	NO(A)EL [mg a.s. kg/bw/d]	TER_{LT}	Trigger
		Appl. rate [kg a.s./ha]	SV_m	MAF_m	f_{twa}				
Isoxaflutole									
Bare soil	Small granivorous mammal	0.1	6.6	1	0.56	0.3	20	57.2	5
Maize	Small herbivorous mammal		72.3			3.8		5.2	5

Long-term risk assessment for mammals drinking contaminated water

The puddle scenario is relevant for the long-term risk assessment.

Table 10.1.2- 5: Evaluation of potential concern for exposure of mammals drinking water

Crop	Koc [L/kg]	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
Isoxaflutole						
Bare soil/maize	112	1 × 100	20	5	**≤ 50**	No concern

Overall, there is no unacceptable risk for mammals from the acute or chronic exposure to isoxaflutole.

RISK ASSESSMENT OF SECONDARY POISONING

As outlined in Point 10.1.1 a risk assessment of secondary poisoning is not deemed necessary.

CP 10.1.2.1 Acute oral toxicity to mammals

For studies already evaluated during the first EU review of isoxaflutole, please refer to corresponding section in the Baseline Dossier (KCP: D-009257-01-1) provided by Bayer CropScience and in the Monograph.

No further studies are required.

CP 10.1.2.2 Higher tier data on mammals

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

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

Not required according to 1107/2009.

CP 10.2 Effects on aquatic organisms

The risk assessment is based on the current Guidance Document on Aquatic Ecotoxicology, SANCO/3268/2001, rev 4 final, 17 October 2002. Some implications of the new Aquatic Guidance Document (EFSA Journal 2013;11(7):3290, 268 pp. doi:10.2903/j.efsa.2013.3290), which is not yet notified, have been taken into consideration as well.

Ecotoxicological endpoints used in risk assessment

Table 10.2- 1: Endpoints used in risk assessment (formulated product)

Test substance	Test species	Endpoint	Reference
IFT + CSA SC 480	Fish, acute , *Oncorhynchus mykiss*	LC_{50} >100 mg product/L	[redacted] (2007) EBUBP107 M-282479-01-1 KCP 10.2.1/02
	Invertebrate, acute, *Daphnia magna*	EC_{50} >100 mg product/L	[redacted] (2007) EBUBP106 M-284338-01-1 KCP 10.2.1/03
	Algae, chronic, *P. subcapitata*	E_rC_{50} 197 mg product/L	[redacted] (2007) EBUBP104 M-284638-01-1 KCP 10.2.1/04
	Aquatic plant, chronic, *Lemna gibba*	E_rC_{50} 0.0492 mg product/L	[redacted] (2007) EBUBP101 M-284141-01-1 KCP 10.2.1/01

Table 10.2- 2: Endpoints used in risk assessment (active substance)

Test substance	Test species	Endpoint		Reference
Isoxaflutole	Fish, acute , *O. mykiss*	LC_{50}	1.7 mg a.s./L	[redacted] (1993) M-166876-01-1 KCA 8.2.1/02 (EU evaluated)
	Fish, chronic, *P. promelas*	NOEC	0.102 mg a.s./L*	[redacted] (2013) EBISX074 M-469327-01-1 KCA 8.2.2.1/02
	Invertebrate, acute *Americamysis bahia*[1]	EC_{50}	0.077 mg a.s./L	[redacted] (1994) M-227961-02-1 KCA 8.2.4.2/07
	Invertebrate, chronic *Americamysis bahia*[1]	NOEC	0.001 mg a.s./L	[redacted] (1995) M-166884-01-1 KCA 8.2.5.2/01
	Algae, growth inhibition *Pseudokirchneriella subcapitata*	120h-E_dC_{50}	0.12 mg a.s./L [2]	[redacted] (1995) M-166898-01-1 KCA 8.2.6.1/01 (EU evaluated)
	Aquatic plants, growth inhibition *Lemna gibba*	9d E_rC_{50}	0.01439 mg a.s./L [3]	[redacted] (2013) M-449195-01-1 KCA 8.2.7/04
RPA 202248	Fish, acute , *O. mykiss*	LC_{50}	>15 mg p.m./L	[redacted] (1995) M-170804-01-1 KCA 8.2.1/03 (EU evaluated)
	Invertebrate, acute* *Americamysis bahia*	EC_{50}	24 mg p.m./L	[redacted] (1995) M-170861-01-1 KCA 8.2.4.2/09
	Algae, growth inhibition *Pseudokirchneriella subcapitata*	72h-E_dC_{50}	1.9 mg p.m./L [2]	[redacted] (1997) M-166891-01-1 KCA 8.2.6.1/08
	Aquatic plants, growth inhibition *Lemna gibba*	E_bC_{50}	0.055 mg p.m./L	[redacted] (1997) M-166889-01-1 KCA 8.2.7/03 (EU evaluated)
RPA 203328	Fish, acute , *O. mykiss*	LC_{50}	160 mg pm./L	[redacted] (1995) M-170722-01-1 KCA 8.2.1/04 (EU evaluated)
	Invertebrate, acute *Daphnia magna*	EC_{50}	>150 mg p.m./L	[redacted] (1994) M-170649-01-1 KCA 8.2.4.1/03 (EU evaluated)
	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_dC_{50}	>9.4 mg p.m./L [2]	[redacted] (1995) M-170835-01-1 KCA 8.2.6.1/03 (EU evaluated)
	Aquatic plants, growth inhibition *Lemna gibba*	E_bC_{50}	>9.8 mg p.m./L	[redacted] (1997) M-166893-01-1 KCA 8.2.7/06
RPA 205834	Fish, acute , *O. mykiss*	LC_{50}	>35 mg p.m./L	[redacted] (1995) M-213119-01-1 KCA 8.2.1/05

Test substance	Test species	Endpoint		Reference
				(EU evaluated)
	Invertebrate, acute *Daphnia magna*	EC_{50}	>60.1 mg p.m./L	[redacted] (1993) M-170847-01-1 KCA 8.2.4.1/04 (EU evaluated)
	Algae, growth inhibition *Desmodesmus subspicatus*	E_bC_{50}	>15 mg p.m./L[2]	[redacted] (1995) M-213127-01-1 KCA 8.2.6.1/04 (EU evaluated)
	Aquatic plants, growth inhibition *Lemna gibba*	E_bC_{50}	1.1 mg p.m./L	[redacted] (2003) B004501 M-241470-01-1 KCA 8.2.7/07

***** The EU agreed endpoint is 0.08 mg/L derived from a juvenile growth study with *Oncorynchus mykiss*. Due to the new data requirements an ELS study with *Pimephales promelas* has been performed ([redacted], 2013). The endpoint of the ELS study is in the same range as the endpoint derived from the juvenile growth test. The risk assessment is based on the new data requirements, hence the endpoint from the ELS study is used in the TER calculations.

[1] Where the mysid endpoint is lower than the endpoint derived with *Daphnia magna* or *Chironomus riparius* the risk assessment for aquatic invertebrates exposed to isoxaflutole is based on mysid shrimp data although not required by the new data requirements (EC 283/2013) for a herbicide. This clearly is worst case.

[2] As the EU agreed endpoint is lower than the E_rC_{50} of the study with *Skeletonema costatum* (MCA, point CA 8.2.6), the EU agreed endpoint is used for the risk assessment. The algae endpoint E_bC_{50} corresponds to a biomass endpoint, which is generally lower than a growth rate endpoint and can therefore be considered as conservative value. The preferred endpoint as also stated in the new Aquatic Guidance Document (EFSA Journal 2013;11(7):3290, 268 pp. doi:10.2903/j.efsa.2013.3290) is growth rate.

[3] Recalculation of Lemna endpoint; please see MCA point CA 8.2.7.

Predicted environmental concentrations used in risk assessment

Table 10.2- 3: Initial maximum PEC_{sw} values – FOCUS Step 1 and 2

Compound	FOCUS Scenario	Maize
		$PEC_{sw, max}$ [µg/L]
Isoxaflutole	STEP 1	31.05
	STEP 2 - North	0.92
	STEP 2 - South	**0.92**
RPA 202248	STEP 1	32.50
	STEP 2 - North	5.95
	STEP 2 - South	**11.27**
RPA 203328	STEP 1	15.48
	STEP 2 - North	2.49
	STEP 2 - South	**4.91**
RPA 205834	STEP 1	1.02
	STEP 2 - North	0.39
	STEP 2 - South	**0.54**

BOLD – values considered in risk assessment

Table 10.2- 4: Initial maximum PEC_{sw} values – FOCUS Step 3

Compound	FOCUS Scenario	Maize
		$PEC_{sw, max}$ [µg/L]
Isoxaflutole	D3 ditch	**0.524**
	D4 pond	**0.021**
	D4 stream	**0.442**
	D5 pond	**0.021**
	D5 stream	**0.446**
	D6 ditch	**0.524**
	R1 pond	**0.021**
	R1 stream	**0.363**
	R2 stream	**0.482**
	R3 stream	**0.513**
	R4 stream	**0.362**
RPA 202248	D3 ditch	**0.335**
	D4 pond	**0.018**
	D4 stream	**0.012**
	D5 pond	**0.023**
	D5 stream	**0.009**
	D6 ditch	**0.363**
	R1 pond	**0.022**
	R1 stream	**1.688**
	R2 stream	**1.568**
	R3 stream	**0.061**
	R4 stream	**4.208**

BOLD – values considered in risk assessment

Table 10.2- 5: Initial maximum PEC_{sw} and 7d-TWA_{sw} values – FOCUS Step 4 (5 m buffer)

Compound	FOCUS Scenario	Maize	Maize
		$PEC_{sw, max}$ [µg/L]	7d-TWA_{sw}, [µg/L]
Isoxaflutole	D3 ditch	0.172	**0.0170**
	D4 pond	0.019	**0.0056**
	D4 stream	0.186	**0.00203**
	D5 pond	0.019	**0.000077**
	D5 stream	0.188	**0.00121**
	D6 ditch	0.172	**0.000212**
	R1 pond	0.019	**0.00210**
	R1 stream	0.153	**0.00395**
	R2 stream	0.203	**0.00259**
	R3 stream	0.216	**0.00992**
	R4 stream	0.182	**0.0194**

BOLD – values considered in risk assessment

Risk assessment for aquatic organisms

ACUTE RISK ASSESSMENT FOR AQUATIC ORGANISMS

Risk assessment based on active substance endpoints

Table 10.2- 6: TER_A calculations based on FOCUS Step 2

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Maize					
Isoxaflutole	Fish, acute	LC_{50} >1700	0.92	>1848	100
	Invertebrate, acute	LC_{50} 77.		**83.7**	100
RPA 202248	Fish, acute	LC_{50} 15000	11.27	1331	100
	Invertebrate, acute	LC_{50} 24000		2130	100
RPA 203328	Fish, acute	LC_{50} 160000	4.91	32587	100
	Invertebrate, acute	LC_{50} 150000		30550	100
RPA 205834	Fish, acute	LC_{50} 35000	0.54	64815	100
	Invertebrate, acute	LC_{50} 60100		111296	100

Bold values do not meet the trigger

Table 10.2- 7: TER_A calculations based on FOCUS Step 3 (invertebrates)

Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	FOCUS scenario	TER_A	Trigger
Isoxaflutole, maize					
Invertebrate, acute	LC_{50} 77	0.524	D3 ditch	147	100
		0.021	D4 pond	3667	100
		0.442	D4 stream	174	100
		0.021	D5 pond	3667	100
		0.446	D5 stream	173	100
		0.524	D6 ditch	147	100
		0.021	R1 pond	3667	100
		0.363	R1 stream	212	100
		0.482	R2 stream	160	100
		0.513	R3 stream	150	100
		0.362	R4 stream	213	100

There is no unacceptable risk for aquatic invertebrates.

CHRONIC RISK ASSESSMENT FOR AQUATIC ORGANISMS

Risk assessment based on active substance endpoints

Table 10.2- 8: TER_{LT} calculations based on FOCUS Step 2

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Maize					
Isoxaflutole	Fish, chronic	NOEC 102	0.92	110	10
	Invertebrate, chronic	NOEC 1		**1.1**	10
	Green algae, chronic	EC_{50} 120		130	10
	Aquatic plants, chronic	E_rC_{50} 14.39		15.6	10
RPA 202248	Green algae, chronic	EC_{50} 1900	11.27	169	10
	Aquatic plants, chronic	E_rC_{50} 55		**4.9**	10
RPA 203328	Green algae, chronic	EC_{50} 8400	4.91	1914	10
	Aquatic plants, chronic	E_bC_{50} 9800		1996	10
RPA 205834	Green algae, chronic	EC_{50} >15000	0.54	27778	10
	Aquatic plants, chronic	E_bC_{50} 1100		2037	10

Bold values do not meet the trigger

Table 10.2- 9: TER_{LT} calculations based on FOCUS Step 3

Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	FOCUS scenario	TER_{LT}	Trigger
Isoxaflutole, maize					
Invertebrate, chronic	NOEC 1	0.524	D3 ditch	**1.9**	10
		0.021	D4 pond	47.6	10
		0.442	D4 stream	**2.3**	10
		0.021	D5 pond	47.6	10
		0.446	D5 stream	**2.2**	10
		0.524	D6 ditch	**1.9**	10
		0.021	R1 pond	47.6	10
		0.363	R1 stream	**2.8**	10
		0.482	R2 stream	**2.1**	10
		0.513	R3 stream	**1.9**	10
		0.362	R4 stream	**2.8**	10
RPA 202248, maize					
Aquatic plants, chronic	E_bC_{50} 55	0.335	D3 ditch	164	10
		0.018	D4 pond	3056	10
		0.012	D4 stream	4583	10
		0.023	D5 pond	2391	10
		0.009	D5 stream	6111	10
		0.363	D6 ditch	152	10
		0.022	R1 pond	2500	10
		1.688	R1 stream	33	10
		1.568	R2 stream	35	10
		0.661	R3 stream	902	10
		4.208	R4 stream	13.1	10

Bold values do not meet the trigger

Refined long-term risk assessment for *Americamysis bahia* (isoxaflutole)

Table 10.2- 10: TER_{LT} calculations based on FOCUS Step 4 7d-TWA [a]

Species	Endpoint [µg/L]	7d-TWA_{sw} [µg/L]	FOCUS scenario	TER_{LT}	Trigger
Isoxaflutole, maize: 5 m drift buffer					
Invertebrate, chronic	NOEC 1.0	0.0170	D3 ditch	59	10
		0.0056	D4 pond	179	10
		0.00203	D4 stream	493	10
		0.000077	D5 pond	12987	10
		0.00121	D5 stream	826	10
		0.000212	D6 ditch	4717	10
		0.00210	R1 pond	476	10
		0.00395	R1 stream	253	10
		0.00259	R2 stream	386	10
		0.00992	R3 stream	101	10
		0.0194	R4 stream	52	10

[a] Justification for use of TWA approach: please see MCA point CA 8.2.5

The TER values meet the trigger value of 10, indicating an acceptable risk to mysid shrimp for the application of the product in maize, provided that a 5 m drift buffer is kept.

Risk assessment for photo-metabolites M14 and M20 and aquatic macrophytes

The risk assessment approach for the photo-metabolites is described in detail in the MCA Section 8.

Table 10.2- 11: TER_A calculations based on FOCUS Step 2

Compound	Species	Endpoint [µg/L] [a]	$PEC_{sw,max}$ [µg/L] [b]	TER_{LT}	Trigger
Maize					
M14	Aquatic plants, chronic	E_bC_{50} 5.5	0.09	61	10
M20	Aquatic plants, chronic	E_bC_{50} 5.5	0.15	36.7	10

[a] E_bC_{50} of RPA 202248 divided by factor 10 (cf. MCA, CA 8.2.7)
[b] based on maximum PEC_{sw} of isoxaflutole (FOCUS Step 2) considering occurrences of 9.8% and 16.8% of M14 and M20, respectively (cf. MCA, CA 8.2.7)

Overall, there is no unacceptable risk for aquatic organisms from the acute or chronic exposure to isoxaflutole.

CP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Report:	[redacted] ∃; [redacted];2007;M-282479-01
Title:	Acute toxicity of cyprosulfamide & isoxaflutole SC 240+240 to fish (Oncorhynchus mykiss) under static conditions
Report No:	EBUBP107
Document No:	M-282479-01-1
Guidelines:	**EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985) OPPTS 850.1075 (Public Draft, 1996) Directive 92/69/EEC, C.1 (1992) OECD No. 203 (rev.1992)**
Deviations:	none
GLP/GEP:	**yes**

Objective:

The objective of this study was to evaluate the acute toxicity of Isoxaflutole & Cyprosulfamide SC 480 to the rainbow trout (*Oncorhynchus mykiss*). The study was conducted under static conditions for 96 hours.

Materials and Methods:

Test item: Cyprosulfamide & isoxaflutole SC 240+240, analyzed a.s. content: Cyprosulfamide: 20.5 % w / w (245 g / L), isoxaflutole: 20.5 % w / w (246 g / L); nominal a.s. content: 240 g / L specified by batch no.: 2006-001042, tox no.: 07429-00.

Ten fish in each treatment were exposed in duplicate to nominal concentrations of 6.25, 12.5, 25.0, 50.0 and 100 mg formulation/L against a control.

Dissolved oxygen concentrations ranged from 89 to 100 % oxygen saturation, the pH values ranged from 6.8 to 7.3 and the water temperature ranged from 11.5°C to 12.2°C in all aquaria over the whole testing period.

Cyprosulfamide was analyzed in all test levels after 0 h, on day 2 and on day 4 of the exposure period to confirm nominal concentrations. The endpoints were expressed in terms of nominal concentrations.

In the event that 100% mortality was observed in test concentrations prior to the end of the test, the analytical determinations were made at those times.

After 4, 24, 48, 72 and 96 hours fish were observed for mortality and sublethal effects.

Dates of experimental work: September 18 - November 07, 2006

Results:

Validity criteria:

Validity Criteria	Recommended by the guideline	Obtained in this study
Mortality during acclimatization	<5%	<5%
Mortality in the control	≤ 10%	0%
Dissolved oxygen saturation	≥ 60%	90 - 99%
pH variation	≤ 1	0.5

All validity criteria for the study were met.

Analytical results:

Based on analytical determination of cyprosulfamide (in water by HPLC-MS/MS) mean measured values between 109 % and 115 % of nominal were found in all exposure levels over the whole testing period of 96 hours.

Given that the toxicity cannot be attributed to any one of the active ingredients but to the formulation as a whole, all results are given as nominal values of the formulation only.

Biological results:

There were neither any sub-lethal effects nor any mortality in the control group.

There were behavioural observations on fish caused by the test item over the whole exposure period in all test levels ≥ 12.5 mg test item/L. At the test level with 12.5 mg test item/L fish showed the following symptoms after 96h: remained for unusually long periods at the water surface; showed labored respiration; remained for unusually long periods on the bottom of the aquarium; turned dark in coloration; were inactive or displayed abnormally low activity; did not show any abnormal signs.

Cumulative mortality of fish exposed to Isoxaflutole & Cyprosulfamide SC 480 (96 h)

Exposure time	4 h		24 h		48 h		72 h		**96 h**	
Test level mg form. / L	no. of dead	% dead	no. of dead	% dead	no. of dead	% dead	no. of dead	% dead	**no. of dead**	**% dead**
Control	0	0	0	0	0	0	0	0	**0**	**0**
6.25	0	0	0	0	0	0	0	0	**0**	**0**
12.5	0	0	0	0	0	0	0	0	**0**	**0**
25.0	0	0	1	10	2	20	2	20	**2**	**20**
50.0	0	0	0	0	0	0	0	0	**0**	**0**
100	0	0	0	0	0	0	0	0	**2**	**20**

Conclusion:

It was concluded that the 96-hour 50%-lethal concentration (LC_{50}) of the test item in rainbow trout based on nominal concentration was higher than the tested concentration of 100 mg formulation/L.

Report:	[redacted]5; [redacted];2007;M-284338-01
Title:	Acute toxicity of AE 0001789 & Isoxaflutole SC 240+240 to the waterflea Daphnia magna in a static laboratory test system - Limit test
Report No:	EBUBP106
Document No:	M-284338-01-1
Guidelines:	**OECD guideline 202,(2004); EEC Directive 92/69/EWG, part C.2 (1992); U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72 2 (1982), OPPTS Guideline 850.1010 public draft 1996 (modified); JMAFF 12 Nousan No. 8147 (2000)**
Deviations:	None
GLP/GEP:	**yes**

Objective:

The aim of the study was to verify the absence of treatment-related effects on mobility of *Daphnia magna* whilst exposed for 48 hours to a limit concentration of 100 mg form./l in a static test system.

Material and methods:

Test item: Isoxaflutole & Cyprosulfamide SC 240 + 240, Batch No.: batch 2006-001042, specification No.: 102000014305), content: 20.5% w/w Isoxaflutole, 20.5% w/w Cyprosulfamide (TOX 07429-00).
Five daphnids (1st instar; < 24 hours old) were exposed in ten replicates to a nominal limit concentration of 100 mg formulation/L under static conditions without feeding. In addition, a control (dilution water) was tested.
The endpoints were expressed in terms of nominal concentrations.
Dilution water was fortified well water with a pH of 8.2. Water temperature was 18 - 22 °C during the test, the photoperiod was 16 hours of light and 8 hours dark. The light intensity was max. 1500 lux.
After 24 and 48 hours, behaviour of the water fleas was visually evaluated by counting mobile daphnids. Additionally all possible signs of sublethal effects had to be recorded.
At test initiation (day 0) and at test termination (day 2) samples of the test solutions and control vessels were removed, pooled and analyzed.

Dates of experimental work: August 22 to August 25, 2006

Results:

Analytical results

The recoveries of the a.s. component cyprosulfamide as measured for samples from start and end of exposure ranged well within the given limits of 80 and 120% of nominal (108% of nominal for day 0 and 114% of nominal for day 2).

Biological results

During 48 hours of static exposure, no immobilities or other effects on behaviour occurred at the tested limit concentration of 100 mg form./l (nominally).

Immobilization of *Daphnia magna* during 48 h exposure to Isoxaflutole & Cyprosulfamide SC 480

Nominal test concentration [mg formulation/L]	Exposed daphnids (n)	Immobilised daphnids	
		24 h (n)	48 h (n)
Control	50	0	0
100	50	0	0

Conclusion:

It was concluded that the 48-hour 50%-lethal concentration (LC_{50}) of the test item in *Daphnia magna* based on nominal concentration was higher than the tested concentration of 100 mg formulation/L.

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Report:	[redacted];[redacted];2007;M-284638-01
Title:	Pseudokirchneriella subcapitata growth inhibition test with cyprosulfamide & isoxaflutole SC 480 (240 + 240) G
Report No:	EBUBP104
Document No:	M-284638-01-1
Guidelines:	**OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (March 23, 2006);** **US EPA OPPTS Guideline No. 850.4400**
Deviations:	None
GLP/GEP:	**yes**

Objectives:

The objective of this study was to determine the effect of Isoxaflutole & Cyprosulfamide SC 480 on the growth of the freshwater green alga, *Pseudokirchneriella subcapitata.*

Materials and Methods:

Test material: Cyprosulfamide & Isoxaflutole SC 240 + 240 analysed content: cyprosulfamide: 20.5 % and isoxaflutole: 20.5 % was tested, specified by batch no.: 2006-001042, sample description: TOX 07429-00 and specification no.: 102000014305).

Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multi-generation test for 72 hours under static exposure conditions to the nominal concentrations of 0.954, 3.05, 9.77, 31.3 and 100 mg formulation/L in comparison to a control.

The test system consisted of 3 replicate vessels per test level 6 replicate vessels in the control group. The initial cell number was 10 000 cells/mL.

Growth inhibition was calculated using algae biomass per volume. The surrogate for biomass was cell density (used as response parameter).

The pH values ranged from 8.0 to 8.2 in the controls and the incubation temperature ranged from 21.5°C to 22.4°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7478 lux.

Quantitative amounts of cyprosulfamide were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period.

Dates of experimental work: October 13 to November 21 2006

Results:

Validity criteria:

Validity Criteria	Recommended by the guideline	Obtained in this study
Biomass increase in the control	>16-fold	19.9-fold
Mean coefficient of variation for specific growth rate in the control (section-by-section)	≤ 35%	32.8%
Coefficient of variation of average specific growth rate between control replicates	≤ 7%	6.9
Increase of pH value of the control during the test	≤ 1.5	0.4

All validity criteria for the study were met

Analytical results:

The analytical findings of cyprosulfamide in the treatment levels found on day 0 were 100 to 110 % of nominal (average 105.2 %). On day 3 analytical findings of 100 to 110 % of nominal (average 104.4 %) were found. Given that the toxicity cannot be attributed to anyone of the a.s. compounds but to the formulation as a whole, all results are based on nominal test concentrations of the formulation.

Biological results:

Effect of Isoxaflutole & Cyprosulfamide SC 480 on Freshwater Algae (*Pseudokirchneriella subcapitata*) in a 72 h growth inhibition test

Nominal Concentration [mg form./L]	Cell Number after 72 h (means) per mL	(0-72h)-Average Specific Growth Rates [days^{-1}]	Inhibition of Average Specific Growth Rate [%]	Doubling Time of Algae Cells [days]
control	199,000	0.991	--	0.699
0.954	228,000	1.043	-5.2	0.665
3.05	128,000	0.849	14.4	0.816
9.77	60,000	0.595	40.0	1.16
31.3	30,000	0.363	63.4	1.91
100	23,000	0.261	73.6	2.66

test initiation with 10,000 cells/mL

Conclusions:

The (0 - 72h)-E_rC_{50} for Cyprosulfamide & Isoxaflutole SC 240 + 240 is 19.7 mg formulation/L and the (0 - 72h)-NOE_rC is 0.954 mg form./L.

Report:	[redacted]i; [redacted];2007;M-284141-01
Title:	Lemna gibba G3 - Growth inhibition test with AE 0001789 & isoxaflutole SC 240 + 240 under static conditions (spec No.: 101000014305)
Report No:	EBUBP101
Document No:	M-284141-01-1
Guidelines:	**OECD 221 Lemna sp. Growth Inhibition Test Revised Proposal for a New Guideline (October 2004);** **US EPA OPPTS Guideline No. 850.4400;none**
Deviations:	None
GLP/GEP:	**yes**

Objective:

The objective of this study was to evaluate the influence of Isoxaflutole & Cyprosulfamide SC 480 on exponentially growing *Lemna gibba* G3. The study was performed as a static experiment for 14 days.

Materials and Methods:

Test item: Cyprosulfamide & Isoxaflutole SC 240 + 240 analysed content: cyprosulfamide: 20.5 % and isoxaflutole: 20.5 % was tested, specified by batch no.: 2096-001042, sample description: TOX 07429-00 and specification no.: 101000014305).

3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multigeneration test for 7 days under static exposure conditions to the nominal concentrations of 9.54, 30.5, 97.7, 313, and 1000 µg formulation/L in comparison to control. The pH values ranged from 7.4 to 8.5 in the control and the incubation temperature ranged from 23.7°C to 24.1°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7,800 lux.

On day 0, 3, 5 and 7 after test initiation reduction in frond density, frond area and biomass dry weight were recorded.

Quantitative amounts of cyprosulfamide were measured in all freshly prepared test levels on day 0 and additionally in all aged test levels on day 7 of the exposure period.

The endpoints were expressed in terms of nominal concentrations.

Dates of experimental work: July 31 to September 29, 2006

Results:

Validity criteria:

Validity Criteria	Recommended by the guideline	Obtained in this study
frond number increase in the control	7-fold	7.5-fold

All validity criteria for the study were met.

Analytical results:

The analytical findings of cyprosulfamide found in all freshly prepared test levels on day 0 in reference to nominal concentrations ranged between 107 and 112 % (average 110 %). In aged test levels on days 7 there were analytical findings between 105 and 115 % (average 109 %) of nominal.

Biological results:

Test level (µg formulation/L)	Observations
Control 9.54	no visual effects observed
30.5	Small and single fronds and slight chlorosis on day 3; small fronds and slight chlorosis on days 5 and 7
97.7 313	Single fronds and slight chlorosis on day 3; small fronds and medium chlorosis on days 5 and 7
1000	Single fronds and slight chlorosis on day 3; medium chlorosis and medium necrosis on day 5; small fronds, medium chlorosis, and medium necrosis

Inhibition of *Lemna gibba* during 7-day exposure to Isoxaflutole & Cyprosulfamide SC 480

Nominal test levels [µg form./L]	Final frond no. (replicate means, day 7)	Final total frond area of plants (replicate means) [mm²]	% inhibition*	
			Average growth rate for frond no.	Average growth rate for total frond area of plants
control	85	271	--	--
9.54	86	271	0.8	-0.8
30.5	51	153	36.8	29.3
97.7	17	60	81.5	79.4
313	10	38	113	96.7
1000	12	45	102	88.2

* negative value means growth stimulation

Conclusion:

The most sensitive response variable was frond number resulting in (0-7-day)-E_rC_{50} of 49.2 µg formulation/L in this study. The lowest NOEC (9.54 µg formulation/L) was based on statistical data analysis and visual effects.

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

No new studies were required.

CP 10.2.3 Further testing on aquatic organisms

No new studies were required.

CP 10.3 Effects on arthropods

CP 10.3.1 Effects on bees

The ecotoxicological endpoints of honey bee laboratory studies are provided in the following tables.

Toxicity of isoxaflutole to bees

Details of the honeybee testing with the active substance isoxaflutole are presented in MCA, Section 6, Point 8.3.1, as well as within the existing Review Report for isoxaflutole.

Table 10.3.1- 1: Acute toxicity of isoxaflutole (tech.) to bees

Test substance	Test species	Endpoint	Reference
Isoxaflutole, tech.	Honey Bee	48 h - LD_{50} – oral > 168.7 µg a.s./bee 48 h - LD_{50} – contact > 100 µg a.s./bee	[redacted] (1996) RA05205 M-170632-02-1 KCA 8.3.1.1.1/01 (oral) KCA 8.3.1.1.2/01 (contact)
Isoxaflutole, tech.	Honey bee	48 h - LD_{50} – oral > 108.9 µg a.s./bee 48 h - LD_{50} – contact > 100 µg a.s./bee	[redacted] (2012) 72931035 M-441348-01-1 KCA 8.3.1.1.1/01

Table 10.3.1- 2: Honey bee toxicity data generated with formulated isoxaflutole

Test substance	Test species/ test design	Endpoint	Reference
Acute oral and contact toxicity (laboratory)			
IFT + CSA SC 240 + 240	Honey Bee	48 h - LD_{50} – oral > 110.8 µg a.s./bee 48 h - LD_{50} – contact > 100.00 µg a.s./bee	[redacted] (2006) 20061213/01-BLEU M-278327-01-1 KCP 10.3.1.1.1/01
Chronic toxicity to adult bees (laboratory)			
Isoxaflutole WG 75	Honey Bee, 10 d chronic adult feeding study	LC_{50} > 120 mg a.s./kg NOEC ≥ 120 mg a.s./kg	[redacted] (2013) S13-00146 M-470650-01-1 KCA 8.3.1.2/01
Bee brood feeding test			
Isoxaflutole WG 75	Honey bee brood feeding (Oomen *et al.*, 1992)	No adverse effects on mortality, bee brood development (eggs, young larvae, old larvae, pupae) and colony development by feeding honey bee colonies sugar syrup at a concentration typically present in the spray tank (250 ppm)	[redacted] (2013) EBISX071 M-454689-01-1 KCA 8.3.1.3/01

Risk assessment for bees

ACUTE RISK ASSESSMENT FOR BEES

The maximum label rate of Isoxaflutole & Cyprosulfamide SC 240+240 is 0.417 L product/ha in maize (BBCH 00 - 13). Since the content of isoxaflutole and cyprosulfamide in the formulation is 240 g substance/L, this accounts to a maximum application rate of 100 g isoxaflutole a.s./ha and to 200 g total substance/ha.

Table 10.3.1- 3: Hazard quotients for bees – oral exposure

Test substance	Oral LD_{50} [µg a.s./bee] / *[µg total substance/bee]*	Max. application rate [g a.s./ha] / *[g total substance/ha]*	Hazard quotient Q_{HO}	Trigger	*A-priori* acceptable risk for adult bees
Isoxaflutole, tech.	> 108.9	100	< 1	50	Yes
IFT + CSA SC 240 + 240	*> 110.8*	*200* [a]	< 2	50	Yes

[a] Maximum application rate = 100 g isoxaflutole a.s./ha (maize) via 500 g / 417 mL IFT + CSA SC 240+240 /ha

The hazard quotient for oral exposure is below the validated trigger value for higher tier testing (i.e. $Q_{HO} < 50$).

Table 10.3.1- 4: Hazard quotients for bees – contact exposure

Test substance	Contact LD_{50} [µg a.s./bee] / *[µg total substance/bee]*	Max. application rate [g a.s./ha] / *[g total substance/ha]*	Hazard quotient Q_{HC}	Trigger	*A-priori* acceptable risk for adult bees
Isoxaflutole, tech.	100	100	< 1	50	Yes
IFT + CSA SC 240 + 240	*> 100*	*200* [a]	< 2	50	Yes

[a] Maximum application rate = 100 g isoxaflutole a.s./ha (maize) via 500 g / 417 mL IFT + CSA SC 240+240 /ha

The hazard quotient for contact exposure is below the validated trigger value for higher tier testing (i.e. $Q_{HC} < 50$).

Further considerations for the risk assessment

In addition to acute laboratory studies with adult honey bees, isoxaflutole was further subjected to chronic laboratory testing with adult honey bees.

This chronic study was designed as a limit test by exposing adult honey bees for 10 consecutive days to a concentration of nominally 120 mg isoxaflutole a.s./kg in aqueous sugar solution. As isoxaflutole is only slightly soluble in water (6.2 mg/l at 20 °C), the test was conducted by using the formulated product Isoxaflutole WG 75. The nominal test concentration as such equals about 20× the water

solubility of isoxaflutole. No adverse lethal-, sub-lethal, behavioural or delayed effects were found by exposing adult honey bees for ten consecutive days exclusively to sugar solution, containing 120 ppm isoxaflutole (nominal).

In order to reveal whether isoxaflutole poses a risk to immature honey bee life stages, a bee brood feeding study has been conducted by following the provisions/method of Oomen P.A., de Ruijter, A. & van der Steen, J. (OEPP/EPPO Bulletin 22:613-616 (1992)), which require, amongst other parameters to "*...use formulated products only... products are fed at a concentration recommended for high-volume use...*". The honey bee brood feeding test is a worst-case screening test, by feeding the honey bees directly in the hive with a treated sugar solution which contains the test substance at a concentration typically present in the spray tank (and as such at a very high concentration) and by investigating the development of eggs, young and old larvae by employing digital photo imaging technology.

This particular study was conducted by mixing formulated isoxaflutole via Isoxaflutole WG 75 (together with formulated cyprosulfamide, as Cyprosulfamide SC 500), and the tested concentration corresponded to a typical concentration of isoxaflutole (and cyprosulfamide) via Isoxaflutole & Cyprosulfamide SC 240+240 present in the spray tank. The actual test concentration of isoxaflutole (and cyprosulfamide) was 250 mg/L. The administration of 1 litre sugar solution per colony, containing 250 ppm isoxaflutole (and cyprosulfamide) has not resulted in adverse effects. There were neither adverse acute or chronic effects on adult honey bees nor adverse effects on immature honey bee life stages (eggs, young larvae, old larvae, pupae) or on the colony itself. Neither mortality of worker bees and pupae (as assessed via dead bee traps) nor the termination rate of eggs, young larvae and old larvae (as assessed via digital imaging of individual marked cells) was statistically significantly different from the untreated control.

Synopsis

The calculated Hazard Quotients for both, isoxaflutole and Isoxaflutole & Cyprosulfamide SC 240+240 are well below the validated trigger value which would indicate the need for a refined risk assessment; no adverse effects on honey bee mortality are to be expected. This conclusion is confirmed by the results of the bee brood feeding study.

Regarding potential side effects of isoxaflutole (and cyprosulfamide) on immature honey bee life stages as well as on colony development, 250 ppm isoxaflutole (and cyprosulfamide), a concentration which corresponds to a typical concentration of isoxaflutole (and cyprosulfamide) via Isoxaflutole & Cyprosulfamide SC 240+240 present in the spray tank, has not resulted in adverse/statistically significant effects on mortality of worker bees and pupae nor in adverse/statistically significant effects on the termination rate of eggs, young larvae and old larvae (as assessed via digital imaging of individually marked cells) in the bee brood feeding study on colony level. Even at this very high concentration under the worst case conditions of the honey bee brood feeding test, no adverse effects on immature honey bee life stages were found; the findings in this study regarding the absence of chronic/delayed effects on adults honey bees are in line with the absence of adverse chronic effects on adult bees in the chronic 10 day laboratory feeding test with adult honey bees under laboratory conditions (at 120 ppm).

Overall, it can be concluded that isoxaflutole, when applied at the maximum application rate of 100 g a.s./ha (together with 100 g cyprosulfamide/ha acting as a herbicide safener) even during the flowering period of potentially bee-attractive weeds inside the cropping does not pose an unacceptable risk to honey bees and honey bee colonies.

CP 10.3.1.1 Acute toxicity to bees

CP 10.3.1.1.1 Acute oral toxicity to bees

Report:	[redacted]h; [redacted];2006, M-278327-01
Title:	Assessment of side effects of Isoxaflutole & Cyprosulfamide SC 240+240 g/L to the honey bee, Apis mellifera L., in the laboratory
Report No:	20061213/01-BLEU
Document No:	M-278327-01-1
Guidelines:	**OECD Guideline No. 213 and No. 214 (1998); US EPA OPPTS Guideline No. 850.3020**
Deviations:	Minor: For the contact toxicity test a 2 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item.
GLP/GEP:	**yes**

Objective:

The objective of this study was to determine the effect of the test item Isoxaflutole & Cyprosulfamide SC 240+240 g/L on the honey bee, *Apis mellifera* L., from oral and contact exposure.

Materials and Methods:

Test item: Isoxaflutole & Cyprosulfamide SC 240 + 240 g/L was tested [analysed content of active ingredients: Isoxaflutole: 20.5%w/w % and Cyprosulfamide: 20.5%w/w; Batch number: 2006-001042; density: 1.198 g/ml].

Under laboratory conditions, *Apis mellifera* (50 worker bees per dose; 10 individuals in 5 replicates per test item dose level, controls and reference item doses) were exposed for 48 hours to a single dose of 100.0 µg a.s./bee by topical application (contact limit test) and to a single dose of 110.76 µg a.s./bee by feeding (oral limit test; value based on the actual intake of the test item). In addition a control group (tap water in the contact test and 50% (w/v) aqueous sucrose solution) and a reference item (Perfekthion EC (= 400 g/L dimethoate) was tested. The test was conducted in the dark, temperature during the test was 25 to 26°C and relative humidity 58 to 62%. Biological observations including mortality and behavioural changes were recorded at 4, 24 and 48 hours after dosing.

Oral toxicity study

For the oral toxicity test Isoxaflutole & Cyprosulfamide SC 240+240 g/L was dissolved in tap water in order to get a stock solution. The final doses were prepared by mixing a defined amount of stock solution with a defined amount of a 50 % aqueous sucrose solution such that the intended nominal dose which was calculated for one bee was found in 20 µL. The amount of test item feeding solution was intentionally set 25 % higher as needed to achieve the nominal dose with the quantity of 250 µL offered per cage to compensate for a potential decrease in food uptake of bees frequently observed in such tests. Before the feeding started the bees starved for 2 hours. A quantity of 250 µL of test solution

was offered in Eppendorf cups for a minimum of 4 hours to each cage of 10 bees to ensure a sufficient intake of feeding solution. After 4 h and 30 minutes the total amount of feeding solution offered was consumed and replaced by untreated 50 % aqueous sucrose solution. The bees in one cage shared the test solution and so received similar doses. The amount of solution consumed (mean value of 10 bees) was determined by weighing the feeders before and after feeding. After the feeding period, the bees in the test cages were supplied *ad libitum* with a pure untreated 50 % aqueous sucrose solution.

Contact toxicity study

For the contact toxicity test Isoxaflutole & Cyprosulfamide SC 240+240 g/L was dissolved in tap water in order to get a stock solution. Bees were treated individually by topical application with a microapplicator. 2 µL of test item or reference item solution were applied dorsally to the thorax of each bee. After application the bees were returned to the test cages and fed with a 50 % aqueous sucrose solution *ad libitum*.

Dates of experimental work: July 07-July 13, 2006

Results:

Validity Criteria	**Recommended by the guideline**	**Obtained in this study**
Mortality in water control	≤ 10%	4%
Mortality in solvent control (sugar solution)	≤ 10%	0%
Contact test LD_{50} (24 h) of reference item	0.10 – 0.30 µg a.s./bee	0.17 µg a.s./bee
Oral test LD_{50} (24 h) of reference item	0.10 – 0.35 µg a.s./bee	0.12 µg a.s./bee

All validity criteria for the study were met.

Mortality and corrected mortality in the contact toxicity test with Isoxaflutole & Cyprosulfamide SC 240+240 g/L

dosage [µg a.s./bee]	Mortality [%]		Corrected mortality [%]	
	after 24 hours	**after 48 hours**	**after 24 hours**	**after 48 hours**
Control (water)	4.0	4.0	-	-
Test item	24.0	28.0	20.8	25.0
reference item				
0.10	2.0	6.0	-2.1	2.1
0.14	34.0	40.0	31.3	37.5
0.22	72.0	74.0	70.8	72.9
0.34	90.0	90.0	89.6	89.6

Mortality of the bees in the oral toxicity test with Isoxaflutole & Cyprosulfamide SC 240+240 g/L

Dosage [µg a.s./bee]	Intake of the reference item [µg a.s./bee]	Mortality [%] after 24 hours	Mortality [%] after 48 hours
Control (sugar solution)	-	0.0	0.0
Test item	110.76	0.0	4.0
reference item			
0.08	0.09	10	14
0.10	0.11	34	44
0.14	0.15	60	70
0.21	0.23	96	98

Toxicity to Honey Bees; laboratory tests

Test Item	**Isoxaflutole & Cyprosulfamide SC 480**	
Test object	***Apis mellifera***	
Application rate [µg a.s./bee]	**110.76**	**100.0**
Exposure	**oral**	**contact**
LD_{50} [µg a.s./bee]	> 110.76	> 100.0

Observations:

In the test item group the bees showed after 4 hours behaviour abnormalities in comparison to the control group. The bees were apathetic and showed uncoordinated movements.
At the assessments done after 24 and 48 hours no behaviour abnormalities could be attributed to the exposure of the test organisms to the test item.

Contact toxicity test:
At the dose of 100 µg a.s./bee which was tested in the contact toxicity test 28.0 % mortality (corrected mortality 25.0 %) was observed after 48 hours. In the control group 4.0 % mortality was observed after 48 hours.

Oral toxicity test:
In the oral toxicity test the dose of 100 µg a.s./bee corresponded to an actual intake of 110.76 µg a.s./bee. At this dose a mortality of 4.0 % was observed after 48 hours. In the control group which was fed with sugar solution no mortality occurred.

Conclusion:

The contact LD_{50} (48 h) was > 100.0 µg a.s./bee and the oral LD_{50} (48 h) was > 110.76 µg a.s./bee.

CP 10.3.1.1.2 Acute contact toxicity to bees

See point 10.3.1.1.1.

CP 10.3.1.2 Chronic toxicity to bees

A 10 day chronic oral toxicity study was conducted with Isoxaflutole WG 75, the corresponding summary is filed under KCA, point 8.3.1.2/01.

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

A honey bee brood feeding study ([redacted] *et al.*) has been conducted ([redacted], 2013, M-454689-01-1, filed under KCA 8.3.1.3 /01) by mixing formulated isoxaflutole as Isoxaflutole WG 75 together with formulated cyprosulfamide, the corresponding summary is filed under KCA, point 8.3.1.2/01.

CP 10.3.1.4 Sub-lethal effects

There is no particular study design / test guideline to assess "sub-lethal effects" in honey bees. 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

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

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[1]).

Table 10.3.2- 1: Endpoints used for risk assessment

Test species,	Tested Formulation, study type, exposure	Ecotoxicological Endpoint	Reference
Aphidius rhopalosiphi	CSA + IFT SC 480 Laboratory, glass plates 26 mL prod./ha 52 mL prod./ha 104 mL prod./ha 209 mL prod./ha 420 mL prod./ha	LR_{50} > 420 mL prod./ha Corr. Mortality [%] Effect on Reproduction [%] 12.1 25.2 3.4 -12.6[A] 5.2 27.6 17.2 46.0 17.2 72.4	[redacted], A., 2006 CW06/048 M-274830-01-1 KCP 10.3.2.1/01
Typhlodromus pyri	CSA + IFT SC 480 Laboratory, glass plates 26 mL prod./ha 52 mL prod./ha 104 mL prod./ha 209 mL prod./ha 420 mL prod./ha	LR_{50} > 420 mL prod./ha Corr. Mortality [%] Effect on Reproduction [%] -4.5[B] 2.8 5.7 -7.5[A] 6.8 -20.4[A] 1.1 -5.4[A] 17.0 -27.6	[redacted], A., 2006 M-279415-01-1 KCP 10.3.2.1/02
Aphidius rhopalosiphi	CSA + IFT SC 480 Extended Lab. exposure on potted barley plants 26 mL prod./ha 52 mL prod./ha 104 mL prod./ha 209 mL prod./ha 420 mL prod./ha	LR_{50} > 420 mL prod./ha Corr. Mortality [%] Effect on Reproduction [%] 0.0 -9.2 7.1 -7.6[A] 0.0 -68.4[A] 0.0 -46.8[A] 10.7 18.4	[redacted], A., 2006 M-279708-01-1 KCP 10.3.2.2/01
Chrysoperla carnea	CSA + IFT SC 480 Extended Lab. exposure on detached maize leaves Control 26 mL prod./ha 52 mL prod./ha 104 mL prod./ha 209 mL prod./ha 420 mL prod./ha	LR_{50} > 420 mL prod./ha Corr. Mortality [%] / Eggs/Female /Day / Hatching [%] - 28.0 99.0 -6.3[B] 22.9 97.4 3.8 21.4 99.3 -9.4[B] 21.8 98.1 -18.6[B] 18.7 97.8 -12.5[B] 23.4 96.5	[redacted], A., 2006 M-279861-01-1 KCP 10.3.2.2/02

A: A negative value indicates a higher reproduction rate in the treatment than in the control.
B: A negative value indicates a lower mortality in the treatment than in the control
C: A negative value indicates a higher percentage of wasps found on plants in the treatment than in the control.
sign.: statistically significant
n.sign.: not statistically significant

[1] 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

RISK ASSESSMENT FOR OTHER NON-TARGET ARTHROPODS

The tier 1 laboratory studies for *Aphidius rhopalosiphi* and for *Typhlodromus pyri* resulted in LR_{50} values >420 mL prod./ha. These values were used for the tier 1 risk assessment.

Table 10.3.2- 2: HQ for terrestrial non-target arthropods for the in-field scenario

Crop	Species	Appl. rate [mL/ha]	MAF	LR_{50} [mL/ha]	HQ	Trigger
Maize	*T. pyri*	417	1	>420	< 1.0	2
Maize	*A. rhopalosiphi*	417	1	>420	< 1.0	2

Table 10.3.2- 3: HQ for terrestrial non-target arthropods for the off-field scenario

Crop	Species	Appl. rate [mL/ha]	MAF	Drift [%]	VDF	Correc-tion factor	LR_{50} [mL/ha]	HQ	Trigger
Maize	*T. pyri*	417	1	2.77	10	10	>420	0.03	2
Maize	*A. rhopalosiphi*	417	1	2.77	10	10	>420	0.03	2

The HQ values calculated for the in-field and for the off-field risk assessment are below the trigger value of 2 indicating that no unacceptable adverse effects are to be expected from the use of IFT + CSA SC 480 according to the proposed use pattern.

Since reproduction effects of 72.4% were observed for *Aphidius rhopalosiphi* in the tier 1 study at 420 mL prod./ha additional extended lab studies were conducted for *Aphidius rhopalosiphi* and for *Chrysoperla carnea*, even though the tier 1 risk assessment based on the LR_{50} is considered to be protective concerning potential sublethal effects. These 2 additional extended laboratory studies confirmed the conclusion of the tier 1 risk assessment, since no relevant adverse effects on mortality or reproduction were observed up to and including the highest test rate of 420 mL prod./ha.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

Report:	[redacted]t; [redacted];2006;M-274830-01
Title:	Toxicity to the parasitoid wasp Aphidius rhopalosiphi (DeStephani-Perez) (Hymenoptera; Braconidae) in the laboratory - isoxaflutole & cyprosulfamide SC 240 + 240 g/l
Report No:	CW06/048
Document No:	M-274830-01-1
Guidelines:	**MEAD-BRIGGS ET AL. (2000), CANDOLFI ET AL. (2001)**
Deviations:	none
GLP/GEP:	**yes**

Objective:

The aim of the study was to determine the toxicity of freshly dried residues of Isoxaflutole & Cyprosulfamide SC 240 + 240 g/l applied onto glass cover slides to the parasitoid wasp *Aphidius rhopalosiphi*.

Materials and Methods:

Test item: A SC formulation of Isoxaflutole & Cyprosulfamide SC 240 + 240 g/l was tested, specified by batch number [analysed content of active ingredients: Isoxaflutole: 20.5%w/w % and Cyprosulfamide: 20.5%w/w; date of completed analysis:27 MAR 2006; Batch number: 2006-001042; specification number: 102000014305; TOX no.: 07429-00; density: 1.198 g/ml].

The test item was applied at rates of 26; 52; 104; 209 and 420 ml product/ha and the effects were compared to a toxic reference (a.i.: dimethoate) applied at 0.0003 l product/ha, and a water treated control.

Mortality of 60 adults was assessed 2, 24 and 48 hours after exposure.

From the water control and all dose rates of Isoxaflutole & Cyprosulfamide SC 240 + 240 g/l, 15 impartially chosen females per treatment were each transferred to a cylinder containing untreated cereal plants infested with *Rhopalosiphum padi* for a period of 24 hours. The number of mummies was assessed 14 days later.

Dates of experimental work: April 24 to May 09, 2006

Results:

Validity Criteria	Recommended by the guideline	Obtained in this study
Mortality in water control	≤ 13%	3.3%
Corrected mortality reference item	50 - 100%	100%
Mean reproduction per female in water control	≥ 5	5.8
No more than 2 wasps producing zero reproduction in water control	≤ 2	2

All validity criteria for the study were met.

Mortality (48 h after treatment) and reproduction

	Mortality [%]			Reproduction		
Treatment [L prod./ha]	**Uncorr.**	**Corr.**	**P-value ***	**Rate**	**Red. Rel. to control [%]**	**Wilcoxon statistic (#)**
control	3.3	0.0	-	5.8	0	
0.026	15.0	12.1	0.160 n.s.	4.3	25.3	n.s.
0.052	6.7	3.4	0.877 n.s.	6.5	-12.6	n.s.
0.104	8.3	5.2	0.877 n.s.	4.2	27.6	n.s.
0.209	20.0	17.2	0.041 s.	3.1	46	n.s.
0.420	20.0	17.2	0.041 s.	1.6	72.4	s.
Reference item (0.0003)	100.0	100.0	-	n.d.	n.d.	-

LD_{50}: > 0.420 l product/ha
* Fisher`s Exact test, two-sided, p-values are adjusted according to Bonferroni-Holm
# Wilcoxon Signed-Rank Test (significance level 0.05)
HOLLANDER, M. & WOLFE, D.A. (1999): Nonparametric statistical methods. - Wiley, New York.
n.d. not detected
n.s. not significant
s. significant

Observations:
In the highest dose rates of 420 and 209 ml product/ha 17.2% corrected mortality was observed. At the lower rates of 104 and 52 l product/ha 5.2% and 3.4% mortality were detected. At the rate of 26 ml product/ha 12.1% corrected mortality occurred.
The reduction in reproductive success relative to the control at the 420 ml and 209 ml product/ha rate was 72.4% and 46%. A reduction of 27.6%; -12.6% and 25.3% was detected at the 104; 52 and 26 ml product/ha rate of the test item.

Conclusion:

The LD_{50} was estimated to be > 0.420 ml product/ha.

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Report:	[redacted];2006;M-279415-01
Title:	Toxicity to the predatory mite Typhlodromus pyri SCHEUTEN (Acari, Phytoseiidae) in the laboratory; Isoxaflutole & Cyprosulfamide SC 240 + 240 g/l
Report No:	CW06049
Document No:	M-279415-01-1
Guidelines:	**BLUEMEL ET AL. (2000), CANDOLFI ET AL. (2001); US EPA OPPTS Guideline No. 850.SUPP**
Deviations:	none
GLP/GEP:	**yes**

Objective:

The aim of the study was to determine the toxicity of freshly dried residues of Isoxaflutole & Cyprosulfamide SC 240 + 240 g/l applied onto glass cover slides to the predatory mite *Typhlodromus pyri*.

Materials and Methods:

Test item: A SC formulation of Isoxaflutole & Cyprosulfamide SC 240 + 240 g/l was tested, specified by batch number [analysed content of active ingredients: Isoxaflutole: 20.5%w/w % and Cyprosulfamide: 20.5%w/w; date of completed analysis:27 MAR 2006; Batch number: 2006-001042; specification number: 102000014305; TOX no.: 07429-00; density: 1.198 g/ml].

The test item was applied at rates of 26; 52; 104; 209 and 420 ml product/ha and the effects were compared to a water control. A toxic reference (a.i.: dimethoate) applied at 10.2 ml product/ha was included to indicate the relative susceptibility of the test organisms and the test system.

Mortality of 100 protonymphs was assessed 1, 4, 7, 10, 12 and 14 days after exposure by counting the number of living and dead mites. The number of escaped mites was calculated as the difference from the total number exposed.

The reproduction rate of surviving mites was then evaluated over the period of 7-14 days after treatment by counting the total number of offspring (eggs and larvae) produced.

Dates of experimental work: July 27 to August 10, 2006

Results:

Validity Criteria	Recommended by the guideline	Obtained in this study
Mortality/Escape-rate in control group (day 7)	≤ 20%	12.0%
Average mortality in the reference item	50 - 100%	90.9%
Average number of eggs/female (calculated as sum of 4 assessment dates – from day 7	≥ 4	4.8

All validity criteria for the study were met

A summary of effects of Isoxaflutole & Cyprosulfamide SC 240 + 240 g/l on mortality and reproduction of *Typhlodromus pyri*

	Mortality [%]			Reproduction		
Treatment [L prod./ha]	**Uncorr.**	**Corr.**	**P-value ***	**Rate**	**Red. Rel. to control [%]**	**P-value #**
control	12.0	0.0	-	4.8	0	-
0.026	8.0	-4.5	1.000 n.s.	4.7	2.8	0.999 n.s.
0.052	17.0	5.7	1.000 n.s.	5.1	-7.5	0.973 n.s.
0.104	18.0	6.8	1.000 n.s.	6.1	-26.4	0.27 n.s.
0.209	13.0	1.1	1.000 n.s.	5.0	-5.4	0.992 n.s.
0.420	27.0	17.0	0.059 n.s.	6.1	-27.7	0.253 n.s.
Reference item (0.0102)	92.0	90.9	-	n.d.	n.d.	-

LD_{50}: > 0.420 l product/ha
* Fisher`s Exact test, two-sided, p-values are adjusted according to Bonferroni-Holm
# one-way ANOVA, p-values are adjusted according to Dunnett
n.d. not detected
n.s. not significant

Observations:

In the highest dose rate of 420 ml prod./ha of Isoxaflutole & Cyprosulfamide SC 240 + 240 g/l there was 17% corrected mortality. The reduction in reproductive success relative to the control at this rate was -27.7%. At the lower rates of 209, 104, 52 and 26 g product test item/ha 1.1, 6.8, 5.7 and – 4.5% corrected mortality were found and the reduction of reproduction was -5.4, -26.4, -7.5 and 2.8%.

Conclusion:

The LD_{50} was estimated to be > 0.420 ml product/ha.

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

Report:	[redacted];2006;M-279708-01
Title:	Toxicity to the parasitoid wasp Aphidius rhopalosiphi (DESTEPHANIPEREZ) (Hymenoptera: Braconidae) using an extended laboratory test; Isoxaflutole & Cyprosulfamide SC 240 + 240 g/l
Report No:	CW06/064
Document No:	M-279708-01-1
Guidelines:	**MEAD-BRIGGS ET AL. (2000), MEAD-BRIGGS ET AL. (draft 2006), CANDOLFI ET AL. (2001); US EPA OPPTS Guideline No. 850.SUPP**
Deviations:	none
GLP/GEP:	**yes**

Objective:

The aim of the study was to determine the toxicity of freshly dried residues of Isoxaflutole & Cyprosulfamide SC 240 + 240 g/l applied onto barley plants, to the parasitoid wasp *Aphidius rhopalosiphi*.

Materials and Methods:

Test item: A SC formulation of Isoxaflutole & Cyprosulfamide SC 240 + 240 g/l was tested, specified by batch number [analysed content of active ingredients: Isoxaflutole: 20.5%w/w % and Cyprosulfamide: 20.5%w/w; date of completed analysis:27 MAR 2006; Batch number: 2006-001042; specification number: 102000014305; TOX no.: 07429-00; density: 1.198 g/ml].

The test item was applied at rates of 26; 52; 104; 209 and 420 ml product/ha and the effects were compared to a water treated control. A toxic reference (a.i.: dimethoate) applied at 6.4 ml product/ha was included to indicate the relative susceptibility of the test organisms and the test system.

Mortality of 30 females was assessed 2, 24 and 48 hours after exposure.

Repellency of the test item was determined during the initial 2 h after the release of the females. Five separate observations were made at 30-minute intervals starting 0-15 minutes after the introduction of all wasps.

From the water control and all dose rates of Isoxaflutole & Cyprosulfamide SC 240 + 240 g/l, 15 impartially chosen females per treatment were each transferred to a cylinder containing untreated cereal plants infested with *Rhopalosiphum padi* for a period of 24 hours. The number of mummies was assessed 12 days later.

Dates of experimental work: September 04 to September 18, 2006

Results:

Validity Criteria	Recommended by the guideline	Obtained in this study
Mortality in water control	≤ 10%	6.7%
Corrected mortality reference item	> 50%	82.1%
Mean reproduction per female in water control	≥ 5	8.5
No more than 2 wasps producing zero reproduction in water control	≤ 2	0

All validity criteria for the study were met.

Mortality (48 h after treatment) and reproduction and repellency

	Mortality [%]			Reproduction		Repellency	
Treatment [L prod./ha]	**Uncorr.**	**Corr.**	**P-value ***	**Rate**	**Red. Rel. to control [%] #**	**% wasps on plant**	**Rel to control [%] #**
control	6.7	0	-	18.5	0	75.2	0
0.026	6.7	0	1.000 n.s.	11.5	-9.5 n.s.	87.3	-16.2 s.
0.052	13.3	7.1	1.000 n.s.	11.3	-7.6 n.s.	80.3	-6.9 n.s.
0.104	6.7	0	1.000 n.s.	17.7	-68.4 n.s.	80.7	-7.3 n.s.
0.209	6.7	0	1.000 n.s.	15.5	-46.8 n.s.	65.7	12.6 s.
0.420	16.7	10.7	1.000 n.s.	8.6	18.4 n.s.	85.2	-13.4 s.
Reference item (0.0003)	83.3	82.1		n.d.	n.d.	75	0.2

LD_{50}: > 0.420 l product/ha
* Fisher`s Exact test, two-sided, p-values are adjusted according to Bonferroni-Holm
# one-way ANOVA, p-values are adjusted according to Dunnett
n.d. not detected
n.s. not significant
s. significant

Observations:
In the dose rates of 0.420 and 0.052 l product/ha 10.7 and 7.1% corrected mortality was observed. At the rates of 0.209, 0.104 and 0.026 l product/ha no mortality was detected. The reduction in reproductive success relative to the control at the 0.420 l product/ha rate was 18.4%. No reduction was detected at all lower product/ha rates.
No statistically significant dose related repellent effect of the test item was observed.

Conclusion:

The LD_{50} was estimated to be > 0.420 ml product/ha.

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Report:	[redacted];2006;M-279861-01
Title:	Toxicity to the green lacewing Chrysoperla carnea STEPH. (Neuroptera, Chrysopidae) using an extended laboratory test; Isoxaflutole & Cyprosulfamide SC 240 + 240 g/l
Report No:	CW06/065
Document No:	M-279861-01-1
Guidelines:	**IOBC (Vogt et al, 2000);** **US EPA OPPTS Guideline No. 850.SUPP**
Deviations:	none
GLP/GEP:	**yes**

Objective:

The aim of the study was to determine the toxicity of freshly dried residues of Isoxaflutole & Cyprosulfamide SC 240 + 240 g/l applied onto detached maize leaves, to the green lacewing *Chrysoperla carnea*.

Materials and Methods:

Test item: A SC formulation of Isoxaflutole & Cyprosulfamide SC 240 + 240 g/l was tested, specified by batch number [analysed content of active ingredients: Isoxaflutole: 20.5%w/w % and Cyprosulfamide: 20.5%w/w; date of completed analysis:27 MAR 2006; Batch number: 2006-001042; specification number: 102000014305; TOX no.: 07429-00; density: 1.198 g/ml].
The test item was applied to maize leaves at rates of 26; 52; 104; 209 and 420 ml product/ha and the effects were compared to a water treated control. A toxic reference (a.i.: dimethoate) applied at 180 ml product/ha was included to indicate the relative susceptibility of the test organisms and the test system.
The preimaginal mortality was monitored over the duration of the study. The toxicity of the test item residues to the larvae and pupae are summarised on next page.
The fertility and fecundity of the surviving hatched adults were then evaluated over the period of one week.

Dates of experimental work: July 20 to August 23, 2006

Results:

Validity Criteria	Recommended by the guideline	Obtained in this study
Mortality in water control	≤ 20%	20.0%
Corrected mortality reference item	≥ 50%	62.5%
Mean number of eggs per female and day in water control	≥ 15	28.0
Mean hatching rate of the eggs (fertility) in water control	≥ 70%	99.0%

All validity criteria for the study were met.

Effects of IFT + CSA SC 480 on preimaginal mortality and reproduction of *Chrysoperla carnea* exposed on maize leaves

	Mortality [%]			Reproduction	
Treatment [L prod./ha]	**Uncorr.**	**Corr.**	**P-value ***	**Fertile eggs per female and day**	**Fertility [hatching rate in %]**
control	20.0	0.0	-	27.7	99
0.026	15.0	-6.3	1.000 n.s.	22.3	97.4
0.052	23.1	3.8	1.000 n.s.	21.3	99.3
0.104	12.5	-9.4	1.000 n.s.	21.4	98.1
0.209	5.1	-18.6	0.435 n.s.	18.1	97.8
0.420	10.0	-12.5	1.000 n.s.	22.5	96.5
Reference item (0.18)	70.0	62.5	-	n.d.	n.d.

LD_{50}: > 0.420 l product/ha
* Fisher`s Exact test, two-sided, p-values are adjusted according to Bonferroni-Holm.
n.d. not detected
n.s. not significant

Mortality:

In all tested rates of the test item more than 32 larvae pupated and more than 29 developed into adults.
In the rates of 26 and 52 ml product/ha 36 and 33 larvae pupated; 32, respectively 30 of them developed into adults. In the rates of 104, 209 and 420 ml product/ha 38, 38 and 36 larvae pupated. Of these pupae, 35, 37 and 36 hatched successfully.
In the control 33 larvae pupated and 32 pupae developed successfully into adults. In the reference item 13 larvae pupated and 11 developed into adult lacewings.
When preimaginal mortality was corrected for control mortality, the corrected figures for all rates of the test item were below 4%.
For the rates of 420 ml product/ha and 209 ml product/ha the corrected mortality was -12.5 and -18.6%. For the rate of 104 ml product/ha it was -9.4%. And for 52 ml and 26 ml product/ha the corrected mortality was 3.8 and -6.3%.
For the reference item 62.5% preimaginal mortality occurred.

Reproduction:

The mean number of fertile eggs per female and day for the control during the test period was 27.7. The hatching rate (= fertility) of the eggs was 99.0 %.
The mean number of fertile eggs per female and day for the 26 ml product/ha rate was 22.3 with a hatching rate of 97.4%. In the rate 52 ml product/ha 21.3 fertile eggs were laid with a hatching rate of 99.3%. The mean number of fertile eggs in the rates 104 product/ha and 209 ml product/ha was 21.4, resp. 18.1 with hatching rates of 98.1% and 97.8%. In the highest rate of 420 ml product/ha 22.5 fertile eggs per female and day were laid with a hatching rate of 96.5%.

Conclusion:

The LD_{50} was estimated to be > 0.420 ml product/ha.

Report:	[redacted]5; [redacted]; [redacted]; [redacted]; [redacted]; [redacted]; [redacted];2012;M-462928-01
Title:	Reproductive and toxicological impacts of herbicides used in Eucalyptus culture in Brazil on the parasitoid Palmistichus elaeisis (Hymenoptera: Eulophidae)
Report No:	M-462928-01-1
Document No:	M-462928-01-1
Guidelines:	**not applicable**
GLP/GEP:	**no**
Classification:	b) supplementary information (EFSA Journal 2011;9(2):2092)

EXECUTIVE SUMMARY

The effect of herbicides used in eucalyptus crops on the parasitoid *Palmistichus elaeisis* Delvare and LaSalle, 1993 (Hymenoptera: Eulophidae) was evaluated in terms of the impact on reproduction and survival. Treatments consisted of commercial doses of the herbicide isoxaflutole with a water-only control. The herbicide was sprayed on the pupae of the alternative host *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae), which were exposed to parasitism by six females of *P. elaeisis* per pupa. Isoxaflutole resulted in higher numbers of individuals and females produced per female; thus this herbicide was less harmful to *P. elaeisis* and maybe used in IPM programmes in eucalyptus plantations.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Product "Fordor" (750 g isoxaflutole/kg)
Active substance(s):	Isoxaflutole
Source of test item:	Bayer S.A.
Lot/Batch number:	Not reported
Purity:	Not reported
Storage conditions:	Not reported

2. Test solutions

Vehicle/solvent:	Fordor as commercial product
Source of vehicle/solvent:	Not reported
Concentration of vehicle/solvent:	200 L/ha of herbicide solution

3. Test organism(s)

Species:	*Palmistichus elaeisis*
Cultivar:	Not reported
Source of test species:	Not reported
Age of test organisms at study initiation / Crop growth stage at treatment:	Pupae of *Tenebrio molitor* (prey species) at 24–72 h of age were each exposed to six parasitoid females for 48 h in a constant environment room. The emerging parasitoids were collected and used in the experiment.
Holding conditions prior to test:	reared in 14 · 2.2 cm glass test tubes, along with a drop of honey and capped with a cotton ball
Acclimatisation:	Not applicable

B. Study design and methods

1. Test procedure

Test system (study type):	Laboratory study
Duration of study:	30 d of parasitism
Treatments:	
Test concentrations	200 L/ha test item
Number of replicates:	Ten replicates
Individuals per replicate:	One pupae of *T. molitor* and six 72-h-olf females of *P. elaeisis*
Test units (type and size):	Sixty T. molitor pupae at 48 h old, with an average weight of 102.33 g and a mean surface area of 7.85×10^{-5} m² were sprayed.
Application / device / nozzles:	electronic pressure sprayer
Water volume:	Not reported
Calibration of sprayer:	spray pressure of 300 kPa and zero wind speed

2. Environmental conditions

Test medium:	polystyrene tray with wheat bran and sugarcane pieces
Temperature / relative humidity:	25 ± 2°C / 70 ± 10%
Photoperiod:	12h
Lighting	500 lux

3. Observations and measurements

Analytical parameters measured:	none
Biological parameters measured:	Longevity was evaluated daily. The duration of the life cycle (egg to adult), the percentage of parasitism (discounting the natural mortality of the host) (Abbott, 1925), the percentage of emergent progeny, number of individuals emerged, number of males and females, sex ratio, width of the head capsule and body length of parasitoids emerging from each pupa of *T. molitor* were obtained from parasitised pupae.
Measurement frequency:	Daily the longevity and other parameters at the end of the test
Statistical analyses:	Anova and Kruskal-Wallis test

RESULTS AND DISCUSSION

1. Validity criteria:

No validity criteria were reported.

2. Biological findings:

The reproductive responses and survival of *P. elaeisis* to isoxaflutole were similar to the water control. A statistically significant reduction of body length in females and males in the isoxaflutole treatment group of 2-3% compared to the control group has been observed.

The results are summarised in the tables below:

Table 1: Reproductive parameters of the first generation of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) from pupae of *Tenebrio molitor* (Coleoptera: Tenebrionidae) treated with isoxaflutole registered for the cultivation of eucalyptus and the water control

	Duration of life cycle [d]	Parasitism [%]	Emergence [%]	No of Indiv per pupae	Females produced by female	Female length [mm]	Male length [mm]	Female longevity [d]	Male longevity [d]	Sex ratio
Isoxaflutole	27.3 +/-0.85	100.0	100.0	91.1 +/-35.1	12.1 +/-0.60	1.92 +/- 0.06*	1.39 +/-0.07*	30.1 +/- 3.78	27.8 +/-3.50	0.81 +/- 0.09
Water	27.0 +/-0.80	100.0	100.0	95.3 +/-38.1	12.9 +/-0.55	1.96 +/- 0.07*	1.43 +/-0.04*	24.2 +/-2.49	19.4 +/-3.21	0.80 +/- 0.08

*significant reduction

Table 2: Survival (%) of *Palmistichus elaeisis* adults from 0 to 96 h after exposure for 48 h to pupae treated with isoxaflutole registered for eucalyptus culture in Brazil

	0	24	48	72	96
Isoxaflutole	100.0	96.7	96.7	96.7	96.7
Water	100.0	100.0	95.0	95.0	95.0

CONCLUSION
Isoxaflutole resulted in higher numbers of individuals and females produced per female; thus this herbicide was less harmful to *P. elaeisis* and maybe used in IPM programmes in eucalyptus plantations.

Comment by the Notifier
Study results indicate a low toxicity of the compound as already indicated by the available regulatory studies and has therefore been classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

CP 10.3.2.3 Semi-field studies with non-target arthropods

No semi-field studies were deemed necessary.

CP 10.3.2.4 Field studies with non-target arthropods

No 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

Table 10.4- 1: Initial maximum PEC_{soil} values

Compound	Maize (pre-emergence)
	$PEC_{soil, max}$ [mg/kg]
IFT + CSA SC 480	0.656 [a]
isoxaflutole	0.1333
RPA 202248	0.1333
RPA 203328	0.0617

[a] PEC_{soil} is calculated based on an application rate of 0.417 L product/ha, a density of 1.18 g/mL, a bulk density of 1.5 g/cm^3 and 0% crop interception

CP 10.4.1 Earthworms

Table 10.4.1- 1: Endpoints used in risk assessment

Test item	Test species, test design	Ecotoxicological endpoint		Reference
IFT + CSA SC 480	*Eisenia fetida* reproduction 56d, mixed	NOEC **$NOEC_{corr}$**	56 mg form./kg dws **28 mg form./kg dws**	[redacted] (2014) M-470232-01-1 KCP 10.4.1.1 /01

Table 10.4.1- 2: Endpoints used in risk assessment

Test item	Test species, test design	Ecotoxicological endpoint		Reference
Isoxaflutole (tech.)	*Eisenia fetida* reproduction 56 d, mixed	NOEC **$NOEC_{corr}$**	17.8 mg a.s./kg dws **8.9 mg a.s./kg dws[a]**	[redacted] (2013) M-450435-01-1 KCA 8.4.1/01
RPA 202248	*Eisenia fetida* reproduction 56 d, mixed	**NOEC**	**16 mg p.m./kg dws**	[redacted] (2012) M-442776-01-1 KCA 8.4.1/02
RPA 203328	*Eisenia fetida* reproduction 56 d, mixed	**NOEC**	**≥1000 mg p.m./kg dws**	[redacted] (2004) M-230530-01-1 KCA 8.4.1/03

Bold values: endpoints used for risk assessment
dws = dry weight soil; a.s. = active substance; prod. = product; corr. = corrected
[a] corrected by factor of 2 due to lipophilic substance (log P_{ow} > 2)

RISK ASSESSMENT FOR EARTHWORMS

Table 10.4.1- 3: TER calculations for earthworms

Compound	Species	Endpoint [mg/kg]	$PEC_{soil,max}$ [mg/kg]	TER_{LT}	Trigger
Bare soil/maize					
IFT + CSA SC 480	Earthworm, reproduction	NOEC 28	0.656	43	5
Isoxaflutole	Earthworm, reproduction	NOEC 8.9[a]	0.1333	67	5
RPA 202248	Earthworm, reproduction	NOEC 16	0.1333	120	5
RPA 203328	Earthworm, reproduction	NOEC ≥ 1000	0.0017	16207	5

[a] Endpoint divided by factor 2

Overall, there is no unacceptable risk for earthworms from the exposure to isoxaflutole.

CP 10.4.1.1 Earthworms sub-lethal effects

Report:	[redacted];2013;M-470232-01
Title:	Isoxaflutole + cyprosulfamide SC 480 (240+240) G: Effects on reproduction and growth of earthworms Eisenia fetida in artificial soil
Report No:	85481022
Document No:	M-470232-01-1
Guidelines:	OECD Guideline for the testing of chemicals No. 222, Earthworm, Reproduction Test (adopted April 13, 2004); ISO Guideline 11268-2, Soil quality - Effects of pollutants on earthworms - Part 2: Determination of effects on reproduction of Eisenia fetida/Eisenia andrei, International Organization for Standardization, 2012
Deviation	**none**
GLP/GEP:	**yes**

Material and Methods:

Test Item: Isoxaflutole + cyprosulfamide SC 480 (240+240) G; short name: IFT+CSA SC 480 (240+240) G; Batch ID.: NK22BX0239; BCS-Code: BCS-AH21981 + BCS-AT21719; content of a.i.s: isoxaflutole (AE B197278): 20.0% w/w (238.8 g/L), cyprosulfamide (AE 0001789): 19.8% w/w (236.5 g/L); density: 1.193 g/ml.

Test Species: Earthworm (*Eisenia fetida*), adult worms (with clitellum and weight range 302 to 600 mg), approximately 8 months old,

Source: From an in-house culture.

Test Design: 56-day test in treated artificial soil prepared according to OECD 222; different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments and 8 replicates for the control with 10 worms each.

Assessment of adult worm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult worms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application).

Endpoints: Mortality, weight change, feeding activity and reproduction rate were determined.

Reference Item:	Luxan Carbendazim 500 FC (Carbendazim, 500 g/L nominal). The effects of the reference item were investigated in a separate study.
Test Concentrations:	Control, 32, 56, 100, 178 and 316 mg isoxaflutole + cyprosulfamide SC 480 (240+240) G/kg soil[2].
Test Conditions:	Artificial soil according to OECD 222; initial pH 5.5 to 5.8, pH at experimental end 6.0 to 6.2; water content 30.3% to 31.0% (55.1% to 56.4% of maximum water holding capacity, WHC) at experimental start and 30.7% to 34.2% (55.7% to 62.2% of the maximum WHC) at experimental end; temperature: within the range of 18 °C to 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 400 lux to 800 lux.
Statistics:	Standard procedures, Fisher's Exact Test (mortality), Williams t-test (weight changes and reproduction), Probit Analysis (EC_{10} and EC_{20}).

Results and Discussion:

All study validity criteria were met.
No statistically significant mortality was observed in any treatment group compared to the control.
The body weight changes of the earthworms after 4 weeks exposure to isoxaflutole + cyprosulfamide SC 480 (240+240) G were not statistically significantly different compared to the control up to and including the test concentration of 56 mg test item/kg soil (Williams t-test, α = 0.05, two-sided). At the concentration of 100 mg test item/kg soil and above the body weight change was statistically significantly reduced compared to the control.
The reproduction rates were not statistically significantly different compared to the control up to and including the test concentration of 56 mg test item/kg soil (Williams t-test, α = 0.05, one-sided smaller). At the concentration of 100 mg test item/kg soil and above the reproduction was statistically significantly reduced compared to the control.
No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control (see Table 1).

Table 1. Effect of Isoxaflutole + cyprosulfamide SC 480 (240+240) G on earthworms (*Eisenia fetida*) in a 56-day reproduction study

Isoxaflutole + cyprosulfamide SC 480 (240+240) G [mg/kg soil dry weight]	**Control**	**32**	**56**	**100**	**178**	**316**
Mortality (day 28) [%]	0.0	0.0	0.0	0.0	2.5	2.5
Significance [1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.
Weight change (day 28) [%]	+ 28.5	+ 27.3	+ 21.0	+ 14.5	+ 7.4	+ 5.1
Significance [2)]	-	n.s.	n.s.	*	*	*
Mean No. of juveniles (day 56)	340	328	326	285	239	224
Significance [1) 2) 3)]	-	n.s.	n.s.	*	*	*
Reproduction in [%] of control (day 56)	-	96.5	95.8	83.7	70.1	65.9
Food consumption [g]	25.0	25.0	25.0	24.0	23.5	22.3

[2] All concentrations are indicated per kg soil dry weight.

Table 1. Effect of Isoxaflutole + cyprosulfamide SC 480 (240+240) G on earthworms (*Eisenia fetida*) in a 56-day reproduction study

Isoxaflutole + cyprosulfamide SC 480 (240+240) G [mg/kg soil dry weight]	Control	32	56	100	178	316
Endpoints [mg/kg soil dry weight]						
NOEC (day 28 mortality and weight)	56					
NOEC (day 56 reproduction)	56					
EC Values (reproduction) 3)	EC_{10}			EC_{20}		
	66.4			136.2		

- = not applicable
n.s. = not significantly different compared to the control
* = significantly different compared to the control
1) Fisher's Exact Test, α = 0.05, one-sided greater
2) Williams t-test, α = 0.05, two-sided for weight changes and one-sided smaller for reproduction
3) Probit analysis

Conclusion:

In an earthworm reproduction and growth study with isoxaflutole + cyprosulfamide SC 480 (240+240) G the No Observed Effect Concentration (NOEC) for mortality, growth, reproduction and feeding activity of the earthworm *Eisenia fetida* was determined to be 56 mg test item/kg soil dry weight. The EC10 was determined to be 66.4 mg test item/kg soil dry weight (95% confidence limits of 10.6 to 109.4 mg test item/kg soil dry weight) and the EC20 was determined to be 136.2 mg test item/kg soil dry weight (95% confidence limits of 62.8 to 199.9 mg test item/kg soil dry weight).

Reference Item Test:

In the most recent test with the reference item Luxan Carbendazim 500 FC (performed under IBACON Study Number 46646022 from August 2013 to October 2013), there were statistically significant effects on reproduction at a concentration of 1.30 mg carbendazim/kg soil and higher; the EC50 for reproduction was calculated as 1.32 mg carbendazim/kg soil. The results are shown in Appendix 2.

CP 10.4.1.2 Earthworms field studies

Based on the isoxaflutole results presented above no field studies were necessary.

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

Testing on springtails (*Folsomia candida*) and soil mites (*Hypoaspis aculeifer*) was performed with the parent compound and two soil metabolites of isoxaflutole. The corresponding summaries are provided below under point 8.4.2.1.

RPA 205834 is a major soil metabolite only in anaerobic soil. Isoxaflutole is only applied in the spring/summer months when anaerobic conditions would not occur. Since RPA 205834 is only formed direct from isoxaflutole and isoxaflutole is rapidly degraded in soil no formation of RPA 205834

would be likely in the winter period when anaerobic conditions could occur. Therefore no studies with soil organisms are considered necessary and no risk assessment is performed.

Table 10.4.2- 1: Endpoints used in risk assessment

Test item	Test species, test design	Ecotoxicological endpoint		Reference
Collembola, reproduction				
Isoxaflutole (tech.)	*Folsomia candida* reproduction 28 d, mixed	NOEC	≥1000 mg a.s./kg dws	[redacted] (2011) M-416012-01-1 KCA 8.4.2/02
		$NOEC_{corr}$	**≥500 mg a.s./kg dws[A]**	
RPA 203328	*Folsomia candida* reproduction 28 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] (2011) M-420062-01-1 KCA 8.4.2/06
RPA 202248	*Folsomia candida* reproduction 28 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] (2011) M-420112-01-1 KCA 8.4.2/04
Soil mites, reproduction				
Isoxaflutole (tech.)	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC	562 mg a.s./kg dws	[redacted] (2011) M-416751-01-1 KCA 8.4.2/03
		$NOEC_{corr}$	**281 mg a.s./kg dws[A]**	
RPA 203328	*Hypoaspis aculeifer* reproduction 14 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] (2011) M-419849-01-1 KCA 8.4.2/07
RPA 202248	*Hypoaspis aculeifer* reproduction 14 d, mixed	**NOEC**	**≥100 mg/kg dws**	[redacted] (2011) M-417912-01-1 KCA 8.4.2/05

dws = dry weight soil; a.s. = active substance; corr. = corrected
Bold values: endpoints used for risk assessment
[A] Corrected by factor of 2 due to lipophilic substance (log P_{ow} > 2)

RISK ASSESSMENT FOR OTHER NON-TARGET SOIL MESO- AND MACROFAUNA (OTHER THAN EARTHWORMS)

Table 10.4.2- 2: TER calculations for other non-target soil meso- and macrofauna

Compound	Species	Endpoint [mg/kg]		$PEC_{soil,max}$ [mg/kg]	TER_{LT}	Trigger
Bare soil/maize (pre-emergence)						
isoxaflutole	*Folsomia candida*	NOEC	≥ 500[a]	0.1333	3750	5
	Hypoaspis aculeifer	NOEC	281[a]	0.1333	2108	
RPA 202248	*Folsomia candida*	NOEC	≥ 100	0.1333	750	5
	Hypoaspis aculeifer	NOEC	≥ 100	0.1333	750	
RPA 203328	*Folsomia candida*	NOEC	≥ 100	0.0617	1621	5
	Hypoaspis aculeifer	NOEC	≥ 100	0.0617	1621	

[a] Endpoint divided by factor 2

Overall, there is no unacceptable risk for collembola and soil mites from exposure to isoxaflutole.

CP 10.4.2.1 Species level testing

No studies are required.

CP 10.4.2.2 Higher tier testing

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

CP 10.5 Effects on soil nitrogen transformation

Table 10.5- 1: Endpoints used in risk assessment

Test substance	Test species	Endpoint		Reference
IFT + CSA SC 480	Nitrogen transformation, 28 d	No influence	0.67 mg prod./kg soil [a] 3.35 mg prod./kg soil [a]	[redacted] (2006) M-280499-01-1 KCP 10.5/01
Isoxaflutole	Nitrogen transformation, 28 d	No influence	0.2 mg a.s./kg soil 1.0 mg a.s./kg soil	[redacted] (1994) M-213129-01-1 KCA 8.5/01
RPA 202248	Nitrogen transformation, 84 d	No influence	0.13 mg a.s./kg soil 0.67 mg a.s./kg soil	[redacted] (2013) M-469915-01-1 KCA 8.5/04
RPA 203328	Nitrogen transformation, 28 d	No influence	0.1 mg a.s./kg soil	[redacted], A. (1997) M-158741-01-1 KCA 8.5/02

[a] based on product density of 1.198 g/mL

RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION

Table 10.5- 2: Risk Assessment for soil micro-organisms

Compound	Species	Endpoint [mg/kg]	$PEC_{soil,max}$ [mg/kg]	Refinement required
IFT + CSA SC 480	Soil micro-organisms	3.35	0.696	No
Isoxaflutole	Soil micro-organisms	1.0	0.1333	No
RPA 202248	Soil micro-organisms	0.67	0.1333	No
RPA 203328	Soil micro-organisms	0.1	0.0617	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:	[redacted]; [redacted]; 2006; M-280499-01
Title:	Isoxaflutole & cyprosulfamide SC 240+240: Determination of effects on nitrogen transformation in soil
Report No:	LRT-N-76/06
Document No:	M-280499-01-1
Guidelines:	**OECD/OCDE No. 216; adopted: 21st January 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test; US EPA OPPTS Guideline No. 850.SUPP**
Deviations:	none
GLP/GEP:	**yes**

Objective:

The aim of the study was to determine the influence of Isoxaflutole & Cyprosulfamide SC 240+240/kg dry weight soil on nitrogen transformation in an agricultural soil.

Materials and Methods:

Test item: Isoxaflutole & Cyprosulfamide SC 240+240 (analytical findings: Isoxaflutole, 246 g/L, Cyprosulfamide, 245 g/L; Specification No.: 102000014305, batch No.: 2006-001042, Master recipe ID: 0034474-001, TOX-No.: 07429-00).
A silty sand soil was exposed for 28 d to 0.56 µL and 2.80 µL Isoxaflutole & Cyprosulfamide SC 240+240/kg dry weight soil. Application rates were equivalent to 0.42 L and 2.1 L Isoxaflutole & Cyprosulfamide SC 240+240/ha. Lucerne-grass-green meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation.

Dates of experimental work: August 15 to September 20, 2006

Results:

Validity criteria:

Validity Criteria	Recommended by the guideline	Obtained in this study
coefficient of variation (CV) between nitrate-N concentration in replicate control samples	≤ 15%	12%

All validity criteria for the study were met.

Effects of IFT + CSA SC 480 on non-target soil microorganisms

Test item	Isoxaflutole & Cyprosulfamide SC 240+240	
Test object	Soil Micro-organisms Nitrogen-Transformation (silty sand soil)	
Exposure	28 days	
µL test item/kg dry weight soil	0.56	2.80
Final results: Difference in rates of nitrogen formation (%) between control and treatment groups	-6 n.s.)	-12 n.s.)

n.s.) No statistically significant difference to the control (t-test, α= 0.05)

During the 28-day test, the single application rate of Isoxaflutole & Cyprosulfamide SC 204+240 and the 5-fold dose of the compound had a temporary influence on nitrogen transformation in a silty sand soil supplemented with Lucerne-grass-green meal (0-7 and 7-14 day intervals). At the end of the study (14-28 day interval) differences in the nitrate rates between control soil samples and treated soil samples are < 25 % and meet the trigger values of the above mentioned guideline for a termination of the study.

Conclusion:

If used as recommended, Isoxaflutole & Cyprosulfamide SC 240+240 should not have an impact on nitrogen transformation in soils.

CP 10.6 Effects on terrestrial non-target higher plants

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev2 final, 2002). It is restricted to off-field situations as non-target plants are non-crop plants located outside the treated area. Spray drift from treated areas may lead to residues of a product in adjacent off-crop areas.

RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS

Table 10.6- 1: Endpoints used in risk assessment

Test organism	Study type	Lowest ER_{50} (mL prod/ha)	Most sensitive species	Parameter	References
Terrestrial non-target plants; 11 species	vegetative vigour; Tier 2 dose response 21 days	> 6.6	sugar beet	dry weight	[redacted] et al., 2007; M-283816-01-1 KCP 10.6.2/01
Terrestrial non-target plants; 11 species	seedling emergence; Tier 2 dose response 21 days	19.7	sugar beet	dry weight	[redacted] et al., 2007; M-283723-01-1 KCP 10.6.2/02

The lowest ER_{50} of > 6.6 mL prod./ha was obtained in the vegetative vigour study. Moreover, endpoints in the vegetative vigour study were generally lower than those in the seedling emergence test. With seedling emergence, interception by off-crop vegetation will further reduce exposure. For these reasons, the risk assessment is clearly driven by vegetative vigour and subsequent TER calculations are confined to the lowest endpoint of the vegetative vigour test.

Table 10.6- 2: TER calculations for non-target terrestrial plants based on the lowest ER_{50} of > 0.0066 L product/ha (vegetative vigour)

Distance from the field edge [m]	Drift with conventional spraying equipment [%]	PER obtained with conventional spraying equipment [L/ha]	TERs			
			conv.	50% red.	75% red.	90% red.
Application rate 0.417 L product/ha, single application						
1	2.77	0.0115	**0.6**	**1.1**	**2.3**	5.7
5	0.57	0.0024	**2.8**	5.6	11.1	27.8
10	0.29	0.0012	5.5	10.9	21.8	54.6

It is concluded that the use of the product will not cause unacceptable effects on terrestrial non-target plants growing near treated fields if one of the following mitigation measures is applied: 1) 90 % drift reducing nozzles, no in-crop buffer required; 2) 50% drift reducing nozzles + 5 m in-crop buffer; 3) conventional nozzles + 10 m in-crop buffer.

CP 10.6.1 Summary of screening data

No new studies were deemed necessary.

CP 10.6.2 Testing on non-target plants

Report:	[redacted]u; [redacted]; [redacted]; [redacted]; [redacted];2007;M-283816-01
Title:	Isoxaflutole + cyprosulfamide SC 240 + 240 g/L - effects on eleven species of non-target terrestrial plants: vegetative vigour test (Tier 2)
Report No:	VV 06/034
Document No:	M-283816-01-1
Guidelines:	OECD 227 (2005) FIFRA Guideline 123-1
Deviations:	none
GLP/GEP:	**yes**

Objective:

The aim of the study was to determine the effect of Isoxaflutole + Cyprosulfamide SC 240 + 240 g/L on the vegetative vigour of eleven plant species representing a broad range of both dicotyledonuous and monocotyledonous plant families over a 21 day period.

Materials and methods:

Test item: Isoxaflutole & Cyprosulfamide SC 240+240 (analytical findings: Isoxaflutole, 246 g/L, Cyprosulfamide, 245 g/L; Specification No.: 10200001-4305, batch No.: 2006-001042, Master recipe ID: 0034474-001, TOX-No.: 07429-00).

Plants at the 2-4 leaf stage of eleven species of non-target terrestrial plants (4 monocots and 7 dicots) were sprayed with Isoxaflutole & Cyprosulfamide SC 240 + 240 g/L. The seven dicotyledonous species are: sugar beet (*Beta vulgaris*), oilseed rape (*Brassica napus*), cucumber (*Cucumis sativus*), buckwheat (*Fagopyrum esculentum*), soybean (*Glycine max*), sunflower (*Helianthus annuus*) and tomato (*Lycopersicum esculentum*) plus four monocotyledonous species: oat (*Avena sativa*), ryegrass (*Lolium perenne*), corn (*Zea mays*) and onion (*Allium cepa*).

Solutions of Isoxaflutole + Cyprosulfamide SC 240 + 240 g/L and serial dilutions were sprayed on the plants surface with application rates ranging from 420 mL product/ha down to 0.2 mL product/ha at a volume rate of 100 L/ha.

Four or five plants were grown in 13 cm diameter pots and there were 8 or 10 pots (replicates for each species) giving a total of 40 plants per treatment level. The test duration was 21 days following treatment of the test item. Spray treatments were applied once to each species at test initiation with a laboratory track sprayer set at the nominal spray volume of 100 litres/ha. Control plants were sprayed with deionised water.

For corn, ryegrass, tomato and oat the employed rates were: 420, 210, 105, 52.5, 26.3 and 13.1 mL product/ha; for cucumber: 210, 105, 52.5, 26.3, 13.1 and 6.6 mL product/ha; for onion, oilseed rape and buckwheat: 105, 52.5, 26.3, 13.1, 6.6 and 3.3 mL product/ha. For sunflower: 105, 52.5, 26.3, 13.1, 6.6, 3.3, 1.64, 0.82 and 0.41 mL product/ha. For soybean: 52.5, 26.3, 13.1, 6.6, 3.3, 1.64, 0.82 and 0.41 mL product/ha. For sugar beet: 26.3, 13.1, 6.6, 3.3, 1.64, 0.82, 0.41 and 0.2 mL product/ha.

Pots were maintained under glasshouse conditions with a temperature control set at 23 ± 8°C during day and 18 ± 8°C at night with a 16 h photoperiod.

Visual observations for survival, phytotoxicity were made on test days 7, 14 and 21. Final assessments were made growth stage, shoot length and shoot biomass (dry weight) 21 days after application against the untreated controls.

Dates of experimental work: September 13, 2006 to January 29, 2007

Results:

Analysis of the highest application rate revealed it to be 93.9 – 94.9% of nominal.
This vegetative vigour study was valid with NOERs obtained for 11 species and with all endpoints and the fulfilled criteria for individual percent of emergence and 90% survival of emerged seedlings during the study period of the controls for all species.
Typical symptoms with Isoxaflutole & Cyprosulfamide SC 240 + 240 g/L observed in this study were necrosis, bleaching and leaf deformation. However in less sensitive species they did not occur at all or were only apparent on the higher treatment levels tested.

Effects of Isoxaflutole & Cyprosulfamide SC 480 on vegetative vigour of 11 plant species

Plant species	mL product / ha					
	Survival		Shoot lenght		Shoot dry weight	
	NOER	EC_{50}	NOER	EC_{50}	NOER	EC_{50}
Buckwheat	105	>105	105	>105	105	>105
Cucumber	210	>210	210	>210	105	>210
Oilseed rape	105	>105	26.3	>105	6.6	43.0
Soybean	26.3	>26.3	26.3	>26.3	1.6	>26.3
Sugar beet	26.3	>26.3	26.3	>26.3	0.8	>6.6
Sunflower	105	>105	6.6	>105	6.6	65.3
Tomato	420	>420	420	>420	105	>420
Corn	420	>420	420	>420	420	>420
Oat	420	>420	420	>420	105	>420
Onion	105	105	105	>105	13.1	>105
Ryegrass	420	>420	420	>420	420	>420

Conclusion:

The most sensitive ER_{50} was obtained for biomass of sugar beet with a value of >6.6 mL product/ha.
The lowest NOER was 0.8 mL product/ha, for sugar beet biomass.

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Report:	[redacted];2007;M-283723-01
Title:	Isoxaflutole + cyprosulfamide SC 240 + 240 g/L - effects on eleven species of non-target terrestrial plants: seedling emergence and seedling growth test (tier 2)
Report No:	SE 06/033
Document No:	M-283723-01-1
Guidelines:	**US EPA Subdivision J, §123-1** **OECD 208 (revised draft March 2005)**
Deviations:	none
GLP/GEP:	**yes**

Objective:

The aim of the study was to determine the effect of Isoxaflutole + Cyprosulfamide SC 240 + 240 g/L on the seedling emergence and seedling growth of eleven plant species representing a broad range of both dicotyledonuous and monocotyledonous plant families over a 21 day period.

Materials and methods:

Test item: Isoxaflutole & Cyprosulfamide SC 240+240 (analytical findings: Isoxaflutole, 246 g/L, Cyprosulfamide, 245 g/L; Specification No.: 102000014305, batch No.: 2006-00-1042, Master recipe ID: 0034474-001, TOX-No.: 07429-00).

In total, seeds of eleven species were tested, including seven dicotyledonous species: sugar beet (*Beta vulgaris*), oilseed rape (*Brassica napus*), cucumber (*Cucumis sativus*), buckwheat (*Fagopyrum esculentum*), soybean (*Glycine max*), sunflower (*Helianthus annuus*) and tomato (*Lycopersicum esculentum*) plus four monocotyledonous species: oat (*Avena sativa*), ryegrass (*Lolium perenne*), corn (*Zea mays*) and barley (*Hordeum vulgare*). All plants were sown and grown in pots in the glasshouse. Solutions of Isoxaflutole + Cyprosulfamide SC 240 + 240 g/L and serial dilutions were sprayed onto the soil surface with application rates ranging from 420 mL product/ha down to 0.82 mL product/ha using a laboratory track sprayer at a volume rate of 100 L/ha. There were six treatment levels for each species.

For corn and oat the employed rates were: 420, 210, 105, 52.5, 26.3 and 13.1 mL product/ha; for barley, soybean, sunflower and tomato: 210, 105, 52.5, 26.3, 13.1 and 6.6 mL product/ha; for ryegrass, oilseed rape, cucumber, buckwheat: 105, 52.5, 26.3, 13.1, 6.6 and 3.3 mL product/ha and sugar beet: 105, 52.5, 26.3, 13.1, 6.6 and 3.3, 1.64 and 0.8 mL product/ha.

Pots were maintained under glasshouse conditions with a temperature control set at 23 ± 8°C during day and 18 ± 8°C at night with a 16 h photoperiod.

Five seeds were sown in each 10 cm diameter pots and there were 8 pots (replicates for each species) giving a total of 40 seeds per treatment level. The test duration was 21 days following treatment of the test item. Spray treatments were applied once to each species at test initiation with a laboratory track sprayer set at the nominal spray volume of 100 litres/ha. Control plants were sprayed with deionised water.

All pots were randomised on benches within the glasshouse after treatment.

Visual observations for phytotoxicity were made on test days 7, 14 and 21. Final assessments were made for seedling emergence, growth stage, plant survival, shoot length and shoot biomass (dry weight) 21 days after application against the untreated controls.

Dates of experimental work: September 18, 2006 to January 26, 2007

Results:

Analysis of the highest application rate revealed it to be 93.2 - 101.1% of nominal.

This study can be considered valid as the validity criteria of individual percent of emergence and 90% survival of emerged seedlings during the study period of the controls was achieved for all species. Onion failed the validity criteria, but this species was replaced by barley to ensure the required number of test species in this study.

Typical symptoms with Isoxaflutole + Cyprosulfamide SC 240 + 240 g/L observed in this study were bleaching, chlorosis, necrosis, leaf deformation and stunting. Some or all of these symptoms were exhibited in six of the tested species, however in less sensitive species they were only apparent on the higher treatment levels tested. Buckwheat, soybean, barley, corn and ryegrass showed no symptoms of phytotocixity.

Effects of Isoxaflutole & Cyprosulfamide SC 480 on seedling emergence of 11 plant species

Plant species	mL product / ha					
	Survival		Shoot lenght		Shoot dry weight	
	NOER	EC_{50}	NOER	EC_{50}	NOER	EC_{50}
Buckwheat	105	>105	105	>105	105	>105
Cucumber	26.3	>105	3.3	>105	105	>105
Oilseed rape	105	>105	52.5	>105	52.5	>105
Soybean	210	>210	13.1	>210	105	>210
Sugar beet	6.6	21.6	13.1	59.4	13.1	19.7
Sunflower	210	>210	26.2	>210	52.5	>210
Tomato	26.3	>210	210	>210	210	>210
Barley	210	>210	210	>210	210	>210
Corn	420	>420	420	>420	420	>420
Oat	420	>420	105	>420	420	>420

Conclusion:

The most sensitive ER_{50} was obtained for biomass to sugar beet with a value of 19.7 mL product/ha. The lowest NOER was 3.3 mL product/ha for shoot length to cucumber.

CP 10.6.3 Extended laboratory studies on non-target plants

No studies are necessary.

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

No studies are necessary.

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

No studies are necessary.

CP 10.8 Monitoring data

No studies are necessary.