Document Title

Summary of the ecotoxicological studies

Ethephon SL 480 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
2016-01-08	Initial document submitted for Annex I renewal Ethephon	M-544534-01-1
2017-07-21	Statement on toxicity of plant metabolites to birds (p.8), mammals (p.12) and bees (p.28) included. Aquatic endpoints have been recalculated (p.17) and summaries of the recalculation have been included for the corresponding studies. Insertion of summary of chronic toxicity studies copied from CA Dossier. Summary of publication included [redacted]; 2014; M-520562-01-1; CP 10.3.2.2, p. 50. Reference to toxic reference item on nitrogen transformation in soil included (CP 10.5, p. 63). Change of legal entity from Bayer CropScience AG to Bayer AG Crop Science Division	M-544534-02-1

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

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CP 10 ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

Use pattern considered in this risk assessment

Table 10- 1: Intended application pattern

Crop	Timing of application (range)	Number of applications	Application interval [days]	Maximum label rate [L pr/ha]	Maximum application rate, individual treatment [kg a.s./ha] ethephon
Winter wheat Winter barley S-EU	BBCH 37-39	1	-	1.0	0.48
Winter wheat Winter barley C-EU	BBCH 41-51	1	-	1.0	0.48
Spring barley S-EU	BBCH 37-39	1	-	0.75	0.36
Spring barley C-EU	BBCH 41-51	1	-	0.75	0.36

CP 10.1 Effects on birds and other terrestrial vertebrates

The risk assessment in this section has been performed according to the EFSA Guidance Document on Risk Assessment for Birds & Mammals (EFSA Journal 2009; 7(12):1438), referred to in the following as "EFSA GD 2009".

CP 10.1.1 Effects on birds

Studies on birds that have been conducted for the active substance are presented in Table 10.1.1- 1. Endpoints from studies evaluated in the previous EU review are stated in grey text to distinguish them from new studies. Values selected for use in the risk assessment are stated in **bold**.

Table 10.1.1- 1: Ethephon: Endpoints from toxicity studies on birds

Test substance	Test species	Endpoint	Reference
Ethephon	Acute oral toxicity *Colinus virginianus*	LD_{50} 764 mg a.s./kg bw	LoEP KCA 8.1.1.1/01 M-187798-01-1
	Acute oral toxicity *Anas platyrhynchos*	LD_{50} 1423 mg a.s./kg bw	LoEP KCA 8.1.1.1/02 M-187802-01-1
	Acute oral toxicity *Serinus canaria*	LD_{50} 1636 mg/kg bw	[redacted] (2013) KCA 8.1.1.1/03 M-457648-01-1
	Geometric mean of the LD_{50} values for the three species above**	**$LD_{50\ geomean}$ 1212 mg a.s./kg bw**	-
	Reproduction study *Coturnix japonica*	$NOAEL_{repro}$ 1000 mg a.s./kg diet 154 mg a.s./kg bw/d	LoEP KCA 8.1.1.3/01 M-203557-01-2
	Reproduction study *Anas platyrhynchos*	$NOAEL_{repro}$ 1000 mg a.s./kg diet* 88 mg/kg bw/d	[redacted] (2014) KCA 8.1.1.3/02 M-474649-01-1
	Reproduction study *Colinus virginianus*	**$NOAEL_{repro}$** 1000 mg a.s./kg diet* **87 mg/kg bw/d**	[redacted] (2014) KCA 8.1.1.3/03 M-478412-01-1

* Highest treatment level.
** Geometric mean calculated as recommended by EFSA GD 2009 as more than one species has been tested.

According to EFSA GD 2009, the geometric mean of all available LD50 values should be used as the endpoint for the acute risk assessment, if more than one species has been tested. As studies with Bobwhite quail (*Colinus virginianus*), Mallard duck (*Anas platyrhynchos*) and Atlantic canary (*Serinus canaria*) have been conducted for ethephon, the following acute risk assessment is based on the $LD_{50\ geomean}$. The endpoint for the long-term risk assessment is the lowest endpoint derived either from the reproduction study, i.e. the NOAEL, or from the acute study, i.e. $LD_{50}/10$. For ethephon, the NOAEL of 87 mg a.s./kg bw/d is lower than the $LD_{50\ geomean}/10$ of 121.2 mg a.s./kg bw. Thus, the NOAEL is used for the long-term TER calculations.

Table 10.1.1- 2: Generic focal species for Tier 1 risk assessment according to EFSA GD 2009

Crop	Scenario	Generic focal species	Representative species	Short cut values	
				mean RUD	90th centile RUD
Cereals	BBCH 30-39	Small omnivorous bird "lark"	Woodlark	5.4	12.0
	BBCH ≥ 40	Small omnivorous bird "lark"	Woodlark	3.3	7.2

[1] EFSA Scientific Report (2008) 174: Conclusion on the peer review of ethephon; List of Endpoints

ACUTE DIETARY RISK ASSESSMENT

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

Crop scenario	Generic focal species	DDD			DDD	LD_{50} [mg a.s./ kg bw]	TER_A	Trigger
		Appl. rate [kg a.s./ha]	SV	MAF				
Cereals BBCH 30-39	Small omnivorous bird "lark"	0.48	12.0	1.0	5.76	1212	210	10
Cereals BBCH ≥ 40			7.2		3.46		351	

The TER_A values calculated in the acute risk assessment on Tier 1 level exceed the trigger of 10. Thus, the acute risk to birds can be considered as low and acceptable.

As requested by the RMS, the risk of plant metabolites to birds via food is addressed in addition.
Birds might be exposed to metabolites that are formed in plants when consumed as food items. For the major metabolite HEPA, maximum residues of 72% (DAT 14) were found in wheat. Therefore, this value will be considered in the dietary exposure to the metabolite.
For birds, no acute oral toxicity study is available. However, in a metabolism study with ethephon on laying hens, 14 to 18% TRR of the metabolite were found in muscle, liver and kidney after 4 days (Byrd, 1992, dRAR 09 CA B7, point 7.2.1.1). No mortalities or other effects on the test animals were observed in this study. This indicates that, although HEPA is formed to a moderate level in birds, no effects occur.
Nevertheless, as an illustration, an acute risk assessment for birds based on worst-case assumptions is presented below. (As ethephon rapidly degrades into the major metabolite HEPA, a potential chronic exposure to HEPA is considered to be covered by the risk assessment for the parent.)

Crop scenario	Generic focal species	adapted appl. rate [kg p.m./ha]	SV_{90}	MAF	DDD	LD_{50} [mg p.m/ kg bw]	TER_A	Trigger
Cereals BBCH 30-39	Small omnivorous bird "lark"	0.48 × 0.72 [a] = 0.346	12.0	1.0	4.1	121.2[b]	29.2	10
Cereals BBCH ≥ 40	Small omnivorous bird "lark"		7.2		2.5		48.7	

[a] 72 % max. TRR found in plants according to residue section
[b] as a worst-case approach, the metabolite is considered to be 10-times more toxic than the parent (LD_{50}/10 = 1212 mg a.s./kg bw /10 = 121.2 mg p.m./kg bw)

The TER_A values calculated in the acute risk assessment on Tier 1 level exceed the trigger of 10. Thus, the acute risk form the metabolite HEPA to birds can be considered as low.

Acute risk assessment for birds drinking contaminated water

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

- Leaf scenario: Birds taking water that is collected in leaf whorls after application of a pesticide to a crop and subsequent rainfall or irrigation.

- Puddle scenario. Birds and mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil.

For the crops under assessment in this evaluation (cereals) the leaf scenario is not considered relevant.

Acute risk assessment for the puddle scenario

An "escape clause" recommended in the EFSA GD 2009 allows for screening the need for a quantitative risk assessment by a comparison between the application rate and the toxicity of the respective substance. This escape clause specifies (on p66) that *"due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals ..., no specific calculations of exposure and TER are necessary when the ratio of effective application rate* (= application rate x MAF) *(in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg)".*

Table 10.1.1- 4: Ethephon: Evaluation of potential concern for exposure of birds via drinking water

Crop	K_{oc} [L/kg]	Single application rate × MAF [g a.s./ha]	LD_{50} [mg a.s./ kg bw]	Ratio (Application rate × MAF) / LD_{50}	"Escape clause" No concern if ratio	Conclusion
Cereals	200*	480	1212	0.4	≤ 50	No concern

*For ethephon this value is a 'pseudo K_{oc}'. This is because adsorption of ethephon is predominantly based on non-specific interactions with the soil and the binding to the soil organic matter is a subordinated process only.

This evaluation confirms that the acute risk to birds from drinking water that may contain residues from the use of ethephon is acceptable.

LONG-TERM REPRODUCTIVE ASSESSMENT

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

Crop scenario	Generic focal species	DDD			DDD	NOAEL [mg a.s./ kg bw/d]	TER_{ET}	Trigger
		Appl. rate [kg a.s./ha]	SV	f_{TWA}				
Cereals, BBCH 30-39	Small omnivorous bird "lark"	0.48	5.4	0.53	1.37	87	63.5	5
Cereals BBCH ≥ 40			3.3		0.84		104	

The TER_{LT} values calculated in the reproductive risk assessment on Tier 1 level exceed the trigger of 5 for all evaluated scenarios in cereals. Thus, the reproductive risk to birds can be considered as low and acceptable.

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

Table 10.1.1- 6: Ethephon: Evaluation of potential concern for exposure of birds via drinking water

Crop	K_{oc} [L/kg]	Single application rate × MAF [g a.s./ha]	NOAEL [mg a.s./ kg bw/d]	Ratio (Application rate × MAF) / NOAEL	"Escape clause" No concern if ratio	Conclusion
Cereals	200*	480	87	5.5	≤ 50	No concern

*For ethephon this value is a 'pseudo K_{oc}'. This is because adsorption of ethephon is predominantly based on non-specific interactions with the soil and the binding to the soil organic matter is a subordinated process only.

This evaluation confirms that the long term risk for birds from drinking water that may contain residues from the use of ethephon is acceptable.

RISK ASSESSMENT OF SECONDARY POISONING

Substances with a high bioaccumulation potential could theoretically pose a risk of secondary poisoning for birds if feeding on contaminated prey like fish or earthworms. For organic chemicals, an octanol-water partition coefficient (log P_{ow}) > 3 is used to trigger an in-depth evaluation of the potential for bioaccumulation.

Table 10.1.1- 7: Log P_{ow} values of ethephon and the major metabolite in soil (HEPA)

Substance	log P_{ow}	Reference
Ethephon	- 0.63 (pH 2) - 1.89 (pH 7) - 1.81 (pH 10)	MCA, Section 2, point 2.7
HEPA	- 4.0 (pH 5) - 4.7 (pH 7) < - 4.7 (pH 9)	

The log P_{ow} values of ethephon and its major metabolite in soil are well below the trigger value of 3. From this any potential for bioaccumulation can be excluded and an in-depth assessment of the secondary poisoning risk is not needed.

CP 10.1.1.1 Acute oral toxicity

A study on the formulated product is not required. Endpoints from studies on the active substance are stated in
Table 10.1.1- 1.

CP 10.1.1.2 Higher tier data on birds

In view of the results presented above, no higher tier studies were deemed necessary.

CP 10.1.2 Effects on terrestrial vertebrates other than birds

Endpoints from studies on mammals that have been conducted for the active substance are presented in Table 10.1.2- 1. All relevant studies were evaluated during the previous EU review. Hence, all endpoints are stated in grey text.

Table 10.1.2- 1: Ethephon: Endpoints for use in the risk assessment for mammals

Test substance	Exposure	Species/Origin	Endpoint	Reference
Ethephon	Acute risk assessment	Rat	LD_{50} 1564 mg a.s./kg bw	LoEP KCA 5.2.1 /01 M-187938-01-1
	Long-term risk assessment	Rat	NOAEL 22.8 mg a.s./kg bw/d	LoEP KCA 5.6.1/01 M-187771-01-1

Table 10.1.2- 2: Generic focal species for Tier 1 risk assessment according to EFSA GD 2009

Crop	Scenario	Generic focal species	Representative species	Short cut values 90th centile RUD	Short cut values Mean RUD
Cereals	BBCH ≥ 20	Small insectivorous mammal "shrew"	Common shrew	5.4	1.9
	BBCH ≥ 40	Small herbivorous mammal "vole"	Common vole	40.9	21.7
	BBCH 30-39	Small omnivorous mammal "mouse"	Wood mouse	8.6	3.9
	BBCH ≥ 40	Small omnivorous mammal "mouse"	Wood mouse	5.2	2.3

ACUTE DIETARY RISK ASSESSMENT

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

Crop scenario	Generic focal species	DDD			DDD	LD_{50} [mg a.s./ kg bw]	TER_A	Trigger
		Appl. rate [kg a.s./ha]	SV	MAF				
Cereals BBCH ≥ 20	Small insectivorous mammal "shrew"	0.48	5.4	1.0	2.6	1564	603	10
Cereals BBCH ≥ 40	Small herbivorous mammal "vole"		40.9		19.6		79.7	
Cereals BBCH 30-39	Small omnivorous mammal "mouse"		8.6		4.1		379	
Cereals BBCH ≥ 40	Small omnivorous mammal "mouse"		5.2		2.5		627	

The TER_A values calculated in the Tier 1 acute risk assessment for wild mammals exceed the trigger of 10 for all evaluated scenarios. Thus, the acute risk to wild mammals can be considered as low and acceptable.

As requested by the RMS, the risk of plant metabolites to mammals via food is addressed in addition. Mammals might be exposed to metabolites that are formed in plants when consumed as food items. For the major metabolite HEPA, maximum residues of 72% (DAT 14) were found in wheat. Therefore, this value will be considered in the dietary exposure to the metabolite.
The toxicity of HEPA was tested in an oral acute study in rat (Denton, 2001, dRAR 08 CA B6, point 6.8.1). The resulting LD_{50} of >2000 mg/kg bw is higher than the available acute endpoints of 764, 1425 and 1636 mg a.s./kg bw for ethephon. Thus, demonstrating the metabolite is less toxic than the parent substance. Overall, a low toxicity is assumed for HEPA and consequently the risk from exposure to the metabolite is considered covered by the risk assessment for ethephon.
Nevertheless, as an illustration, an acute risk assessment for mammals based on worst-case assumptions is presented below (as ethephon rapidly degrades into the major metabolite HEPA, a potential chronic exposure to HEPA is considered to be covered by the risk assessment for the parent).

Crop scenario	Generic focal species	adapted appl. rate [kg p.m./ha]	SV_{90}	MAF	DDD	LD_{50} [mg p.m./ kg bw]	TER_A	Trigger
Cereals BBCH ≥ 20	Small insectivorous mammal "shrew"	0.48 × 0.72[a] = 0.346	5.4	1.0	1.9	>2000	1072	10
Cereals BBCH ≥ 40	Small herbivorous mammal "vole"		40.9		14.1		141	
Cereals BBCH 30-39	Small omnivorous mammal "mouse"		8.6		3.0		673	
Cereals BBCH ≥ 40	Small omnivorous mammal "mouse"		5.2		1.8		1113	

[a] 72 % max. TRR found in plants according to residue section

All TER values are well above the trigger value of 10 indicating acceptable risk from the metabolite to mammals.

Acute risk assessment for mammals drinking contaminated water

For further details, reference is made to Point 10.1.1 of this dossier. However, according to EFSA GD 2009, unlike for birds the scenario of pools formed in leaf axils is not relevant for mammals. Therefore the risk assessment for mammals is limited to the scenario of puddles formed on the ground after application.

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

Crop	K_{oc} [L/kg]	Single application rate × MAF [g a.s./ha]	LD_{50} [mg a.s./ kg bw]	Ratio (Application rate × MAF) / LD_{50}	"Escape clause" No concern if ratio	Conclusion
Cereals	200*	480	1564	0.3	50	No concern

*For ethephon this value is a 'pseudo K_{oc}'. This is because adsorption of ethephon is predominantly based on non-specific interactions with the soil and the binding to the soil organic matter is a subordinated process only.

This evaluation confirms that the acute risk for mammals from drinking water that may contain residues from the use of ethephon is acceptable.

LONG-TERM REPRODUCTIVE ASSESSMENT

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

Crop scenario	Generic focal species	DDD				DDD	NOAEL [mg a.s./kg bw/d]	TER_{LT}	Trigger
		Appl. rate [kg a.s./ha]	SV	MAF	f_{TWA}				
Cereals BBCH ≥ 20	Small insectivorous mammal "shrew"	0.48	1.9	1.0	0.53	0.48	22.8	47.2	5
Cereals BBCH ≥ 40	Small herbivorous mammal "vole"		21.7			5.5		**4.1**	
Cereals BBCH 30-39	Small omnivorous mammal "mouse"		3.9			1.0		23.0	
Cereals BBCH ≥ 40	Small omnivorous mammal "mouse"		2.3			0.6		39.0	

Bold values do not meet the trigger

The TER_{LT} values calculated in the reproductive risk assessment at Tier 1 do not exceed the trigger of 5 for the small herbivorous mammal scenario. Thus, a refined risk assessment for this scenario is presented below based on measured residues of ethephon on treated cereal plants.

Refined long-term risk assessment for small herbivorous mammals feeding in cereal fields

Refinement of RUD

With ethephon, many residue studies have been conducted on cereals, providing residue values of the compound on plants immediately after application at rates around 0.480 kg a.s./ha. New residue studies are available conducted on wheat and barley with application at BBCH 39 (end of stem elongation) and one study with barley at BBCH 43, which include analysis of green shoots on the day of application ('Day 0'). The Day 0 data from these studies (including 8 trials each for wheat and barley) are considered relevant for estimating residues on grass, and for the refinement of RUD. These data are summarised in Table 10.1.2- 6.

Table 10.1.2- 6: Residue levels of ethephon in mg/kg directly after the application ('day 0')

Plant sample	BBCH	Rate [kg a.s./ha]	Initial residue [mg/kg]	RUD	Reference
Barley shoots	39	0.480	4.5	9.38	KCA 6.3.1/03 [redacted] (2015) M-529491-01-1
			4.2	8.75	
			5.9	12.3	
			3.5	7.29	
			5.6	11.7	KCA 6.3.1/04 [redacted] (2015) M-532463-01-1
			6.6	16.1	
			3.3	6.88	
			8.2	17.1	
Wheat shoots	39	0.480	5.7	11.9	KCA 6.3.2/03 [redacted] (2015) M-529488-01-1
		0.520	17	32.7	
		0.480	6.9	14.4	
		0.480	5.6	11.7	
		0.480	7.1	14.8	KCA 6.3.2/04 [redacted] (2015) M-532272-01-1
		0.480	6.4	13.3	
		0.480	10	20.8	
		0.480	16	33.3	
			Mean	**15.2**	

Foliar interception

Crop interception should be taken into account according to the BBCH growth stage, as recommended by FOCUS (2014)[2]. For cereals the following interception values are used:

- bare-emergence (BBCH 00-09): 0%,
- leaf development (BBCH 10-19): 0%,
- tillering (BBCH 20-29): 20%,
- stem elongation (BBCH 30-39): 80%,
- **flowering (BBCH 40-69): 90%**
- BBCH 70-89: 80%,
- senescence ripening (BBCH 90-99): 80%

For Ethephon SL 480, the application in cereals is intended earliest at growth stage BBCH 39. As the small herbivorous mammal "vole" becomes relevant only at BBCH ≥ 40, an interception value of 90% (10% deposition) is used to calculate a use-specific RUD. The refined RUD is 15.2 × 0.1 = **1.52 for cereals.** From this the refined DDD is calculated as follows:

Table 10.1.2- 7: Ethephon: Refined reproductive risk assessment for wild mammals

Crop scenario	Generic focal species	DDD					DDD	NOAEL [mg a.s./kg bw/d]	TER_{LT}	Trigger
		Appl. rate [kg a.s./ha]	FIR/bw [g/d] a)	RUD (refined)	MAF	f_{TWA}				
Cereals BBCH ≥ 40	Small herbivorous mammal "vole"	0.48	1.33	1.52	1.0	0.53	0.5	22.8	44.3	5

a) According to Appendix A of EFSA GD 2009

[2] FOCUS Groundwater (2014): Generic Guidance for Tier 1 FOCUS Groundwater Assessments, Version 2.2.

This refinement allows the overall conclusion that the use of Ethephon SL 480 in cereals is safe for all generic focal species including the herbivorous vole.

Long-term risk assessment for mammals drinking contaminated water

Table 10.1.2- 8: Ethephon: Evaluation of potential concern for exposure of mammals via drinking water

Crop	K_{oc} [L/kg]	Single application rate × MAF [g a.s./ha]	NOAEL [mg a.s./ kg bw/d]	Ratio (Application rate × MAF) / NOAEL	"Escape clause" No concern if ratio	Conclusion
Cereals	200*	480	22.8	21.1	< 50	No concern

*For ethephon this value is a 'pseudo K_{oc}'. This is because adsorption of ethephon is predominantly based on non-specific interactions with the soil and the binding to the soil organic matter is a subordinated process only.

This evaluation confirms that the long term risk for mammals from drinking water that may contain residues from the use of ethephon is acceptable.

RISK ASSESSMENT OF SECONDARY POISONING

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

As presented in Table 10.1.1- 7, log P_{ow} values are far below the trigger value indicating a low risk of secondary poisoning.

CP 10.1.2.1 Acute oral toxicity to mammals

Study already evaluated during the first Annex I inclusion. No new studies were required.

CP 10.1.2.2 Higher tier data on mammals

In view of the results presented above, no higher tier studies were deemed necessary.

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

Information on effects of ethephon on reptiles or amphibians is not available. No guidelines for studies with terrestrial amphibian life stages and reptiles are available and no risk assessment schemes are established so far.

CP 10.2 Effects on aquatic organisms

The risk assessment is based on Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013; 11(7):3290, 268 pp, hereafter referred to as EFSA GD 2013. Studies on aquatic organisms have been conducted for the active substance and the formulation Ethephon SL 480. The endpoints from these studies are presented in Table 10.2- 1 on the following page. Rows for studies evaluated during the previous EU review contain grey text. This is to distinguish them from rows for additional studies which contain black text. Endpoints selected for use in the risk assessment are stated in **bold** ~~black~~ text.

Table 10.2- 1: Ethephon and Ethephon SL 480: Endpoints from studies on aquatic organisms

Test substance	Test species	Endpoint		Reference
Ethephon SL 480	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_rC_{50} E_yC_{50} E_bC_{50}	>75.8 mg product/L >75.8 mg product/L[2] >75.8 mg product/L[2]	[redacted] (2015) KCP 10.2.1/03 M-526336-01-1
	Algae, growth inhibition *Scenedesmus subspicatus*	E_rC_{50}	98 mg product/L	[redacted] (2009) KCP 10.2.1/01 M-179329-01-1
	Aquatic plants, growth inhibition *Lemna gibba*	E_rC_{50}	>100 mg product/L	[redacted] (2014) KCP 10.2.1/04 M-505517-01-1
Ethephon	Fish, acute, *Cyprinus carpio*	LC_{50}	**>100 mg a.s./L**[3]	LoEP KCA 8.2.1/03 M-187823-01-1
	Fish, acute, *Cyprinodon variegatus*[1]	LC_{50}	>102 mg a.s./L	[redacted] (2013) KCA 8.2.1/04 M-444829-01-1
	Fish, chronic (ELS) *Pimephales promelas*	NOEC	**43 mg a.s./L**[3, 4]	LoEP KCA 8.2.2.1/01 M-205148-01-1
	Invertebrate, acute *Daphnia magna*	EC_{50}	**>90.4 mg a.s./L**	[redacted] (2015) KCA 8.2.4.1/02 M-524938-01-1
	Invertebrate, acute *Crassostrea virginica*[1] (Eastern oyster)	EC_{50} shell growth	60 mg a.s./L	[redacted] (1989) KCA 8.2.4.2/01 M-187969-01-1
	Invertebrate, chronic *Daphnia magna*	21d LC_{50} NOEC EC_{10} EC_{20}	>160 mg a.s./L[2, 3] 67 mg a.s./L[3] **122.4 mg a.s./L**[3, 5] 136 mg a.s./L[5]	LoEP KCA 8.2.5.1/01 M-187833-01-1
	Algae, growth inhibition *Chlorella vulgaris*	E_rC_{50}	10.9 mg a.s./L	LoEP KCA 8.2.6.1/01 M-187835-01-1
	Algae, growth inhibition *Selenastrum capricornutum*	E_bC_{50}	>1.4 mg a.s./L[3, 6]	LoEP KCA 8.2.6.1/02 M-187839-01-1
	Algae, growth inhibition *Navicula pelliculosa*	E_bC_{50}	>1.5 mg a.s./L [7]	LoEP KCA 8.2.6.1/03 M-187837-01-1
	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_bC_{50}	**7.1 mg a.s./L** [8]	LoEP KCA 8.2.6.1/04 M-236983-01-1
	Algae, growth inhibition *Navicula pelliculosa*	E_rC_{50}	>2.86 mg a.s./L	[redacted] (2015) KCA 8.2.6.1/05 M-534339-01-1
	Algae, growth inhibition *Skeletonema costatum*[1]	E_bC_{50}	>1.8 mg a.s./L [7]	[redacted] (1990) KCA 8.2.6.1/06 M-187843-01-1
	Algae, growth inhibition *Anabaena flos aquae*	E_bC_{50}	>1.8 mg a.s./L [6]	LoEP KCA 8.2.6.2/01 ~~M-236983-01-1~~ M-187841-01-1
	Aquatic plants, growth inhibition *Lemna gibba*	E_bC_{50}	**>1.6 mg a.s./L**[3, 9]	LoEP KCA 8.2.7/01 M-187845-01-1

Test substance	Test species	Endpoint	Reference
	Aquatic plants, growth inhibition *Myriophyllum spicatum*	E_rC_{50} >100 mg a.s./L	[redacted] (2015) KCA 8.2.7/02 M-537257-01-1

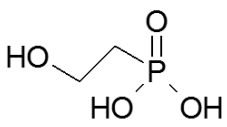
[1] Estuarine/marine species, tested in salt water; [2] LC_{50} for parental *Daphnia*. This is the agreed *acute* endpoint from the previous EU review (at that time the 48h acute study was deemed invalid). A new acute toxicity study has been conducted for the current EU review; [3] Risk assessment endpoint in previous EU review;
[2] As requested by the RMS, E_bC_{50} and E_yC_{50} values should be determined as additional endpoints to this study.
[3] Risk assessment endpoints selected in current EU review are stated in **bold**.
[4] As requested by the RMS, EC_{10} and EC_{20} values should be determined as additional endpoints to this study. However, due to the lack of a concentration response, it was not possible to derive valid EC_{10} and EC_{20} from the results of the study.
[5] As requested by the RMS, EC_{10} and EC_{20} values should be determined as additional endpoints to this study. According to the new aquatic Guidance Document (EFSA, 2013, Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290), the EC_{10} is the more relevant endpoint compared to the NOEC and is therefore used in the risk assessment.
[6] The study was considered valid at the time of the original inclusion of ethephon. However, according to the current test guidelines and due to statistical reasons, a re-evaluation of the study endpoints is not reasonable. Results of the study are not used in the risk assessment.
[7] The RMS asked to calculate additional endpoints for growth rate and yield. The limit study was considered valid at the time of the original inclusion of ethephon. However, endpoint recalculation is not possible due to a high coefficient of variation exceeding the validity criterion of 35%. In addition, no EC_{50} value can be derived from a limit test. Results of the study are not used in the risk assessment.
[8] The E_rC_{50} of 9.3 mg a.s./L derived in this study is not an accurate endpoint as it does not fit into the statistical model (extrapolation). Therefore, and as recommended by the RMS, the E_bC_{50} is used in the risk assessment.
[9] The RMS requested to calculate the endpoints for growth rate and yield. However, due to mathematical reasons, it was not possible to derive valid endpoints from the results of the study.

Note on metabolite HEPA

HEPA (2-hydroxyethylphosphonic acid) is classed as a 'major' metabolite of ethephon in soil, having been detected at 10.6% (i.e. >10%) of applied radioactivity in a soil photolysis study on ethephon (MCA Section 7). PEC_{sw} values are presented in MCP Section 9 (highest PEC_{sw} for HEPA is 0.805 µg/L). In accordance with EFSA GD 2013, the 'relevance' of HEPA to the risk assessment needs to be considered. The molecular structure of ethephon and HEPA are shown below.

Ethephon:

HEPA:



Given that the structure of HEPA is very similar to ethephon (which is of low toxicity) and the molecule has no toxophore, HEPA is concluded to be 'non-relevant' for the risk assessment. Therefore, *a priori*, by reference to EFSA GD 2013, the acute and chronic toxicity of HEPA is equal to the toxicity of ethephon for all first tier taxonomic groups and accordingly, the risk to aquatic organisms from this metabolite can be concluded as low.

Predicted environmental concentrations for ethephon used in the risk assessment

Table 10.2- 2 Initial max PEC_{sw} values for use in alkaline & acidic soils, FOCUS Steps 1 & 2: winter cereals

Compound	FOCUS Scenario	Use in alkaline soils		Use in acidic soils	
		Winter cereals 1 × 0.48 kg a.s./ha, average crop cover	Winter cereals 1 × 0.48 kg a.s./ha, full canopy	Winter cereals 1 × 0.48 kg a.s./ha, average crop cover	Winter cereals 1 × 0.48 kg a.s./ha, full canopy
		$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]
Ethephon	STEP 1	130.7	130.7	130.7	130.7
	STEP 2 – North	11.13	5.041	20.46	8.540
	STEP 2 - South	20.87	8.695	**39.54**	15.69

Bold values are worst case values and are used in the risk assessment

Table 10.2- 3 Initial max PEC_{sw} values for use in alkaline & acidic soils FOCUS Step 1 & 2: spring cereals

Compound	FOCUS Scenario	Use in alkaline soils		Use in acidic soils	
		Spring cereals 1 × 0.36 kg a.s./ha, average crop cover	Spring cereals 1 × 0.36 kg a.s./ha, full canopy	Spring cereals 1 × 0.36 kg a.s./ha, average crop cover	Spring cereals 1 × 0.36 kg a.s./ha, full canopy
		$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]
Ethephon	STEP 1	130.7	130.7	130.7	130.7
	STEP 2 – North	5.041	5.041	8.540	8.540
	STEP 2 - South	8.695	8.695	15.69	15.69

As the use in winter cereals results in higher PEC_{sw} values than the use in spring cereals, the following risk assessment is based on the maximum PEC_{sw} values in winter cereals, as a worst case.

Risk assessment for aquatic organisms

ACUTE RISK ASSESSMENT

Table 10.2- 4: Ethephon / winter cereals: TER_A calculations based on PEC_{sw} values from FOCUS Step 2

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Ethephon	Fish, acute	LC_{50}	>100000	39.54	>2529	100
	Invertebrate, acute	EC_{50}	> 90400		>2286	

Table 10.2- 5: Ethephon / winter cereals: $RAC^*_{sw,ac}$ compared with PEC_{sw} values from FOCUS Step 2

Compound	Species	$RAC_{sw,ac}$ ($L(E)C_{50}$/100) [µg/L]	$PEC_{sw,max}$ [µg/L]	$RAC_{sw,ac} \geq PEC_{sw,max}$?
Ethephon	Fish, acute	>1000	39.54	yes
	Invertebrate, acute	> 904		yes

* RAC = Regulatory Acceptable Concentration

CHRONIC RISK ASSESSMENT

Table 10.2- 6: Ethephon / winter cereals: TER_{LT} calculations based on PEC_{sw} values from FOCUS Step 2

Compound	Species	Endpoint [µg/L]		$PEC_{sw.max}$ [µg/L]	TER_{LT}	Trigger
Ethephon	Fish, chronic	NOEC	43000	39.54	4088	10
	Invertebrate, chronic	~~NOEC~~ EC_{10}	~~67000~~ 122700		~~1694~~ 3103	
	~~Green algae, chronic~~	~~EC_{50}~~	~~>1400~~		~~>35.4~~	
	Green algae, chronic[a]	E_bC_{50}	7100		180	
	Aquatic plant, chronic	EC_{50}	>1600		>40.5	

[a] new algae endpoint based on of reassessment of all ecotoxicological data available for this organism group

Table 10.2- 7: Ethephon / winter cereals: $RAC_{sw,ch}$ compared with PEC_{sw} values from FOCUS Step 2

Compound	Species	$RAC_{sw,ch}$ (NOEC/10 or EC_{50}/10) [µg/L]	$PEC_{sw.max}$ [µg/L]	$RAC_{sw,ch} \geq PEC_{sw,max}$?
Ethephon	Fish, chronic	4300	39.54	yes
	Invertebrate, chronic	~~6700~~ 12270		yes
	~~Green algae, chronic~~	~~>140~~		~~yes~~
	Green algae, chronic[a]	710		yes
	Aquatic plant, chronic	>160		yes

[a] new algae endpoint based on of reassessment of all ecotoxicological data available for this organism group

All TER values are greater than the relevant trigger values. Similarly, RAC values are always greater than the PEC_{sw} values. Hence, there is a low risk to aquatic organisms.

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

Report:	KCP 10.2.1/03; [redacted]; 2015; M-526336-01-1
Title:	Pseudokirchneriella subcapitata growth inhibition test with ethephon SL 480 G - Final report
Report No.:	E 201 4786-8
Document No.:	M-526336-01-1
Guideline(s):	Directive 91/414/EEC; Regulation (EC) No 1107/2009
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objectives:

To determine the influence of the test item on exponentially growing *Pseudokirchneriella subcapitata* expressed as NOEC, LOEC and EC_X for growth rate of algal biomass (cells per volume).

Material and Methods:

The test item was Ethephon SL 480 (analysed: 41.0% w/w a.s. or 492.3 g a.s./L) from batch no. B3090017. *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) was exposed in a chronic multi-generation test for 72 hours under static conditions to a geometric mean measured (nominal) concentration of 75.8 (100) mg form./L in comparison to a control. The test consisted of six replicate vessels for the test item and for the control. The initial cell density was 10,000 cells/mL. Growth inhibition was calculated based on biomass per volume. The surrogate for biomass was cell density (used as response parameter). pH was 7.9 in the control replicates and the temperature was 22.3 - 23.5 °C (measured in an additional incubated vessel) at a continuous illumination of 4.67 kLux (mean). The concentration of ethephon was analysed on day 0 and 3. The mean geometric measured concentration was 31.1 mg a.s./L.

Results:

All the validity criteria in the OECD Guideline were met. Hence, the assay was valid:

Validity Criteria:	Obtained in this study:
Increase in biomass:	Biomass increased in the control by 64.1-fold, thus more than 16-fold, within the evaluation period.
Sectional growth rate in control:	Mean percent coefficient of variation of sectional growth rates from day 0-1, day 1-2, and day 2-3 in the control is 23%, thus not exceeding 35%
Between-replicate variation of growth rate in control:	Percent coefficient of variation of the average growth rate in control replicates is 1.3%, thus not exceeding 7%

The analysis of ethephon in medium of the treatment on day 0 was 110% of nominal. After 72 hours, analysed levels were 52.7% of nominal. No morphological change in algae was observed.

Effect of ethephon on Freshwater Algae (*Pseudokirchneriella subcapitata*) in a 72 h growth inhibition test

Geom. mean measured 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 [%]
Control	641000	1.387	0.0
75.8	647000	1.390	-0.2

-% inhibition: *increase* in growth relative to control. No significant difference was found based on Student-t-test.

Conclusions:
The (0 - 72h)-E_rC_{50} for Ethephon SL 480 was >75.8 mg form./L (>31.1 mg a.s./L).

As requested by the RMS, E_yC_{50} and E_bC_{50} values and corresponding NOEC and LOEC values should be determined as additional endpoints to the algae study ([redacted], 2015, M-526336-01-1). Results were reevaluated in a separate statistical report, which can be provided on request. A summary is presented below.

Introduction
A statistical evaluation addressing the calculation of NOEC and LOEC values was conducted with the results of the study M-526336-01-1, ([redacted], 2015) to fulfill the data requirements according to regulation EU 283/2013.

Statistical evaluation
The study M-526336-01-1 ([redacted], 2015) was statistically evaluated for the effects of Ethephon SL 480 G on the algae *Pseudokirchneriella subcapitata*. The organisms were exposed for 72 hours to a single concentration (limit test) of Ethephon 480 G: 75.8 mg product/L. Additionally, a control was tested in parallel. The data used for this evaluation were obtained from original study report.
Only the effects on biomass change of the algae were used for the statistical evaluation. In order to derive No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) effects on biomass of the test subjects, a Welch-t test (24h) or a Student-t-test (48 and 72h) was performed with the software ToxRatPro Version 3.2.1 ([redacted], 2015). for each of the sampled intervals individually.

Results
According to the statistical parameters; p(t) >0.05 for all sampling points the NOEC and LOEC for biomass change should be considered valid.
The obtained NOEC and LOEC values are presented in the table below.

No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) calculated by ToxRatPro Version 3.2.1 as change in biomass of *Pseudokirchneriella subcapitata* on the three sampling periods, 24, 48 and 72h after exposure to Ethephon SL 480 G.

Toxicity	**72h NOEC** (mg product/L)	**72h LOEC** (mg product/L)
Effect on biomass change	≥ 75.800	> 75.800
Effect on growth rate change*	≥ 75.800	> 75.800
Effect on yield change*	≥ 75.800	> 75.800

* Value obtained from the statistical evaluation on the original study report ([redacted], 2015).

Conclusions

The calculated 72h NOEC and LOEC values are ≥ 75.8 mg product/L. The statistical parameters presented above showed that these values can be considered reliable. Due to the test design (limit test) it is not possible to calculate Effect Concentrations with 50% (E_bC_{50}) effect on the biomass change.

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Report:	KCP 10.2.1/04; [redacted]; 2014; M-505517-01-1
Title:	Lemna gibba G3 - Growth inhibition test with ethephon SL 480 A-G under semi-static conditions
Report No.:	EBETN002
Document No.:	M-505517-01-1
Guideline(s):	OECD Guideline 221 (March 23, 2006) US EPA OCSPP 850.4400
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

To determine the influence of the test item on exponentially growing *Lemna gibba* expressed as NOEC, LOEC and EC_x for growth rate of the response variables, frond number, and total frond area.

Material and Methods:

The test item was Ethephon SL 480 (analysed 41.0% w/w a.s. or 492.3 g a.s./L) from batch no. B3090017. Four replicates of 12 fronds of *L. gibba* per test concentration were exposed in a chronic multi-generation test for 7 days under semi-static conditions to nominal concentrations of 0.0320, 0.160, 0.800, 4.00, 20.0 and 100 mg form./L in comparison to a control. The pH ranged from 7.5 to 8.7 in the control and temperature ranged from 24.6 to 24.9°C (measured in an additional incubated vessel) at a continuous illumination of 6781 lux (average). Concentrations of ethephon were measured in freshly prepared media on day 0, 3, and 5 and in aged media on day 3, 5, and 7.

Results:

The doubling time of frond number in the control was 1.9 days, corresponding to a 13.4 fold increase. Therefore the study met all validity criteria of the OECD Guideline. The analysed concentrations of ethephon in fresh media on day 0, 3, and 5 were 98 - 109% of nominal. In aged media on day 3, 5, and 7, analysed concentrations were 11 - 24% of nominal. The correct dosing was confirmed in all freshly prepared test media. Hence, results are based on nominal concentrations.

Nominal conc. [mg form./L]	Frond no. (day 7) mean of 4 replicates	Total frond area (day 7) mean of 4 replicates [mm^2]	% Inhibition	
			Mean growth rate for frond no.	Mean growth rate for total frond area
control	160	1807	--	--
0.0320	165	1780	-1.0	0.6
0.160	156	1749	1.2	-0.9
0.800	133	1588	7.2 *	3.1 *
4.00	125	1429	9.6 *	5.6 *
20.0	136	1523	6.4 *	5.6 *
100	132	1597	7.6 *	2.5

-% inhibition: *increase* in growth relative to the control
* Results which were significantly different (Williams Multiple sequential t-test Procedure) from the control

Only minor effects on frond number and area occurred with inhibition of <10%. There was no clear dose-response relationship. There were some separated fronds on day 7 at 4, 20 and 100 mg form./L

Endpoint (0-7 day)	Effect on mean growth rate of frond no. [mg form./L]	Effect on mean growth rate of total frond area [mg form./L]
E_rC_{50} (CI 95%)	**>100**	**>100**
LOE_rC	0.800	0.800
NOE_rC	0.160	0.160

Conclusions:

The E_rC_{50} for frond number and frond area was >100 mg form./L (>41 mg a.s./L). The NOE_rC for both parameters was 0.160 mg form./L based on minor effects at 0.8 mg form./L (NOE_rC).

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

No studies are required.

CP 10.2.3 Further testing on aquatic organisms

No studies are required.

CP 10.3 Effects on arthropods

CP 10.3.1 Effects on bees

The risk assessment in this section has been performed according to the existing guidance in force at the time of the preparation and submission of this dossier namely the EU Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2) and EPPO Standard PP 3/10 (3) Environmental Risk Assessment Scheme for Plant Protection Products - Chapter 10: Honey bees.

Previously-evaluated data indicate that ethephon has a low acute oral and contact toxicity to bees (LD_{50} >100 µg a.s./bee). Also, data on other non-target arthropods (CA 8.3.2; CP 10.3.2) do not show any insecticidal activity for ethephon. For completeness, several additional studies on bees have been conducted for this current EU review, in order to fulfil the data requirements under Regulation 1107/2009 (Ref: Data requirements Regulations 283/2013 and 284/2013, 1st March 2013).

New and previously-evaluated studies on bees for the active substance are summarised in MCA Section 8.3.1. This section includes summaries of two studies conducted using Ethephon SL 480 as the test item, which was used as a means of testing the active substance (in accordance with Point 4 on page 54 of Regulation 283/2013). One of these studies was a honey bee brood feeding study (KCA 8.3.1.3/02) conducted in 2013.

The honey bee brood feeding study (KCA 8.3.1.3/02) showed a higher Brood Termination Rate (BTR) in treated colonies than in the control colonies. However, after finalisation of the study it was realised that the treated sucrose solution, which contained 2.4 g a.s./L, should have been pH-buffered. The pH of a 2.4 g a.s./L aqueous solution of Ethephon SL 480 is 2.0 ([redacted], 2015, M-542286-01-1, KCA 8.3.1.3/03). Uptake of 1 L of treated sucrose solution by each colony was clearly slower than uptake of untreated sucrose solution by control colonies. This was probably related to the acidity of the dosing solution. The possibility of consequent experimental artefacts could not be excluded. Hence, the study was concluded as unreliable. Subsequently, to replace the study, an acute larval toxicity study ([redacted], 2015) and a honeybee tunnel test ([redacted], 2015) were done. The acute larval study was conducted in order to assess *inherent toxicity* of ethephon to brood. The tunnel test was performed to provide a *realistic* worst case, in terms exposure of honey bee colonies (in contrast to the very high exposure concentration and direct dosing in the brood feeding study).

The tunnel test ([redacted], 2015) was based on OECD Guidance Document no. 75. Honey bee colonies were exposed by the spraying of flowering *Phacelia tanacetifolia* at 120 or 480 g a.s./ha whilst worker bees were foraging for pollen and nectar. The rationale for conducting this study was to provide: 1) Information on the effects of ethephon on foraging behaviour, brood development and colony-condition, and 2) quantitative data on residues of ethephon in larvae, pollen and nectar. The study is summarised later in this section.

Endpoints from the available studies on bees for ethephon and Ethephon SL 480 are compiled in Table 10.3.1- 1. Studies evaluated in the previous EU review are stated in grey text. Additional studies submitted for the current EU review are stated in black text.

Table 10.3.1- 1: Endpoints from toxicity studies on bees for ethephon and Ethephon SL 480

Test substance	study type	Endpoint	References
Ethephon	Honey bee, 48 h	Oral. LC_{50} >116.5 µg a.s./bee Contact: LC_{50} >100 µg a.s./bee	LoEP KCA 8.3.1.1.1/01 M-172533-01-2
Ethephon	Honey bee, 48h	Oral. LD_{50} >111.0 µg a.s./bee Contact: LD_{50} >100.0 µg a.s./bee	[redacted] (2015) KCA 8.3.1.1.1/02 M-514214-01-1
Ethephon	Bumble bee, 48 h	Oral: LD_{50} >167.0 µg a.s./bee	[redacted] (2015a) KCA 8.3.1.1.1/03 M-534551-01-1
Ethephon	Bumble bee, 48 h	Contact: LD_{50} >100.0 µg a.s./bee	[redacted] (2015b) KCA 8.3.1.1.1/04 M-525423-01-1
Ethephon SL 480	Honey bee, 10 days	LDD_{50} >95.53 µg a.s./bee/day NOEDD 95.53 µg a.s./bee/day	[redacted] (2015) KCA 8.3.1.2/01 KCP 10.3.1.2/01 M-534554-01-1
Ethephon SL 480	Honey bee brood feeding study	3 colonies each fed 1 L sucrose sol. containing 2.4 g a.s./L. Due to oversight, dosing solution was not pH-buffered. Uptake slower in test item colonies than control, probably due to acidity (pH 2.0). BTR higher for test item than control. Study is unreliable*.	[redacted] (2015) KCA 8.3.1.3/01 KCP 10.3.1.3/01 M-528291-01-1
Ethephon	Honey bee larvae, acute, 7 days	LD_{50} >100 µg a.s./larva NOED 100 µg a.s./larva	[redacted] (2015) KCA 8.3.1.3/02 M-540682-01-1
Ethephon SL 480	Honey bee, 48h	Oral: LD_{50} >110.7 µg a.s./bee Contact: LD_{50} >100 µg a.s./bee	[redacted] (2014) KCP 10.3.1.1.1/01 M-504112-01-1
Ethephon SL 480	Honey bee tunnel test, OECD Guidance Document No. 75	No effects on adults, brood or colonies for sprays of 120 & 480 g a.s./ha to flowering *Phacelia* during bee flight. Highest measured residues in pollen & nectar from foragers were 28 and 3 mg a.s./kg, respectively (day 0). Subsequent samples from foragers & combs indicated a rapid decline in concentrations.	[redacted] (2015) KCP 10.3.1.5/01 M-540667-01-1

*Study not suitable for use in risk assessment. To replace this study an acute larval toxicity study ([redacted], 2015) and a honey bee tunnel test assessing brood ([redacted], 2015) were subsequently conducted.
BTR: Brood Termination Rate.

Risk assessment for bees

The endpoints and results from laboratory studies in Table 10.3.1- 1 are for larvae and adult honey bees, with the latter also exposed for a chronic duration (10 days). Bumble bees have also been tested as a representative 'non-*Apis*' species. Overall, the results indicate that ethephon has a low toxicity.

The only study which showed effects was the brood feeding study ([redacted], 2015). The main effect was on BTR for cells monitored from the egg stage (Mean BTR of 31.33% in the test item dosed colonies compared with 11.67% in the control colonies). However, this study is unreliable due to the acidity (pH 2.0) of the dosing solution. When considering the low pH, coupled with the substantial volume of solution provided to each colony (1 L), an artefactual 'physico-chemical' effect cannot be excluded. In the subsequent tunnel test ([redacted], 2015), the highest measured concentration in nectar was 3 mg a.s./kg (day 0). This realistic worst-case concentration is 800x lower than the concentration

in the sugar solution used in the brood feeding study (2400 mg a.s./L). Hence, *in hindsight*, the exposure concentration in the brood feeding study can be regarded as completely unrealistic. An acute toxicity study on honey bee larvae (KCA 8.3.1.3/01, [redacted], 2015) showed no effects for a limit dose of 100 µg a.s./larva. This supports the notion that the results of the brood feeding study are unreliable.

In the tunnel test ([redacted], 2015) Ethephon SL 480 was sprayed onto flowering *Phacelia* at 120 or 480 g a.s./ha in the presence of one colony per tunnel. The test was a *more realistic* experiment than the brood feeding study, as the colony in each tunnel would have been exposed to residues in/on pollen and nectar brought to the colony by foraging bees. No treatment-related effects on adults or brood were seen. No residues of ethephon were detected in larvae. In pollen and nectar samples, the highest levels were detected nearest to the time of application, and declined rapidly thereafter. The highest measured residue in pollen from foragers after application at 480 g a.s./ha was 28 mg a.s./kg, from a sample taken on the day of application. Subsequent samples of pollen from foragers and pollen from combs indicated a rapid decline in the concentration. The pattern was the same in terms of residues in nectar. The highest measured residue in nectar from foragers after application at 480 g a.s./ha was 3 mg a.s./kg, from a sample taken on the day of application. Subsequent samples of nectar from foragers and nectar from combs indicated a rapid decline in the concentration.

It is clear from all the laboratory studies and the tunnel test that ethephon has a low toxicity to bees. Ethephon SL 480 is proposed for use in cereals at 480 g a.s./ha. This crop is not attractive to bees. Hence, both acute and chronic exposure of foragers is likely to be negligible. In turn, this means that it is highly unlikely that any residues in/on pollen or nectar would be carried to the colony by foraging bees. Therefore, exposure of the colony, including larvae, can also be assumed to be negligible. Overall, no effects would be envisaged from an application at the GAP rate of 480 g a.s./ha to cereals. This is also confirmed by the acute Hazard Quotients which are calculated below, which are very much less than the trigger of 50.

Hazard Quotients:

The risk assessment for bees is based on the maximum rate of application in the GAP of 480 g a.s./ha. This is for application to cereals, which are in any case unlikely to be foraged substantially by bees. The critical endpoints (LD_{50} values) in the Table 10.3.1- 1 are the LD_{50} of >111 and >100 µg a.s./bee for oral and contact exposure, respectively.

The risk assessment is based on the Hazard Quotient approach (Q_H) by calculating the ratio between the application rate (expressed in g a.s./ha) and the laboratory contact and oral LD_{50} (expressed in µg a.s./bee). Q_H values higher than 50 indicate the need of a higher tier assessment.

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

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

Table 10.3.1- 2: Hazard quotients for bees – oral exposure

Compound	Oral LD_{50} [µg a.s./bee]	Max. application rate [g a.s./ha]	Hazard quotient Q_{HO}	Trigger	*A-priori* acceptable risk for adult bees
Ethephon	>111	480	<4.3	50	yes

The Q_H for oral exposure is below the validated trigger value of 50, indicating a low risk.

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

Compound	Contact LD_{50} [µg a.s./bee]	Max. application rate [g a.s./ha]	Hazard quotient Q_{HC}	Trigger	*A-priori* acceptable risk for adult bees
Ethephon	> 100	480	<4.8	50	yes

The Q_H for contact exposure is below the validated trigger value of 50, indicating a low risk.

As requested by the RMS, the risk of plant metabolites to bees is addressed in addition.
Bees might be exposed to metabolites that are formed in plants when consumed as food items (nectar, pollen). For the major metabolite HEPA, a worst-case approach was applied including the application rate of the parent. In addition, the metabolite is considered to be 10 times more toxic than the parent.
The acute risk assessment (HQ approach) for bees based is presented below.

Exposure scenario	Appl. rate [g a.s./ha]	LD_{50} [µg p.m./bee]	HQ	Trigger
Oral	480	>11.1 [a]	<43	50
Contact	480	>10 [a]	<48	50

[a] Assuming a ten times higher toxicity of HEPA compared to the parent ethephon and not including molar mass correction

The resulting hazard quotients are below the trigger value indicating low risk from the metabolite HEPA.

Overall conclusions for bees

The Hazard Quotients are well below the validated trigger value of 50. This indicates that the risk to foraging bees is low. This was also confirmed by the lack of effects on foragers in a tunnel test, for an application rate of 480 g a.s./ha. A laboratory study on honey bee larvae, and brood assessments in the tunnel test, showed no effects, indicating a low risk to bee brood.

Overall, it can be concluded that ethephon, when applied at the maximum application rate of 480 g a.s./ha, does not pose an unacceptable risk to foraging bees and their colonies. Hence, the risk from the uses on cereals according to the proposed GAP is low.

CP 10.3.1.1 Acute toxicity to bees

CP 10.3.1.1.1 Acute oral toxicity to bees

A new study to determine the acute oral and contact toxicity of Ethephon SL 480 to honey bees is summarised below.

Report:	KCP 10.3.1.1.1/01; [redacted]; 2014; M-504112-01-1
Title:	Effects of ethephon SL 480A G (Acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	90441035
Document No.:	M-504112-01-1
Guideline(s):	OECD 213 and 214 (1998)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

To determine the acute contact and oral toxicity of Ethephon SL 480 to the honey bee (*A. mellifera*).

Material and Methods:

The test item was Ethephon SL 480 (analysed: 41.0% w/w a.s. or 492.3 g a.s./L) from batch no. B3090017. Under laboratory conditions 50 worker bees were exposed for 48 hours to a single dose of 100 µg a.s./bee by topical application (contact limit test) and 50 worker bees were exposed for 48 hours to a single dose of 110.7 µg a.s./bee by feeding (oral limit test, value based on the actual intake of the test item).

Results:

Contact Test: At the end of the test (48 hours after application), there was 0% mortality in the 100 µg a.s./bee group. Also no mortality occurred in the control group (water + 0.5% Adhäsit). No behavioural abnormalities were observed.

Oral Test: The nominal test level of Ethephon SL 480 (100 µg a.s./bee) corresponded to an actual intake of 110.7 µg a.s./bee. This dose led to 0% mortality after 48 hours. Also no mortality occurred in the control (50% w/v sucrose solution). No behavioural abnormalities were observed.

Acute toxicity of Ethephon SL 480 to Honey Bees in the laboratory:

Test Item	Ethephon SL 480	
Exposure	contact (solution in Adhäsit (0.5 %)/water)	oral (50 % w/v sucrose solution)
Dose µg a.s./bee	100	110.7
LD_{50} µg a.s./bee	> 100	> 110.7
NOED µg a.s./bee*	100	110.7

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

Validity criteria:

Mortality of the honey bees in the control (contact test):	0 % (required: ≤ 10%)
Mortality of the honey bees in the control (oral test):	0 % (required: ≤ 10%)
LD_{50} of Reference Item (24 hrs), Contact test: LD_{50} of Reference Item (24 hrs), Oral test:	0.29 µg a.s./ bee (required: 0.10-0.30 µg a.s./ bee) 0.17 µg a.s./ bee (required: 0.10 - 0.35 µg a.s./bee)

The contact and oral tests are considered valid as the control mortality in each case was < 10% and the LD_{50} values obtained with the reference item (dimethoate) were within the required ranges.

Conclusions:

The contact LD_{50} (48 h) was > 100 µg a.s./bee. The oral LD_{50} (48 h) was > 110.7 µg a.s./bee.

CP 10.3.1.1.2 Acute contact toxicity to bees

Please refer to the previous section CP 10.3.1.1.1.

CP 10.3.1.2 Chronic toxicity to bees

A new 10 day chronic study on adult honey bees is summarized in MCA Section 8.3.1.2 and as requested by RMS below, and the endpoints are stated in Table 10.3.1-1.

Report: KCP 10.3.1.2/01 [redacted]; 2015; M-534554-01-1
Title: Ethephon SL 480A G - Assessment of effects on the honeybee, Apis mellifera L., in a 10 days chronic feeding test under laboratory conditions
Report No.: S14-00179
Document No.: M-534554-01-1
Guideline(s): No specific guideline available. Based on OECD Guideline No. 213 (1998), CEB No. 230 (2013) and OECD Guideline Proposal (2013)
Guideline deviation(s): none
GLP/GEP: yes

Objective:

To determine the effect of Ethephon SL 480 on the honey bee in a 10-day chronic feeding test.

Material and Methods:

The test item was Ethephon SL 480 (492.3 g a.s./L; 41.0 % w/w a.s.) of batch no. B3090017. During 10 days, bees were exposed to 50 % w/v sucrose solution with nominal concentrations of 187.5, 375, 750, 1500 and 3000 mg a.s./kg by continuous and *ad libitum* feeding. The control was exposed to untreated sucrose solution. Mortality and sub-lethal effects were assessed daily. The consumption of sucrose solution, the mean intake of test item and the accumulated mean intake of test item were determined. Solutions were prepared freshly every day throughout the 10-day period. Samples were taken daily for analysis for ethephon. This analysis was performed around *one year* after the in-life phase and no stability data are available. Hence, the analytical results are considered to be supporting information only. [In-life: 27 May to 24 June 2014; chemical analysis: 22 April to 12 May 2015]

Results:

No control mortality was observed. The cumulative mortality at 187.5, 375, 750, 1500 and 3000 mg a.s./kg solution was 0.0, 0.0, 2.5, 0.0 and 5.0 %, respectively at the final assessment. In the reference item group, mortality was 87.5 %. The study was considered valid because the mean mortality in the control was ≤15% and the mortality for the reference item was ≥50 %. In the control and at all test item treatment levels no sub-lethal effects were observed. Overall mean daily consumption of feeding solution (i.e. the average consumption/bee over 10 days) was at the highest concentration of 3000 mg a.s./kg statistically significantly lower than to the untreated control. Results are in the following table.

Results of a chronic feeding study on adult honeybees:

Treatment mg a.s./kg feeding solution	10-day cumulative mortality %	Overall mean consumption of feeding solution mg/bee/day	Dietary dose (DD) µg a.s./bee/day	Accumulated mean uptake µg a.s./bee
C[1] (0.0)	0.0	40.9	-	-
R[2] (0.8)	87.5	35.4	0.029	0.29
Ethephon SL 480[3]				
187.5	0.0	39.7	7.44	74.39
375	0.0	42.3	15.85	158.52
750	2.5	42.5	31.90	319.02
1500	0.0	38.5	57.70	577.03
3000	5.0	31.8*	95.53	955.29
LC_{50}	>3000 mg a.s./kg feeding solution			
LDD_{50}	> 95.53 µg a.s./bee/day			
NOEC	3000 mg a.s./kg feeding solution			
NOEDD	95.53 µg a.s./bee/day			

[1] Feeding solution: 50 % w/v aqueous sucrose solution
[2] Feeding solution: 50 % w/v aqueous sucrose solution containing Perfekthion (a.s. dimethoate)
[3] Feeding solution: 50 % w/v aqueous sucrose solution containing Ethephon SL 480
* 22% lower than the control, which was statistically significant (Williams t-test α = 0.05)
LDD_{50} = Median Lethal Dietary Dose

Analytical Results: The analysed concentration of ethephon for 10 consecutive days per individual test item treatment level was within the range of 74 – 85 % of the nominal concentration. No residues of ethephon above the LOQ (10 µg/kg) were found in any of the control samples.

Conclusions:

The LC_{50} for 10 days of continuous exposure was >3000 mg a.s./kg feeding solution. The corresponding LDD_{50}, based on the actual consumption, was >95.53 µg a.s./bee/day The NOEC for mortality after 10 days was 3000 mg a.s./kg feeding solution. The corresponding NOEDD, based on the actual consumption, was 95.53 µg a.s./bee/day. Consumption of sucrose solution containing 3000 mg a.s./kg was 22% lower than that consumption of untreated sucrose solution in the control.

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

A study on acute toxicity to honey bee larvae and a honey bee brood feeding study are summarised in MCA Section 8.3.1.3 and below as requested by RMS. A tunnel test which includes assessment of brood is summarised later in this section.

Report:	KCP 10.3.1.3/01; [redacted]; 2015; M-528291-01-1
Title:	Ethephon SL 480B G - A honey bee brood feeding study to evaluate potential effects on brood development and mortality of the honey bee, Apis mellifera L. (Hymenoptera: Apidae)
Report No.:	20130045
Document No.:	M-528291-01-1
Guideline(s):	EPPO Bulletin 22 ([redacted] et al. 1992)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

After study finalisation, it was realised that the sucrose solution containing 2.4 g a.s./L should have been pH-buffered. The pH of a 2.4 g a.s./L aqueous solution of Ethephon SL 480 is 2.0 ([redacted], 2015, M-542286-01-1, KCA 8.3.1.3/03, KCP 10.3.1.3/02). Uptake of 1 L of the treated sucrose solution by each colony was clearly slower than uptake of untreated sucrose solution by control colonies. This was probably related to acidity. The possibility of consequent experimental artefacts could not be excluded. Hence, the study was concluded as unreliable. Subsequently, to replace the study, an acute larval toxicity study ([redacted], 2015) and a honeybee tunnel test ([redacted], 2015) were done.

In the honey bee tunnel test ([redacted], 2015), Ethephon SL 480 was sprayed onto flowering *Phacelia* at 120 or 480 g a.s./ha in the presence of one colony per tunnel. The nectar from foraging bees was analysed for ethephon. The highest measured concentration in nectar was 3 mg a.s./kg (day 0). This realistic worst-case level of ethephon in nectar is 800x lower than the concentration in the sugar solution used in the brood feeding study (2400 mg a.s./L). Hence, *in hindsight*, the exposure concentration in the brood feeding study can be regarded as completely unrealistic.

Objective:

To investigate the effect of Ethephon SL 480 on honey bee brood when exposed by via the diet.

Material and Methods:

The test item was Ethephon SL 480 (487.7 g a.s./L, analysed) from batch no. NK49CX0211. The test item (4.93 mL) was mixed with each 1 L of 50% (w/v) sucrose solution to give a concentration of 2.4 g a.s./L. One litre of this solution was then fed to each of three colonies per test group. Mortality of adult bees, pupae and larvae was assessed 21 days after introduction of the test item. Also bee brood development (eggs, young and old larvae) was recorded one day before introduction of the test item, and 4, 8, 15 and 21 days after introduction of the test item. Three control colonies were given untreated sucrose solution. 3.0 g of Insegar (25% fenoxycarb) in 1 L of sucrose solution was used as a reference substance (i.e. 0.75 g fenoxycarb/L). The bees were free flying, with access to natural

foraging recourses (e.g. nectar and pollen) in the surroundings. Due to the time of the year, mass-flowering crops was already fading (Dates of experimental work: June 17 to July 12, 2013).

Results:

Ethephon SL 480: Results of a brood feeding study on honey bee (*Apis mellifera*):

Assessment period	Control n=3	Test Item n=3	Reference Item n=3
	Worker Mortality / Colony (Means ± SD)		
Pre-Application (DAT -3 to 0)	22.33 ± 4.68	13.00 ± 2.65	17.67 ± 8.08
Post-Application (DAT 1 to 22)	13.52 ± 4.43	10.29 ± 3.39	18.18 ± 3.09
	Pupal Mortality / Colony (Means ± SD)		
Pre-Application (DAT -3 to 0ba)	0.42 ± 0.38	1.08 ± 0.52	0.25 ± 0.25
Post-Application (DAT 1 to 22)	0.58 ± 0.17	0.52 ± 0.38	53.58 ± 20.07$^{\Delta}$
	Development of selected Eggs (Means ± SD)		
Brood Termination Rate (%) at BFD 22 (DAT 21)	11.67 ± 2.52	31.33 ± 15.95$^{\Delta}$	34.67 ± 23.71$^{\Delta}$
Brood Index at BFD 22 (DAT 21)	4.42 ± 0.13	3.43 ± 0.80	3.27 ± 1.19
Compensation Index at BFD 22 (DAT 21)	4.57 ± 0.09	3.84 ± 0.58	3.36 ± 1.25
	Development of selected Young Larvae (Means ± SD)		
Brood Termination Rate (%) at BFD 22 (DAT 21)	3.33 ± 1.5	9.33 ± 9.24$^{\Delta}$	12.00 ± 6.08$^{\Delta}$
Brood Index at BFD 22 (DAT 21)	4.83 ± 0.08	4.60 ± 0.35	4.40 ± 0.30*
Compensation Index at BFD 22 (DAT 21)	4.85 ± 0.09	4.61 ± 0.36	4.42 ± 0.29*
	Development of selected Old Larvae (Means ± SD)		
Brood Termination Rate (%) at BFD 22 (DAT 21)	1.67 ± 2.08	5.67 ± 4.73$^{\Delta}$	14.67 ± 11.59$^{\Delta}$
Brood Index at BFD 22 (DAT 21)	4.92 ± 0.10	4.72 ± 0.24	4.26 ± 0.58*
Compensation Index at BFD 22 (DAT 21)	4.94 ± 0.07	4.81 ± 0.13	4.30 ± 0.61*

$^{\Delta}$ Statistically significantly greater as compared to the control
* Statistically significantly smaller as compared to the control
DAT Days After Treatment
BFD Brood area Fixing Day

Uptake of sucrose solutions: The results for uptake of the 1 L of sucrose solutions per colony are presented below:

Results for consumption of 1 L of 50 % sucrose solution

Treatment	Replicate	Test solution consumed (Y/N)	Test solution consumed within (h)	Leftover volume (mL)*	No. of dead bees in feeder
Control	1	Y	48	0	0
	2	Y	24	0	0
	3	Y	24	0	0
Test item	1	Y	48	0	1
	2	Y	72	0	0
	3	Y	72	0	4
Reference item	1	Y	48	0	52
	2	Y	48	0	53
	3	Y	48	0	61

* measured on DAT 22; the initial volume of feeding solution per colony was 1000 mL per colony

Two of the colonies presented with sucrose solution containing the test item took 72 hours to take up the complete 1 L volume. This contrasts with the control, for which two colonies took 24 hours to take up the same volume.

Bee behaviour: In all treatments, no abnormal behaviour was observed during the whole study period, except slightly increased aggressiveness in two of the reference item replicates between DAT 10-12.

Colony strength: During the course of the study, the mean colony strength in the control, test item and reference item treatment displayed a relative increase of 30%, 19% and 17%, respectively, at study termination (DAT 22). No statistically significant differences were detected between the treatments.

Brood nest (eggs/larvae/pupae): During the course of the study, the estimated mean comb area comprising brood per colony displayed a relative change of + 16%, - 2% and - 30%, respectively, at study termination (DAT 22). There was a statistically significant negative effect on the relative change of the brood nest size of the reference item treatment as compared to the control.

Stores (pollen/nectar/honey): During the course of the study, the estimated mean comb area comprising food per colony displayed a relative increase of 51%, 63% and 65%, respectively, at study termination (DAT 22). For this parameter, no statistically significant differences were detected between the test item treatment or the reference item treatment, compared with the control. In this study, the major influence of the reference item could be seen as a high level of pupal mortality which is a known effect for this substance.

Vacant cells: During the course of the study, the estimated mean comb area comprising of vacant cells per colony displayed a relative change of - 43%, - 24% and + 17%, for the control, test item and reference item treatment, respectively, at study termination (DAT 22). There was a statistically significant negative effect on the relative change of vacant cells of the reference item treatment as compared to the control.

Brood Termination Rate (BTR): As compared to the control, in the test item treatment a statistically significant increase of BTR was detected for initially selected eggs (from BFD 5 onwards), young larvae (from BFD 9 onwards) and old larvae (from BFD 5 onwards). Although BTR was statistically significantly higher than observed in the control for both young and old larvae in the test item treatment the actual levels were quite low (9.33 and 5.67%, respectively) which may not be biologically significant for the development of the colony. As compared to the control, in the reference item treatment a statistically significant increase of BTR was detected for initially selected eggs (from BFD 16 onwards), young larvae (from BFD 9 onwards) and old larvae (from BFD 9 onwards). Although this supports that the test system was sensitive to detect potential effects of plant protection products on honey bee brood the overall levels of effects on BTR seen in the reference item treatment were relatively low. In this study, the primary indicator of effect was of that on pupal mortality, which was not observed in either the control or test item treatment.

Bee brood index: While the Brood Indices of initially selected young and old larvae in the test item treatment displayed increases comparable to the control, thus indicating a successful development of the brood, the Brood Index of eggs remained lower as compared to the control. Statistical analyses showed that Brood Indices in the test item treatment were not significantly decreased as compared to the control, except for a single assessment at BFD 9, where a statistically significant decrease was detected for eggs. Compared to the control, mean Brood Indices of the reference item treatment were not statistically significantly decreased for selected eggs, but were significantly decreased for young larvae at BFD 22 and for old larvae from BFD 9 onwards.

Brood Compensation Index: Overall, except for selected eggs, the Brood Compensation Indices of the control and test item displayed comparable increases, indicating a successful compensation of previous brood losses. Statistical analyses showed that Brood Compensation Indices in the test item treatment were not significantly decreased after completing a whole brood cycle (i.e. at BFD 22) as compared to the control (although a transient difference was observed between control and test item treatment at BFD 9). In contrast, the mean Brood Compensation Indices of the reference item treatment exhibited a statistically significant decrease as compared to the control for young larvae at BFD 22 and for old larvae from BFD 9 onwards, but not for eggs.

Conclusions:

Overall, according to the results of this study, it seems unlikely that Ethephon SL 480 fed under worst case test conditions at a concentration of 2.4 g a.s./L (2400 mg a.s./L) will cause irreversible adverse effects on honey bee colony vitality or survival.

Evaluator comment:

The BTR for marked eggs was higher in the ethephon-treated colonies than the control. But also, consumption of sucrose solution was also markedly slower in these colonies than in the control. It cannot be excluded that the acidity (pH 2.0) of the ethephon-treated solution had an influence on the uptake rate of the treated solutions. Also, this low pH is likely to have resulted in general 'irritation' of adults and brood in the dosed colonies. These factors had the potential to increase the BTR. As such, the higher BTR in the test item colonies than the control colonies can be regarded as an artefact of the 'physico-chemical' impact of low pH. For this reason, the study was judged to be unreliable. In addition, the study is lacking in *relevance* as the tested concentration in sucrose was 800x higher than *measured realistic* worst-case levels in nectar from foraging bees in the subsequent tunnel test ([redacted], 2015). Overall, the brood feeding study summarised above is not considered suitable for use in the risk assessment.

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. In addition, effects on foraging behaviour were assessed in a honey bee tunnel test (CP 10.3.1.5).

CP 10.3.1.5 Cage and tunnel tests

Existing data show that ethephon has a low toxicity to bees. To ensure that the requirements of Regulation 1107/2009 are satisfied, a honey bee tunnel test has been conducted on Ethephon SL 480. This study is summarised below.

Report: KCP 10.3.1.5/01; [redacted]; 2015; M-540667-01-1
Title: Assessment of side effects of ethephon SL 480A G on the honeybee (Apis mellifera L.) in the semi-field after one application on Phacelia tanacetifolia in 2015
Report No.: B170AMS
Document No.: M-540667-01-1
Guideline(s): OECD Guidance Document No. 75 (2007) and current recommendations of the AG Bienenschutz (PISTORIUS et al., 2012) OEPP/EPPO Guideline No. 170(4) (2010)
Guideline deviation(s): No major deviations (see chapter 7 for the deviations from the study plan)
GLP/GEP: **yes**

Objective:

To determine the effects of Ethephon SL 480 on the honeybee (*Apis mellifera* L) after one application on *Phacelia tanacetifolia* in a semi-field brood study. In particular, the study was to assess mortality, flight intensity, behaviour, condition of colonies, and development of the brood. In addition the aim was to quantify ethephon residues in pollen and nectar from forager bees, and in pollen, nectar and larvae from combs.

Material and Methods:

The test item was Ethephon SL 480 (analysed: 41.0% w/w a.s. or 492.3 g a.s./L) from batch no. B3090017. The study included four treatment groups with four replicates (tunnels) each: one water-treated control group (C), two test-item groups (T1 and T2) and one reference item group (R). In addition there was one extra tunnel each for T1 and T2 to provide samples of matrices for quantification of ethephon concentrations. Each tunnel in this study contained a single colony.

Treatments were applied at full-flowering (BBCH 65) with bees actively foraging on the crop. The target application rate of the test item in T1 was 120 g a.s./ha (actual average rate applied was 125 g a.s./ha) and in T2 it was 480 g a.s./ha (actual average rate applied was 492 g a.s./ha). Tap water was applied in the control group and Insegar was applied in group R at 300 g fenoxycarb/ha. The spray volume was 300 L/ha in all treatment groups.

The initial mean colony sizes per treatment group were in the range of 2795 to 5633 bees. The colonies were placed in the tunnels on 9 July 2015 in the late evening and remained in the tunnels for 12 days. Thereafter they were kept at a monitoring site for further assessments. Colonies were assessed once before set-up, twice whilst inside the tunnel and three times at the monitoring site. The in-life phase of the study was conducted from 9 July to 10 Aug 2015.

The following endpoints were assessed:

- Total and mean number of dead bees (workers and pupae counted separately) on the linen sheets in tunnels, in the dead bee traps, and on the bottom of the hive before and after the spray application in C, T1, T2 and R.
- Flight intensity (mean number of forager bees/m^2 of *Phacelia tanacetifolia*) before and after the spray application in C, T1, T2 and R.
- Behaviour of the bees in the crop and around the hive.
- Condition of the colonies (colony strength and area of the different brood stages and food storage per colony, at each assessment date).
- Development of the brood assessed in individual brood cells. For this assessment >200 individually marked egg cells per colony were selected when possible.
- Determination of residues of ethephon in pollen and nectar from collected forager bees, and in pollen, nectar and larvae from combs.

Results:

Validity of the study:

The application procedure resulted in precise application rates and a uniform distribution of the treatments over the plants. The hives and the crop were in good condition, adequate for the purposes of this study as can be seen by the low termination rates of the control colonies and low background mortality. Foraging intensity during the exposure phase flight activity ensured sufficient exposure to treated flowers. The reference item fenoxycarb produced statistically significant effects on adult bee mortality 1 or 2 days after a full brood cycle starting at the application day and strong lasting effects on pupal survival during the monitoring period. Assessments of the brood termination of a selected egg cohort by image analysis resulted in consistently higher termination rates (although the statistical analysis of the latter was inconclusive). Together these findings showed that the test set-up and the statistical analysis of the results were adequate to detect in a meaningful manner significant effects of the test item on these parameters if these occurred.

Mortality:

	Treatment group	Control (C)	Test item (T1)	Test item (T2)	Reference item (R)
Daily mean mortality (dead worker bees /colony) ± STD	4DBA to 0DBA	9.3 ± 0.7	9.5 ± 3.1	13.1 ± 5.1	11.8 ± 6.1
	0DAA	9.8 ± 2.9	8.5 ± 2.9	12.8 ± 5.3	5.8 ± 2.1
	0DAA to 7DAA[3)]	32.6 ± 16.2	15.9 ± 7.0	17.6 ± 7.7	11.6 ± 4.5
	0DAA to 27DAA	40.1 ± 26.5	30.4 ± 31.2	36.4 ± 26.4	43.0[1)] ± 51.9
Daily mean mortality (dead larvae+pupae /colony) ± STD	4DBA to 0DBA	0.3 ± 0.2	0.7 ± 1.3	0.5 ± 0.5	0.9 ± 0.7
	0DAA	1.0 ± 1.4	0.3 ± 0.5	0.0 ± 0.0	0.3 ± 0.5
	0DAA to 7DAA[3)]	0.3 ± 0.2	0.2 ± 0.2	0.3 ± 0.4	0.1 ± 0.2
	0DAA to 27DAA	0.1 ± 0.2	0.1 ± 0.2	0.2 ± 0.4	4.1[2)] ± 5.9

DAA: days after application; DBA: days before application; STD: standard deviation of daily mean mortality of 4 replicates;
[1)] Statistically significantly higher than control group (1-sided Dunnett t-test) on 22DAA (P=0.014) and 23DAA (P<0.001)
[2)] Statistically significantly higher than control group (Mann-Whitney U-test) with pooled data (P<0.001)
[3)] Mortality assessed on 8DAA in the morning

Throughout the period before exposure, mortality of adult bees across all future treatments was similar indicating comparable acclimatisation of the colonies to restricted conditions in the tunnels. On the application day and during the entire exposure period from day 0 until day 7 after application, mortality of adult bees across all treatments was similar, indicating no effect of the test item. The number of dead adult bees in the test item treatments did not differ statistically from the control treatment in the period 0DAA to 27DAA. The reference item treatment showed a statistically significant difference from mean values in the control at the monitoring site (1-sided Dunnett's t-test, α = 0.05) on 22DAA (P=0.014) and 23DAA (P<0.001).

The number of observed dead pupae and larvae before exposure was similar in all treatments groups. During the exposure period and the whole testing period after the application the mortality values between the test item groups and the control group were comparable. The mean value of the pupal and larval mortality in the reference item treatment was statistically significant over the period 0DAA to 27DAA (P<0.001)(Mann-Whitney U-Test pooled). These effects are expected after exposure of bees to this reference substance confirming exposure and sensitivity of the test system to detect harmful effects. Thus, no relevant test-item related adverse effects on adult bee or pupal mortality were observed.

Flight Intensity

	Treatment group	Control (C)	Test item (T1)	Test item (T2)	Reference Item (R)
Daily mean flight intensity (bees/m²) ± STD	4DBA to 0DBA	7.8 ± 0.6	7.2 ± 0.8	9.7 ± 3.8	6.6 ± 2.0
	0DAA	8.2 ± 0.6	8.9 ± 1.4	10.1 ± 2.8	7.6 ± 3.9
	0DAA to 7DAA	11.8 ± 2.0	11.2 ± 1.6	10.6 ± 2.4	10.4 ± 3.5

DAA: days after application; DBA: days before application; STD: standard deviation of daily mean flight intensity of the 4 replicates

Foraging rates were similar across all treatments before exposure (4DBA and 0DBA). No significant differences were found between future treatment groups and the control group (Dunnett's t-Test, two sided, $\alpha = 0.05$). On the application day in the morning, just before the water application, the average number of foraging bees was 8.8 ± 0.8 in the control, 8.5 ± 0.7 in the T1 treatment, 10.3 ± 3.3 in the T2 treatment and 9.8 ± 2.1 in the reference item treatment.

On the day of application (0DAA) no statistically significantly (Mann-Whitney U-Test pooled data) reduced number of foraging bees was observed in all treatment groups compared to the control group. From 0DAA to 7DAA foraging activity was similar in all treatment groups and no test item and reference item related effects occurred. Thus, no relevant test-item related adverse effects on flight intensity were observed.

Behaviour of the Bees

Bees with locomotion problems, cramping bees, inactive bees and trembling bees were observed during the study, especially during the monitoring phase. The mostly observed abnormal behaviour was cramping bees. This was more noticeable in the treatment groups than in the control. After the application 10 cramping bees in total were observed in the control group, 25 in the T1 group, 38 in the T2 group and 41 in the R group. The highest number of trembling bees was observed in the control group, with a total of 56 records after the application (or more since the assessment was not done on one occasion). In the test item group T1 1 bee was recorded, in the T2 25 and in the R group 15 bees. Other abnormal symptoms occurred only occasionally in all treatment groups. No hanging bees, bees clustering at hive and bees aggressive to other bees were observed during the study period. Behavioural abnormalities occurred but were at a similar level as in the control and were not seen as an effect related to the test item.

Development of Honeybee Brood in Individual Cells

Findings are summarised in the table below.

Replicate	Brood / Compensation indices at x days after brood area fixing day (BFD)					Termination rate (BFD+22)
	0	+6	+10	+17	+22	[%]
Mean C	**1.00 / 1.00**	**3.19 / 3.22**	**3.67 / 3.73**	**3.66 / 3.79**	**4.41 / 4.49**	**11.81**
STD	0.00 / 0.00	0.38 / 0.33	0.21 / 0.10	0.25 / 0.14	0.39 / 0.08	7.87
Mean T1	**1.00 / 1.00**	**3.00 / 3.01**	**3.66 / 3.69**	**3.48 / 3.63**	**4.26 / 4.38**	**14.88**
STD	0.00 / 0.00	0.23 / 0.24	0.20 / 0.20	0.35 / 0.26	0.38 / 0.13	7.56
Mean T2	**1.00 / 1.00**	**2.80 / 2.85**	**3.35 / 3.54**	**3.31 / 3.75**	**4.07 / 4.53**	**18.62**
STD	0.00 / 0.00	0.39 / 0.33	0.41 / 0.24	0.37 / 0.24	0.49 / 0.29	9.84
Mean R	**1.00 / 1.00**	**2.59 / 2.67***	**3.27 / 3.46**	**3.19 / 3.57**	**3.95 / 4.37**	**20.95**
STD	0.00 / 0.00	0.52 / 0.44	0.47 / 0.30	0.54 / 0.24	0.64 / 0.19	12.77

BFD: Brood area fixing day; STD: Standard deviation
*: Mean value for treatment group statistically significantly lower (compensation index) compared to the control

In the control group C, successful development was observed in the majority of the marked brood cells, indicating a healthy development of brood. The mean termination rate at the end of the observation period (BFD+22) was at 11.81%.
In the test item treatment group T1 the brood development and mean termination rates were similar to the control without statistically significant differences. The mean termination rate at the end of the observation period (BFD+22) was at 14.88%.

In the test item treatment group T2 the brood development and mean termination rates were also similar to the control without statistically significant differences. The mean termination rate at the end of the observation period (BFD+22) was at 18.62%.

In the reference item treatment group R, the post treatment mean values of the brood and compensation indices were slightly lower than those observed in the control. The mean termination rate at the end of the observation period (BFD+22) was 20.95 % and slightly higher than in the control, without statistically significant differences. In this study strong effects on the marked brood were not observed due to exposure to the reference item. However, strong effects were noted on pupal mortality with the reference item inducing 20 - 40x higher mortality than the control or test item treatment groups.

Overall, exposure to the test item did not cause any test-item related adverse effects on larval development.

Strength of the Colonies

The overall development of colony strength (number of bees per hive) of all treatment groups showed fluctuations in a typical and normal range. At the start of the test the colony strength of all future treatment groups was comparable to the control group and no statistical significant differences were observed (Dunnett's t-Test, two sided, $\alpha = 0.05$). The mean number of bees in the control and the treatment groups showed the same increasing trend from the first to the last assessment. No statistically significant differences were detected in the post application assessments (Dunnett's t-Test, one sided, $\alpha = 0.05$).

Development of the Brood Area

The mean amount of brood in the colonies (sum of cells containing eggs, larvae, and pupae) was assessed. The mean number of brood stages in the control and the treatment groups showed a similar trend from the first to the last assessment. No statistically significant differences were detected in the post application assessments (followed by Dunnett's t-Test, one sided, $\alpha = 0.05$).

Development of the Food Storage Area

The mean amount of food stores in the colonies (sum of cells containing nectar and pollen) was assessed. The majority of the colonies were well provided during the course of the study. Thus, no treatment related adverse effects on the development of the food storage area were observed.

Residue analysis

Residue analysis of ethephon was performed by using High Performance Liquid Chromatography (HPLC), chromatographed under gradient reversed phase conditions, which was coupled with electrospray and tandem mass spectrometry (MS/MS) detection. The respective Limit of Quantification (LOQ), defined as the lowest validated fortification level, of ethephon was 50 µg/kg. The corresponding respective Limit of Detection (LOD) was 30 µg/kg. Measured concentrations of ethephon in the pre-application samples were below the LOD in all cases.

Findings in the post application collected samples are presented in the table below.

Commodity	Sample interval	Treatment	Date	Timing	Measured conc. [µg/kg]
Larvae	S3	T1e	17 July 2015	3 DAA	<LOD
Larvae	S4	T1e	21 July 2015	7 DAA	<LOD
Larvae	S5	T1e	28 July 2015	14 DAA	<LOD
Larvae	S6	T1e	4 August 2015	21 DAA	<LOD
Larvae	S3	T2e	17 July 2015	3 DAA	<LOD
Larvae	S4	T2e	21 July 2015	7 DAA	<LOD
Larvae	S5	T2e	28 July 2015	14 DAA	<LOD
Larvae	S6	T2e	4 August 2015	21 DAA	<LOD
Pollen from combs	S4	T1e	21 July 2015	7 DAA	255
Pollen from combs	S5	T1e	28 July 2015	14 DAA	Note 1
Pollen from combs	S6	T1e	4 August 2015	21 DAA	<LOD
Pollen from combs	S4	T2e	21 July 2015	7 DAA	1440
Pollen from combs	S5	T2e	28 July 2015	14 DAA	853
Pollen from combs	S6	T2e	4 August 2015	21 DAA	<LOD
Pollen from bees	S2	T1e	14 July 2015	0 DAA	Note 2
Pollen from bees	S3	T1e	17 July 2015	3 DAA	676
Pollen from bees	S4	T1e	21 July 2015	7 DAA	261
Pollen from bees	S2	T2e	14 July 2015	0 DAA	27966
Pollen from bees	S3	T2e	17 July 2015	3 DAA	2751
Pollen from bees	S4	T2e	21 July 2015	7 DAA	257
Nectar from combs	S4	T1e	21 July 2015	7 DAA	<LOQ
Nectar from combs	S5	T1e	28 July 2015	14 DAA	78
Nectar from combs	S6	T1e	4 August 2015	21 DAA	69
Nectar from combs	S4	T2e	21 July 2015	7 DAA	362
Nectar from combs	S5	T2e	28 July 2015	14 DAA	288
Nectar from combs	S6	T2e	4 August 2015	21 DAA	124
Nectar from bees	S2	T1e	14 July 2015	0DAA	759
Nectar from bees	S3	T1e	17 July 2015	3 DAA	<LOD
Nectar from bees	S4	T1e	21 July 2015	7 DAA	<LOQ
Nectar from bees	S2	T2e	14 July 2015	0DAA	3046
Nectar from bees	S3	T2e	17 July 2015	3 DAA	103
Nectar from bees	S4	T2e	21 July 2015	7 DAA	<LOQ

T1e = samples taken from tunnel e applied with ethephon at 120 g/ha; T2e = samples taken from tunnel e applied with ethephon at 480 g/ha.
LOQ = Limit of Quantification (50 µg/kg).
LOD = Limit of Detection (30 µg/kg).
Note 1: Sample not analysed because there was no pollen on 14DAA in the T1e colony.
Note 2: Sample not analysed because not enough sampled material was available.

Conclusions:

Ethephon SL 480 was applied at two rates corresponding to 120 g a.s./ha (treatment T1) and 480 g a.s./ha (treatment T2), at full-flowering *Phacelia tanacetifolia*, during daily honeybee foraging activity. The effects on honeybee colonies under confined conditions considering mortality, flight intensity, behaviour, colony strength, amount of brood and brood cell development were evaluated.

No biologically relevant test-item or rate-response related adverse effects on mortality and flight intensity were observed in T1 and T2. Some unusual behavioural was observed during the study period but at a similar level in all treatment groups and so cannot be considered to be treatment related.

No test-item related adverse effects on colony strength and amount of brood, measured as mean number of cells covered with the different brood stages and of the food storage area were observed in T1 and T2.

The effects on brood development (termination rates, brood and compensation indices) on individually marked cells performed in this study revealed that Ethephon SL 480 did not cause any treatment-related adverse effect on honeybee brood development.

The analytical chemistry confirmed exposure in a dose related manner for foragers. Transport of contaminated food into the hive (and thereby potential exposure of brood) was demonstrated. No residues were found on larvae above the limit of detection. Fast reduction of residue levels was seen on pollen and nectar on foraging bees and pollen stored on combs, but less so on nectar on the combs. In-colony concentrations in pollen and nectar sampled from combs were several orders of magnitude lower than those collected initially from the treated crop by foraging bees.

CP 10.3.1.6 Field tests with honeybees

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

CP 10.3.2 Effects on non-target arthropods other than bees

The risk assessment in this section was performed according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) and according to the Guidance Document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods (ESCORT 2, [redacted] et al. 2000[3]).

For information on studies already evaluated during the previous EU review, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph.

A single-rate glass plate study for *Aphidius rhopalosiphi* and the same for *Typhlodromus pyri*, were evaluated during the previous EU review. In the study on *A. rhopalosiphi* >50% mortality occurred at

[3] [redacted] 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

726 g a.s./ha. Hence, for the current EU review, a new laboratory study on this species has been conducted with a *range* of application rates (including lower rates) in order to derive an LR_{50} ([redacted]; 2015; M-528489-01-1). This study is summarised in MCA 8.3.2 and MCP 10.3.2.1. In addition, extended laboratory studies on *A. rhopalosiphi*, *Coccinella septempunctata*, and *Chrysoperla carnea* have been conducted since the time of the previous EU review. Summaries of these three studies are presented later in this section.

The endpoints from the available studies on non-target arthropods are listed in Table 10.3.2- 1. Endpoints from studies evaluated during the previous EU review are stated in grey text. Endpoints from additional studies submitted for this EU review are stated in black text.

Table 10.3.2- 1: Ethephon (Ethephon SL 480): Endpoints from studies on non-target arthropods

Test species	Study type, application rate	Endpoint	Reference
Aphidius rhopalosiphi	Laboratory, glass plate 726 g a.s./ha	87.2% mortality; 5.4% *increase* of parasitisation efficiency	LoEP KCA 8.3.2.1/01 M-172516-01-1
Aphidius rhopalosiphi	Laboratory, glass plate 5 rates: 48 to 480 g a.s./ha	Rate g a.s./ha: 48 85 152 270 480 Corr. Mort. %: 0 5.0 1.7 5.0 55.0 LR_{50} : 465 g a.s./ha	[redacted] (2015) KCA 8.3.2.1/03 KCP 10.3.2.1/05 M-528489-01-1
Typhlodromus pyri	Laboratory, glass plate 726 g a.s./ha	17.7% mortality. No significant adverse effects on reproduction (R=0.67)	LoEP KCP 8.3.2.2/01 M-172467-01-1
Chrysoperla carnea	Laboratory, glass plate 726 g a.s./ha	-3.4% 'corrected mortality' (i.e. there was less mortality than the control); 60.1% less reproduction than the control	LoEP KCP 10.3.2.1/01 M-179325-01-1
Poecilus cupreus	Laboratory, sand substrate 726 g a.s./ha	0% mortality; 0% effect on reproduction	LoEP KCP 10.3.2.1/02 M-172462-01-1
Aphidius rhopalosiphi	Extended lab, barley seedlings 1440 g a.s./ha [test item: 476 g a.s./L+59 g cyclanilide/L]	0% mortality; 7.1% less reproduction than the control	LoEP KCP 10.3.2.2/01 M-171646-01-1
Typhlodromus pyri	Extended Lab, bean leaves 4 rates: 209 to 1672 g a.s./ha	-0.2% corrected mortality and 17% reduction in repro at 836 g a.s./ha [10.1% corrected mortality and 0.9% reduction in repro at 1672 g a.s./ha]	LoEP KCP 10.3.2.2/02 M-230332-01-1
Chrysoperla carnea	Extended Lab, maize leaves 726 g a.s./ha	-4.1% corrected mortality (i.e. there was less mortality than the control); 1.33% less reproduction than the control	LoEP KCP 10.3.2.2/03 M-179333-01-1
Aphidius rhopalosiphi	Extended Lab, barley plants 5 rates: 633 to 6818 g a.s/ha	0% mortality for control and all rates. 11% less repro at 6818 g/ha than control LR_{50} >6818 g a.s./ha	[redacted] (2008a) KCP 10.3.2.2/04 M-304060-01-1
Coccinella septempunctata	Extended Lab, bean leaves 5 rates: 609 to 4870 g a.s/ha	12.5% corrected mortality and no impact on reproduction at 4870 g a.s./ha. LR_{50} >4870 g a.s./ha	[redacted] (2009) KCP 10.3.2.2/05 M-328138-01-1
Chrysoperla carnea	Extended Lab, bean leaves 5 rates: 609 to 4870 g a.s/ha	5% mortality and no impact on reproduction at 4870 g a.s./ha. LR_{50} >4870 g a.s./ha	[redacted] (2008b) KCP 10.3.2.2/06 M-326982-01-1

Based on the data summarised in Table 10.3.2- 1, it is clear that ethephon has a low toxicity to non-target terrestrial arthropods. There was an effect on survival of *A. rhopalosiphi* in a single-rate glass plate study at 726 g a.s./ha. This has been addressed by a lack of effects on this species in two

extended laboratory studies (KCP 10.3.2.2/01 and [redacted], 2008a) including at a rate of 6818 g a.s./ha (i.e. 14x higher than the GAP rate in cereals). Also, there was a possible effect on reproduction of *C. carnea* in a single-rate glass plate study at 726 g a.s./ha. This has been addressed by a lack of effects on this species in two extended laboratory studies (KCP 10.3.2.2/03 and [redacted], 2008b) including at a rate of 4870 g a.s./ha (i.e. 10x higher than the GAP rate in cereals).

Risk assessment for other non-target arthropods

Table 10.3.2- 2: Tier 1 In-field risk assessment for non-target arthropods

Crop	Species	Appl. rate, g a.s./ha	LR_{50}, g a.s./ha	HQ	Trigger
Cereals	*T. pyri*	480	>726	<0.66	2
	A. rhopalosiphi	480	465	1.03	2

HQ: Hazard Quotient

Table 10.3.2- 3: Tier 1 Off-field risk assessment for non-target arthropods

Crop	Species	Rate g a.s/ha	Drift %	VDF	CF	LR_{50} g a.s./ ha	HQ	Trigger
Cereals	*T. pyri*	480	2.77	10	10	>726	<0.02	2
	A. rhopalosiphi	480	2.77	10	10	465	0.03	2

VDF: 'Vegetation Distribution Factor' (divides exposure estimate by 10).
CF: 'Correction Factor' to account for interspecies variation in sensitivity (multiplies exposure estimate by 10).

For *A. rhopalosiphi* and *T. pyri* the calculated HQ values for the in-field and off-field scenario are below the trigger of concern. Therefore, there is a low risk to non-target arthropods from the proposed use in cereals at 480 g a.s./ha. A range of extended laboratory studies confirm this conclusion, and indicate an even wider margin of safety.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

These data are presented below and in MCA 8.3.2, and the endpoints are listed in Table 10.3.2- 1.

Report:	KCP 10.3.2.1/05; [redacted]; 2015; M-528489-01-1
Title:	Toxicity to the parasitoid wasp Aphidius rhopalosiphi (Hymenoptera: Braconidae) using a laboratory test ethephon SL 480 g/L
Report No.:	CW15/020
Document No.:	M-528489-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP not applicable [redacted] et al. (2000), [redacted] et al. (2001)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

To investigate the toxicity of Ethephon SL 480 to *A. rhopalosiphi* when exposed to treated glass plates.

Material and Methods:

The test item was Ethephon SL 480 (analysed: 41.0% w/w a.s. or 492.3 g a.s./L) from batch no. B3090017. The test item was applied to glass plates at rates of 48, 85, 152, 270 and 480 g a.s./ha, and allowed to dry. The effects on *A. rhopalosiphi* (<48 h old) of contact exposure to these plates was compared to those of a water-treated control. A toxic reference (dimethoate) applied at 0.05 g a.s./ha was also included. There were 4 replicates of 15 wasps, for the treatment group, and for the control. Mortality was assessed 2, 24 and 48 h after the start of exposure. Temperature was 19.5-20.5 °C and relative humidity was 71-83%. The light/dark cycle was 16:8 h with light intensity of 1026-1495 Lux.

Results:

Ethephon SL 480: Results of a laboratory glass-plate rate-response study on *Aphidius rhopalosiphi*

Exposure	Dried spray deposits on glass plates		
Treatment [g a.s./ha]	**Mortality after 48 hours [%]**	**Corrected mortality [%]**	**P-Value[1]**
Control	0.0	-	-
48	0.0	0.0	1.000 ns
85	5.0	5.0	0.487 ns
152	1.7	1.7	1.000 ns
270	5.0	5.0	0.487 ns
480	55.0	55.0	<0.001*
Toxic reference 0.05 g dimethoate/ha	91.7	91.7	-
LR_{50} = 465 g a.s./ha (95% Confidence Interval: 393 – 611, calculated with Probit analysis)			

[1] Fisher´s Exact test (one-sided, α = 0.05); * = statistically significant; ns = not statistically significant

Conclusions:

The LR_{50} for *A. rhopalosiphi* was calculated to be 465 g a.s./ha.

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

Three studies conducted since the time of the previous EU review are summarised below.

Report: KCP 10.3.2.2/04; [redacted]; 2008; M-304060-01-1
Title: Dose-response toxicity (LR50) of Ethephon SL 480 g/L to the parasitic wasp Aphidius rhopalosiphi (DESTEFANI-PEREZ) under laboratory conditions
Report No.: 08 10 48 011 A
Document No.: M-304060-01-1
Guideline(s): IOBC Guideline ([redacted] et al. 2000)
Guideline deviation(s): none
GLP/GEP: yes

Objective:

To determine a dose-response relationship for mortality of adult *A. rhopalosiphi* in an extended laboratory test. Wasps were exposed to dried spray residues of the test item on potted barley plants.

Material and Methods:

The test item was Ethephon SL 480 of batch B7090030 [analysed: 40.5% w/w a.s.; 487.0 g a.s./L]. *A. rhopalosiphi* adults were exposure to fresh dry spray residues of 1.3, 2.4, 4.3, 7.8 and 14.0 L product/ha on potted barley plants. These rates were applied in 400 L water/ha. The control was treated with water (400 L/ha). In terms of active substance, the application rates were: 633, 1168, 2094, 3799 and 6818 g a.s./ha. Dimethoate EC 400 (10 mL product/ha) was used as a toxic reference item. Test organisms were in 6 replicates of 5 female wasps per treatment group for the test item and control group (toxic reference item group had only 1 replicate). During the mortality test, the wasps were fed with aqueous fructose solution (10% w/w). Aphids (*Rhopalosiphum padi*) were used as host organisms. The number of surviving wasps, behaviour and position and the number of parasitised aphids (mummies) were recorded over a period of 14 days. From these data the endpoints for mortality and fecundity were calculated.

Results:

All validity criteria according to the published method for this test were met.

Ethephon SL 480: Results of an extended laboratory study on *Aphidius rhopalosiphi*

Exposure	Dried spray deposits on potted barley plants			
Treatment		Reproduction		
Applied rate, g a.s./ha	Mortality after 48 hours [%]	Mean no. mummies/ female	Relative to control [%]	Reduction relative to control [%]
0 (control)	0	45.5	-	-
633	0	41.6	91.4	8.6 ns
1168	0	44.9	98.7	1.3 ns
2094	0	38.5	84.6	15.4 ns
3799	0	44.3	97.4	2.6 ns
6818	0	40.6	89.2	10.8 ns
LR_{50}	>6818 g a.s./ha			
Toxic Reference:	100			

ns: No statistically significant difference compared to the control (Dunnett`s multiple t-test, 1-sided, $p \leq 0.05$).

The results of the toxic reference item group indicated that the test system was suitably sensitive.

Conclusions:

The LR_{50} and ER_{50} for *Aphidius rhopalosiphi* were > 6818 g a.s./ha.

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Report:	KCP 10.3.2.2/05; [redacted]; 2009; M-328138-01-1
Title:	Dose-response toxicity (LR50) of Ethephon SL 480 g/L to the ladybird Coccinella septempunctata L. under extended laboratory conditions
Report No.:	08 10 48 058 A
Document No.:	M-328138-01-1
Guideline(s):	IOBC Guideline ([redacted] et al. 2000), modified for extended laboratory conditions
Guideline deviation(s):	instead of glass plates detached bean leaves were treated and larvae were exposed under extended laboratory conditions to freshly applied residues on the bean leaves
GLP/GEP:	**yes**

Objective:

To determine a dose-response relationship for mortality of the larvae of *Coccinella septempunctata* in an extended laboratory test. Additionally, fecundity of emerged adults was assessed.

Material and Methods:

The test item was Ethephon SL 480 of batch B7090030 [analysed: 40.5% w/w a.s.; 487.0 g a.s./L]. *C. septempunctata* larvae were exposed to fresh dry spray residues of 1.25, 2.5, 5, 7.5 and 10 L product/ha on beans leaves. These rates were applied in 200 L water/ha onto excised leaves. The control was treated with water (200 L/ha). Application rates in terms of active substance were: 609, 1218, 2435, 3653 and 4870 g a.s./ha. Dimethoate EC 400 (30 mL product/ha) was used as a toxic reference item. Larvae were in 40 replicates of 1 larva per treatment group for the test item, toxic reference item and control groups. During the exposure period larvae were fed with black bean aphid (*Aphis fabae*) and pea aphid (*Acyrthosiphon pisum*). The number of dead larvae and pupae and emerged adults were recorded after 20 days. Adults were transferred to reproduction chambers. The number of eggs laid and larvae hatched (F1) were recorded. From these data the endpoints for mortality and reproductive performance were calculated.

Results:

All validity criteria according the published method for this test were met.

Ethephon SL 480: Results of an extended laboratory study on *Coccinella septempunctata*

Exposure	Dried spray deposits on excised bean leaves					
Treatment		Reproductive performance				
		Fecundity	Fertility			
	Mortality After 20 days [%]	average no. eggs/viable female/day	mean hatching rate %	reduction relative to control %	average no. fertile eggs/ viable female/day	reduction relative to control %
Control:	20.0	6.6	76.0	-	5.0	-
Rate g a.s/ha↓	Corr. Mort. %					
609	0	7.6	75.8	0.3	5.8	0
1218	0	6.6	77.7	0	5.1	0
2435	6.3	5.9	75.0	1.3	4.4	12.0
3653	3.1	6.8	77.3	0	5.3	0
4870	12.5	6.1	76.8	0	4.7	6.0
LR_{50}	>4870 g a.s/ha					
Toxic ref:	71.9	-			-	-

The results of the toxic reference item group indicated that the test system was suitably sensitive. There were no statistically significant differences (Fisher's Exact Binomial Test, 1-sided, $p \leq 0.05$) in mortality observed in all test item treatment groups, compared to the control. The reproductive output was above the lower limit given as validity criterion (average number of fertile eggs per viable female per day in the control of > 2) according to the historical database of the ring-testing group. According to that, this parameter was considered as not impacted by the test item.

Conclusions:

The LR_{50} for *C. septempunctata* was > 4870 g a.s./ha. Reproduction was not impacted.

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Report: KCP 10.3.2.2/06; [redacted]; 2008; M-326982-01-1
Title: Dose-response toxicity (LR50) of Ethephon SL 480 g/L to the green lacewing Chrysoperla carnea STEPH. under extended laboratory conditions
Report No.: 08 10 48 057 A
Document No.: M-326982-01-1
Guideline(s): IOBC Guideline ([redacted] et al. 2000), modified
Guideline deviation(s): none
GLP/GEP: yes

Objective:
To determine a dose-response relationship for mortality of larvae of *Chrysoperla carnea* in an extended laboratory test. Additionally, fecundity of emerged adults was assessed.

Material and Methods:

The test item was Ethephon SL 480 of batch B7090030 [analysed: 40.5% w/w a.s.; 487.0 g a.s./L]. *C. carnea* larvae were exposed to fresh dry spray residues of 1.25, 2.5, 5, 7.5 and 10 L product/ha on bean leaves. These rates had been applied in 200 L/ha of water to excised leaves. The control was treated with water (200 L/ha). Application rates in terms of active substance were: 609, 1218, 2435, 3653 and 4870 g a.s./ha. Dimethoate EC 400 (40 mL product/ha) was used as a toxic reference item.

There were 40 replicates of 1 larva per treatment group for the test item, reference item and control groups. During the assessments the larvae were fed eggs of *Sitotroga cerealella*. The number of surviving larvae and hatched adults was recorded after 21 days. Emerged adults were placed in reproduction chambers. The number of eggs laid and larvae hatched (F1) were recorded. From these data the endpoints for mortality and fecundity were calculated.

Results:

Ethephon SL 480: Results of an extended laboratory study on *Chrysoperla carnea*.

Exposure	dried spray deposits on excised bean leaves		
	Mortality after 21 days [%]	Reproduction	
		Fecundity	Fertility
Applied rate: g a.s./ha		mean no. eggs/female/day	mean hatching rate [%]
0 (control)	0	18.9	78
609	0	20.7	78
1218	2.5	18.7	79
2435	2.5	19.1	78
3653	2.5	18.6	79
4870	5.0	18.2	79
LR_{50}	>4870 g a.s./ha		
Toxic reference:	77.5	-	-

All validity criteria in the published method for this test were met. The results of the toxic reference item group indicated that the test system was suitably sensitive. Mortality was ≤ 5% for all treatment rates of the test item. The reproductive output (mean number of eggs/female/day) in all test item groups was above the lower limit given as validity criterion for the glass plate method (mean fecundity of > 15 eggs/female/day in the first week) according to the historical database of the ring testing group. According to that, this parameter was considered as not impacted by the test item.

Conclusions:

The LR_{50} for *Chrysoperla carnea* was > 4870 g a.s./ha. Reproduction was not impacted.

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Report: KCP 10.3.2.2/07; [redacted]; [redacted]; [redacted]; 2014; M-520562-01-1
Title: Total effects of selected plant protection products applied to different natural substrates on the predatory mite Typhlodromus pyri Sch.
Report No.: M-520562-01-1
Document No.: M-520562-01-1
Guideline(s): not applicable
Guideline deviation(s): not applicable
GLP/GEP: **no**

Executive summary

An extended laboratory study was conducted to evaluate lethal and sublethal effects of ethephon on *Typhlodromus pyri* Sch. (Acari: Phytoseiidae). The endpoints of the studies were mortality after 7 days of exposure and reduction in total egg production after 14 days. Ethephon was tested using the

maximum recommended rate (479.8 g a.s./ha). The study was performed according to the "island method" ([redacted], 2000, J. Appl. Ent. 124: 267-268). *T. pyri* protonymphs were exposed on bean (*Phaseolus vulgaris*) leaf discs, treated with ethephon with the help of the Potter laboratory spray tower on the basis of the application of 200 L of water/ha. A control (distilled water) and a reference item treatment were also included. Total effects (E) of the tested PPPs on T. pyri were determined by combining lethal (mortality) and sublethal effects (reproduction) using the IOBC classification. Ethephon was found to be harmless to *T. pyri*. At the maximum recommended rate, ethephon did neither increase mortality rate nor adversely affected the reproductive capacity of the mites.

Material and methods

A. Material

1. Test material

Test item:	Not specified
Active substance(s):	Ethephon
Chemical state and description:	Not specified
Source of test item:	Not specified
Batch number:	Not specified
Purity:	Not specified
Storage conditions:	Not specified
Solubility at room temperature:	Not specified

2. Test organism(s)

Species:	*Typhlodromus pyri* Sch.
Age of test organisms at study initiation:	Protonymphs
Source of test species:	[redacted]
Culturing conditions:	The mites were reared on the bean in the Laboratory of Apitoxicology at the Institute of Industrial Organic Chemistry, Branch Pszczyna In the laboratory

B. Study design and methods

1. Test procedure

Test system:	Extended laboratory study with *T. pyri*. Application on natural substrate (bean leaf discs, *Phaseolus vulgaris*, Fabaceae).
Duration of study:	14 days
Treatments:	• Control (distilled water) • Ethephon formulation • Reference item (Bi 58 Nowy 400 EC, 400 g of dimethoate/1)
Application rate:	• Ethephon: 479.8 g a.s./ha (max. recommended rate) • Reference item: 0.6 g a.s./ha
Number of replicates:	3
Individuals per replicate:	20
Test conditions:	• Temperature: 23-27 °C • Relative air humidity: 58-98% • Light intensity: 623-894 lx. • 16-hour light and 8-hour dark regime
Feeding:	Pine pollen (*Pinus sp.*) and *T. urticae* were put in the center of each leaf disc as a food source. During the whole experiment, the mites had continuous access to water. Food deficits were supplemented when the need arose.
Application / device / nozzles:	Potter laboratory spray tower

2. Observations and measurements:

Biological parameters measured:	• Mortality: Dead and escaped individuals after 7 days of exposure Reproduction: After 7 days of exposure, the surviving mites were sexed and the sex-ratio determined. The numbers of males, females, eggs, and

larvae were recorded at 10, 12, and 14 days of exposure, but eggs laid until the 7th day were removed from the test units and not counted

Measurement frequency:
- Mortality: 1x (day 7)
- Reproduction: 3x (day 10, 12 and 14)

Statistical analyses: Mortality: The percentage of mortality was corrected according to the Abbott's formula by using natural mortality in the control as a correcting factor. The significance of differences in the number of dead animals between the treatments and the control was analyzed using the $\chi 2$-test. Statistical differences at $p \leq 0.05$ were considered significant. Statistical analyses of the test data were performed using the STATISTICA 10.0.1011.7 software.

Reproduction: The mean numbers of eggs per female (reproduction rates) between the 7th and the 14th day of exposure were calculated for each replicate. Possible changes in the number of females during the reproduction period and the hatching of larvae from eggs between the assessment dates were taken into account by using the following formula:

$$RrX = \frac{nLd10}{nFd7} + \frac{nEd10 + nLd12}{(nFd7 + nFd10)/2} + \frac{nEd12 + nLd14}{(nFd10 + nFd12)/2} + \frac{nEd14}{(nFd12 + nFd14)/2}$$

RrX:	the reproduction rate in replicate X,
X (I, II, III):	the replicate number,
d7, d10, dl2 and dl4:	observation days (y),
nLdy:	the number of larvae (in replicate X) on day y,
nEdy:	the number of eggs (in replicate X) on day y,
nFdy:	the number of females (in replicate X) on day y.

The final effects of ethephon on reproduction of the mites were characterized by two values: the mean reproduction rate (Rr) and the percentage of reproduction reduction (Pr). The mean reproduction rate for the treatment groups was calculated on the basis of the reproduction rates determined for three replicates of this group (RrX). The percentage of reproduction reduction (Pr) relative to the control (after 14 days of exposure) was calculated using the following formula (equation 2):

$Pr = (1 - Rt/Rc) \times 100\%$

Pr: reproduction reduction [%],
Rt: the mean number of eggs per female in the treated group,
Rc : the mean number of eggs per female in the control group.
The mean numbers of eggs/females were analyzed using the Student's t-test to determine differences between the treatments. Assumptions of the Student's t-test were checked using the Shapiro-Wilk's test on normal distribution and the Levene's test on variance homogeneity.

The total effects (E) of each chemical were calculated using the following formula ([redacted], 1982):

$E = 100\% - (100\% - M) \times R$

M: corrected mortality (Abbott, 1925)
R: reproductive capacity

Results

1. Validity criteria:

Validity criteria are no explicitly stated but study fulfils the validity criteria according to the stated guideline for laboratory studies ([redacted] et al., 2000; control mortality ≤ 20%; control reproduction ≥ 4 eggs/female/day; reference mortality >50%.)

2. Biological findings:

The results relating to mortality of *T. pyri* exposed to ethephon are presented in Table 1.
No statistically significant relationship between mortality of the mites and the ethephon was noticed.
The results obtained in the reference item groups indicated that the test organisms were sensitive to the reference item dimethoate.

Table 1: Mortality of *T. pyri* after 7 days of exposure to dry residues of ethephon on leaf discs

Control [%]	Ethephon [%]	Reference item
10.0[a]	7.4*	79.6[b]

Different letters indicate a significant difference (χ2-test, p < 0.05)
* Abbott corrected mortality

The results relating to reproduction of the *T. pyri* exposed to ethephon are presented in Table 2.
At the significance level of 0.05, the total number of eggs assessed in the ethephon treatments was not significantly different from the control (Student's t-test, p > 0.05).

Table 2: Effects of ethephon on reproduction of *T. pyri*

Mean reproduction rate after 14 days [eggs/female ±SE]		Percentage of reproduction reduction
Control	Ethephon	
4.9±0.3	4.8±0.7	2.0%

Results summary

Ethephon was found to be harmless to *T. pyri*. At an application rate of 479.8 g a.s./ha, ethephon did neither increase mortality nor reduce the number of eggs significantly when compared to the control group.

Comment from the applicant: The presented results of the publication are in line with the results of the available regulatory studies that were conducted with *T. pyri*. The regulatory studies indicated under extended laboratory conditions no adverse effects on mortality or reproduction up to and including the highest test rate (1680 g a.s./ha). The publication is therefore considered as supplementary information.

CP 10.3.2.3 Semi-field studies with non-target arthropods

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

CP 10.3.2.4 Field studies with non-target arthropods

In view of the results presented above, no additional 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 in this section is based on the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2 final; 17 October 2002).

Predicted Environmental Concentrations in soil for use in the risk assessment:

Maximum PEC_{soil} values are quoted from MCP Section 9, Point 9.1.3, and are listed in Table 10.4- 1. These values have been calculated for a single application of 1 L product/ha (480 g a.s./ha), assuming a soil depth of 5 cm and a soil density of 1.5 g/cm³.

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

Substance	Winter cereals; 1 L product/ha (480 g a.s./ha)	
	$PEC_{soil, max}$ [mg/kg]	
	Early application (BBCH 37-39) (80% plant interception)	Late application (BBCH 41-51) (90% plant interception)
Ethephon SL 480 [a]	0.321	0.160
Ethephon	0.128	0.064
HEPA[b]	0.012	0.006

[a] Calculated using the product density of 1.203 g/mL; [b] 'Major' metabolite in soil.

CP 10.4.1 Earthworms

For information on studies already evaluated during the previous EU review, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph.

Two new earthworm reproduction studies have been for conducted for the current EU review. Firstly, a study has been performed using the formulation Ethephon SL 480. The formulation was employed as the test item as a means of testing the active substance. The rationale for conducting this study was to confirm the result of the study evaluated during the previous EU review. Secondly, a study has been conducted on the soil metabolite HEPA. This study was performed because HEPA is considered to be a 'major' metabolite in soil in Section CA 7 (Environmental Fate and Behaviour).

The endpoints from toxicity studies on earthworms are presented in Table 10.4.1- 1. Endpoints from studies evaluated during the previous EU review are stated in grey text. Endpoints from new studies are stated in black text. Summaries of the two new studies are provided in MCA Section 8.4.1.

Table 10.4.1- 1: Ethephon and HEPA: Endpoints from earthworm toxicity studies

Test item	Test species, test design	Endpoint		Reference
Ethephon	*Eisenia fetida* acute, 14 d, mixed*	LD_{50}	>165.4 mg a.s./ha equivalent to >60 kg a.s./ha	LoEP KCA 8.4.1/01 M-187830-01-1
Ethephon	*Eisenia fetida* reproduction 56 d, mixed*	NOEC	**200** mg a.s./kg dw soil	LoEP KCA 8.4.1/02 M-200761-01-1
Ethephon SL 480	*Eisenia fetida* reproduction 56 d, mixed*	NOEC EC_{10}	230.4 mg a.s./kg dw soil 112.2 mg a.s./kg dw soil	[redacted] (2014) KCA 8.4.1/03 KCP 10.4.1.1/ 01 M-486043-01-1
HEPA	*Eisenia fetida* reproduction 56 d, mixed*	NOEC	100 mg/kg dw soil	[redacted] (2015) KCA 8.4.1/04 M-528145-01-1

dw = dry weight; *At the start, the test item was mixed into the soil to achieve a homogeneous distribution.

Since no effect was observed at the highest test rate in the earthworm reproduction study with ethephon (KCA 8.4.1/02) the endpoint from the study conducted with Ethephon SL 480 is considered to be the relevant endpoint for the earthworm risk assessment (NOEC 230.4 mg a.s./kg dw soil).

Risk assessment for earthworms

Table 10.4.1- 2: TER calculations for earthworms

Compound	Species, study type	Endpoint [mg a.s./kg]	Worst case $PEC_{soil,max}$ [mg/kg]	TER_{LT}	Trigger
Ethephon SL 480	Earthworm, reproduction	NOEC 230.4	0.128	1800	5
HEPA	Earthworm, reproduction	NOEC 100	0.012	8333	5

All TER values calculated with the worst case $PEC_{soil, max}$ values clearly exceed the trigger value of 5 indicating that no unacceptable adverse effects on earthworms are to be expected from the intended uses of Ethephon SL 480.

CP 10.4.1.1 Earthworms sub-lethal effects

Studies are provided below and under KCA 8.4.1 including a study using Ethephon SL 480 as the test item (KCA 8.4.1/03, [redacted], 2014, M-486043-01-1).

Report:	KCP 10.4.1.1/01; [redacted]; 2014; M-486043-01-1
Title:	Ethephon SL 480A G: Effects on reproduction and growth of earthworms Eisenia fetida in artificial soil
Report No.:	M-486043-01-1
Document No.:	M-486043-01-1
Guideline(s):	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
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of this study was to investigate the effects of Ethephon SL 480 on the survival (% mortality), body weight, feeding activity and reproduction of the earthworm *Eisenia fetida*.

Material and Methods:

The test item was Ethephon SL 480 (analysed: 41.0% w/w a.s. or 492.3 g a.s./L) from batch no. B3090017. Ten worms (clitellate adults, age: approximately 10 months) per replicate (eight replicates for the control, four replicates per test item concentration) were exposed to Ethephon SL 480 in artificial soil. The test item was mixed into the soil before the start of exposure, to achieve a homogenous distribution. Nominal concentrations were 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg dw soil (7.4, 13.1, 23.0, 41.0, 73.0, 129.6, 230.4 and 410 mg a.s./kg dw soil, respectively). Temperature was 18 - 22°C with a 16 h light (400-800 lux)/8 h dark cycle. After 28 days, the adult worms were removed, weighed, counted and the remaining treated artificial soil (without the adult worms) was then returned to the respective test containers for further 28 days. At the end of the test period (i.e. after 56 days) the hatched juvenile worms were extracted from the artificial soil by placing the test units in a water bath at 50 - 60 °C and counting all emerging worms.

Results:

Validity criteria:

Mortality of the adult worms in the control:	0 % (required: ≤ 10%)
Number of juveniles per replicate in the control:	148 to 246 (required: ≥ 30)
Coefficient of variation for the number of juveniles in the control:	15.3% (required: ≤ 30%)

All study validity criteria were met.

No statistically significant mortality was observed in any treatment group. The bodyweight changes at 28 days were not statistically significantly different compared to the control up to and including the highest test concentration of 410 mg a.s./kg soil (Williams t-test, α = 0.05, two-sided). The number of juveniles produced was not statistically significantly different to the control up to and including 230.4 mg a.s./kg dw soil. At the highest test concentration of 410 mg a.s./kg dw soil the number of juveniles was statistically significantly lower than the control (Williams t-test, α = 0.05, one-sided smaller). No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control.

Ethephon SL 480: Effects on Survival (% mortality), Biomass and Reproduction of *Eisenia fetida*

Ethephon SL 480 [mg test item/kg dw soil]	Control	18	32	56	100	178	316	562	1000
ethephon, mg a.s./kg dw soil.	**0**	**7.4**	**13.1**	**23.0**	**41.0**	**73.0**	**129.6**	**230.4**	**410**
Mortality (day 28) [%]	0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.5	0.0
Body weight change (day 28) [%]	30.8	31.8	31.3	29.5	33.4	30.0	35.0	33.9	26.6
Mean No. of juveniles (day 56)	209	183	220	201	202	199	172	203	157*
Reproduction in [%] of control	-	87.6	105.0	95.9	96.6	94.9	82.4	97.2	74.8*
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Endpoints [mg a.s./kg dw soil]									
NOEC day 28 mortality, weight	**410**								
NOEC day 56 reproduction	**230.4**								

Rounded values were calculated from the exact raw data. * = significantly different to the control (α = 0.05)

The EC_{50} (repro) for Carbendazim 500 FC tested as a toxic reference item was 1.32 mg test item/kg soil dw. The effects of carbendazim confirm the suitable sensitivity of the test system.

Conclusions:

In an earthworm reproduction study with Ethephon SL 480 the overall NOEC for mortality, growth, reproduction and feeding activity was 230.4 mg a.s./kg dw soil.

The RMS requested to report the corresponding EC_{10} and EC_{20} values for this study. As stated in the study report the EC_{10} was determined to be 273.7 mg product/kg soil (corresponding to 112.2 mg a.s./kg soil) and the EC_{20} was determined to be 1151.5 mg product/kg soil (corresponding to 472.1 mg a.s./kg soil). Confidence intervals could not be determined.

CP 10.4.1.2 Earthworms field studies

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

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

No studies on soil meso- and macro-fauna other than earthworms were evaluated during the previous EU review. In the active substance data requirements under Regulation 1107/2009, the need for studies on these organisms is not linked with a DT50 or DT90 trigger in soil. Hence, in order to satisfy these requirements, testing on Collembola (*Folsomia candida*) and soil mites (*Hypoaspis aculeifer*) has now been performed. In accordance with Point 4 on p54 of the data requirements, the test item in these studies was the representative plant protection product (Ethephon SL 480).

In addition, testing on collembola and soil mites has been performed for the soil metabolite HEPA. These studies were done because HEPA is considered to be a 'major' metabolite in soil in Section CA 7 (Environmental Fate and Behaviour).

Summaries of the studies are given in MCA Section 8.4.2.1 and endpoints are listed in Table 10.4.2- 1.

Table 10.4.2- 1: Ethephon and HEPA: Endpoints from Collembola and soil mite studies

Test item	Test species, test design	Endpoint	Reference
Collembola, reproduction			
Ethephon SL 480	*Folsomia candida* reproduction, 28 d, mixed*	NOEC 410 mg a.s./kg dw soil	[redacted] (2014) KCA 8.4.2.1/01 KCP 10.4.2.1/01 M-491237-01-1
HEPA	*Folsomia candida* reproduction, 28 d, mixed*	NOEC 100 mg/kg dw soil	[redacted] (2015) KCA 8.4.2.1/03 M-525322-01-1
Soil mites, reproduction			
Ethephon SL 480	*Hypoaspis aculeifer* reproduction, 14 d, mixed*	NOEC 410 mg a.s./kg dw soil	[redacted] (2014) KCA 8.4.2.1/02 KCP 10.4.2.1/02 M-489168-01-1
HEPA	*Hypoaspis aculeifer* reproduction, 14 d, mixed*	NOEC 28.5 mg/kg dw soil	[redacted] (2015) KCA 8.4.2.1/04 M-538939-01-1

* At the start, the test item was mixed into the soil to achieve a homogeneous distribution.

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

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

Compound	Species	Endpoint [mg/kg]		$PEC_{soil,max}$ [mg/kg]	TER_{LT}	Trigger
Ethephon	*Folsomia candida*	NOEC	410	0.128	3203	5
	Hypoaspis aculeifer	NOEC	410		3203	
HEPA	*Folsomia candida*	NOEC	100	0.012	8333	
	Hypoaspis aculeifer	NOEC	28.5		2375	

All TER values calculated with the worst case $PEC_{soil,max}$ values clearly exceed the trigger value of 5 indicating that no unacceptable adverse effects on soil macro-organisms are to be expected from the intended use of Ethephon SL 480.

CP 10.4.2.1 Species level testing

Studies are provided below and in KCA 8.4.2.1.

Report: KCP 10.4.2.1/01; [redacted]; 2014; M-491237-01-1
Title: Ethephon SL 480A G: Effects on reproduction of the Collembola Folsomia candida in artificial soil
Report No.: 90441016
Document No.: M-491237-01-1
Guideline(s): GLP compliant study based on OECD 232, 2009 and ISO 11267, 1999
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:

The purpose of the study was to determine the effects of Ethephon SL 480 on mortality and reproduction of the Collembola *Folsomia candida* in artificial soil.

Material and Methods:

The test item was Ethephon SL 480 (analysed: 41.0% w/w a.s. or 492.3 g a.s./L) from batch no. B3090017. Ten collembolans (10-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (treated with water) and 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg artificial soil dw. In terms of ethephon, these concentrations were 7.4, 13.1, 23.0, 41.0, 73.0, 129.6, 230.4 and 410 mg a.s./kg dw soil, respectively. The test item was mixed into the soil before the start of exposure, to achieve a homogenous distribution. Temperature was 18 to 22°C and lighting was 400-800 lux (16h light: 8h dark). Collembola were fed with approximately 2 mg of dry yeast for each test vessel at the beginning of the test and on day 14. Assessment of adult mortality, behavioural effects and reproduction was performed after 28 days. An additional test with a toxic reference item was also conducted.

Results:

Validity of the study:

	Required	**Achieved**
Control Mortality:	≤ 20%	9%
Control Reproduction (Juveniles per Container):	≥ 100	450 to 685
Coefficient of Variation of the Control Reproduction:	≤ 30%	13.8%

All validity criteria were met.

Mortality: Mortality was not statistically significantly increased in any treatment group compared to the control (Fisher's Exact test, α = 0.05, one-sided greater).

Reproduction: Reproduction was not statistically significantly reduced compared to the control up to and including the highest test concentration of 410 mg a.s./kg dw soil (Williams t-test, α = 0.05).

No behavioural abnormalities were observed in any of the treatment groups.

Ethephon SL 480: Effect on Collembola (*Folsomia candida)* in a 28-day reproduction study

Ethephon SL 480 [mg/kg dw soil]	**Control**	**18**	**32**	**56**	**100**	**178**	**316**	**562**	**1000**
ethephon, mg a.s./kg dw soil.	**0**	**7.4**	**13.1**	**23.0**	**41.0**	**73.0**	**129.6**	**230.4**	**410**
Mortality (day 28) [%]	9	15	3	13	3	5	8	5	8
Statistical significance	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
No. of juveniles (day 28)	543	624	612	538	587	612	552	579	557
Reproduction in [%] of control	-	115	113	99	108	113	102	107	102
Statistical significance	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Endpoints [mg a.s./kg dw soil]								
NOEC (mortality)	410								
NOEC (reproduction)	410								

n.s. = not statistically significantly different compared to the control ($\alpha = 0.05$)

Conclusions:

There were no statistically significant differences from the control for survival (% mortality) and reproduction of *Folsomia candida* up to and including 410 mg a.s./kg dw soil (the highest concentration tested). Hence, the NOEC was 410 mg a.s./kg dw soil.

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Report: KCP 10.4.2.1/02; [redacted]; 2014; M-489168-01-1
Title: Ethephon SL 480A G: Effects on reproduction of the predatory mite Hypoaspis aculeifer in artificial soil

Report No.: 90441089
Document No.: M-489168-01-1
Guideline(s): Guidelines for the testing of chemicals No. 226 Predatory Mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil, adopted October 03, 2008

Guideline deviation(s): none
GLP/GEP: **yes**

Objective:

The purpose of the study was to determine the effects of Ethephon SL 480 on mortality and reproduction of the predatory mite *Hypoaspis aculeifer*.

Material and Methods:

The test item was Ethephon SL 480 (analysed: 41.0% w/w a.s. or 492.3 g a.s./L) from batch no. B3090017. Ten adult female mites per replicate (8 control replicates and 4 replicates for each test item concentration) were exposed to control and treatments in artificial soil. Concentrations of 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg dw soil were tested. In terms of ethephon, these concentrations were 7.4, 13.1, 23.0, 41.0, 73.0, 129.6, 230.4 and 410 mg a.s./kg dw soil, respectively. The test item was mixed into the soil before the start of exposure, to achieve a homogenous distribution. Each test vessel contained 20 g ± 1 g dw artificial soil. The mites were of a uniform age (approx. 9 days after reaching the adult stage). During the test, they were fed with two spatulas of cheese mites (*Tyrophagus putrescentiae*) at the start and 1-2 spatulas on day 2, 5, 7, 8 and 13. Temperature range was 18 to 20°C and the lighting regime was 400–800 Lux with 16 h light:8 h dark.

At 14 days, the surviving adults and the living juveniles were extracted by filling the soil into millipore pots with attached plastic containers for collecting the escaping mites. These extraction units were placed in a Kempson extractor. The soil including the mites was exposed to approximately 25°C and 30°C for around 2 days. Extracted *Hypoaspis* were collected in a fixing liquid (glycol and a detergent) and cooled to 16°C. Mites were counted under a binocular microscope.

Results:

Validity of the study: All validity criteria were met.

Validity criteria	Recommended	Obtained
Adult mortality in controls	≤ 20%	4%
Number of juveniles per replicate in controls	≥ 50	184 to 238
Coefficient of variation for no. of juveniles per replicate in controls	≤ 30%	9.0%

Mortality: A statistically significantly higher mortality of 23% was observed at 73 mg a.s./kg dw soil (Fisher's Exact Test, α = 0.05, one-sided greater). This was not considered to be test item related since no statistically significantly higher mortality was observed in the higher treatment levels up to and including 410 mg a.s./kg dw soil.

Reproduction: Reproduction was not statistically significantly different to the control up to and including the highest test level of 410 mg a.s./kg soil (Williams t-test, α = 0.05, one-sided smaller).

Ethephon SL 480: Effect on predatory mite (*Hypoaspis aculeifer*) in a 14-day study

Exposure	Ethephon SL 480, *Hypoaspis aculeifer*		
mg a.s./kg dw soil	% mortality (adults)[1]	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)[2]
Control	4	199 ± 18	-
7.4	5	204 ± 8	103
13.1	10	187 ± 20	94
23.0	8	187 ± 13	94
41.0	5	183 ± 21	92
73.0	23*	182 ± 22	92
129.6	8	180 ± 18	90
230.4	5	185 ± 19	93
410	8	192 ± 10	96
Endpoints [mg a.s./kg dw soil]			
NOEC (mortality)	410		
NOEC (reproduction)	410		

[1] statistical significance tested with Fisher's Exact Test, α = 0.05, one-sided greater
[2] statistical significance tested with Williams t-test, α = 0.05, one-sided smaller
* statistically significantly different compared to the control.

Conclusions:

There were no test item related effects on survival (% mortality) or reproduction of *Hypoaspis aculeifer* up to and including 410 mg a.s./kg dw soil (highest concentration tested). Hence, the NOEC was 410 mg a.s./kg dw soil.

CP 10.4.2.2 Higher tier testing

In view of the results presented above, no further testing is necessary.

CP 10.5 Effects on soil nitrogen transformation

For information on the study already evaluated during the previous EU review, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph.

Two additional N-transformation studies are available and are submitted for the current EU review. Endpoints from studies on N-transformation are presented in Table 10.5- 1. The endpoints from the study evaluated during the previous EU review are stated in grey text. Endpoints from the additional studies are stated in black text. Summaries of these two studies are provided in MCA Section 8.5.

Table 10.5- 1: Ethephon and HEPA: Endpoints from studies on nitrogen transformation

Test substance	Test species/study type	Endpoint	References
Ethephon	Study duration 28 d	no unacceptable effects at: 2.56 mg a.s./kg dw soil 1.92 kg a.s./ha	LoEP KCA 8.5/01 M-179286-01-1
Ethephon SL 480	Study duration 28 d	no unacceptable effects at*: 11.2 mg a.s./kg dw soil 8.42 kg a.s./ha	[redacted] (2008) KCA 8.5/02 KCP 10.5/01 M-302534-01-1
HEPA	Study duration 28 d	no unacceptable effects at*: 2.93 mg/kg dw soil 2.197 kg/ha	[redacted] (2015) KCA 8.5/03 M-526473-01-1

* i.e. differences from the control were <25%.

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
Ethephon	Soil micro-organisms	2.56	0.128	No
HEPA	Soil micro-organisms	2.93	0.012	No

Endpoints are substantially higher than the $PEC_{soil, max}$ values, indicating a low risk to soil micro-organisms.

Report: KCP 10.5/01; [redacted]; 2008; M-302534-01-1
Title: Ethephon SL 480 G: Determination of effects on nitrogen transformation in soil
Report No.: LRT-N-99/08
Document No.: M-302534-01-1
Guideline(s): OECD 216; adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test.
Guideline deviation(s): none
GLP/GEP: yes

Report: KCP 10.5/02; [redacted]; 2008; M-299147-01-1
Title: Reference chemical sodium chloride: Determination of effects on nitrogen transformation in soil
Report No.: LRT-N-REF-08/08
Document No.: M-299147-01-1
Guideline(s): Guidelines for the Official Testing of Plant Protectants, Part VI, 1-1, Influence on the Activity of the Soil Microflora, [redacted] March 1990 (2nd ed.).
Guideline deviation(s): none
GLP/GEP: **no**

Objective:

To determine the influence of Ethephon SL 480 on nitrogen transformation in an agricultural soil.

Material and Methods:

The test item was Ethephon SL 480 (analysis: 481.2 g a.s./L; Batch No.: 2007-000306). A loamy sand soil was exposed for 28 d to 4.67 µL and 23.33 µL test item/kg dw soil (2.25 and 11.2 mg a.s./kg dw soil, respectively). Application rates were equivalent to 3.5 L and 17.5 L test item/ha (1.68 and 8.42 kg a.s./ha, respectively). Lucerne-grass-green meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation.

Results:

Ethephon SL 480: Effects on non-target soil microorganisms

Test item	Ethephon SL 480	
Test object	Soil Microorganisms; N-Transformation (loamy sand soil)	
Duration	28 days	
µL test item/kg dw soil	4.67	23.33
mg a.s./kg dw soil	2.25	11.2
L test item/ha	3.5	17.5
kg a.s./ha	1.68	8.42
Difference in rates of N formation (%) between control and treatment	2 n.s.	9 *

*statistically significant difference to the control (Welch-t-Test for inhomogeneous variances, α= 0.05)
n.s. : No statistically significant difference to control (Welch-t-Test for inhomogeneous variances, α= 0.05)

In a separate reference test, sodium chloride was used as a reference standard ([redacted], 2008, M-299147-01-1). In tests (non-GLP) with the agricultural soil described above, 16 g NaCl/kg dry weight soil had a distinct and long-term (> 28 days) influence on microbial mineralization of nitrogen. Therefore, the sensitivity of the test system is proven and the results can be used in the risk assessment.

Conclusion:

Differences from the control are <25%. Hence, Ethephon SL 480 should not have an impact on N-transformation in soils at 11.2 mg a.s./kg dw soil (8.42 kg a.s./ha).

CP 10.6 Effects on terrestrial non-target higher plants

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

Studies have been conducted for the current EU review using the representative formulation Ethephon SL 480. These are a seedling emergence study ([redacted], 2015a) and a vegetative vigour study ([redacted], 2015b) following OECD Guidelines no. 208 and 227, respectively. These studies are summarised in CP 10.6.2, and endpoints derived from them are stated in Table 10.6-1.

Table 10.6- 1: Endpoints from non-target plant tests on Ethephon SL 480

Test organism	Study type	Test duration	Lowest ER_{50} (L product/ha)	Most sensitive species	References
Ethephon SL 480					
Terrestrial non-target plants; 10 species	seedling emergence; Tier 2 dose response	21 days	ER_{50} > 10.156	No effect > 50% for any of the species tested	[redacted] (2015a) RCP 10.6.2/01 M-534783-01-1
Terrestrial non-target plants; 10 species	vegetative vigour; Tier 2 dose response	21 days	ER_{50} = 3.0527	tomato (shoot dry weight)	[redacted] (2015b) RCP 10.6.2/02 M-534784-01-1

To assess the risk to terrestrial non-target plants, a TER calculation has been performed for the representative use given in Table 10- 1. The lowest endpoint from the studies on Ethephon SL 480 was used which is the ER_{50} of 3.0527 L product/ha for shoot dry weight of tomato (vegetative vigour study). For a single application to cereals 2.77% of the full application rate of 1 L product/ha is assumed to reach the area at 1 m from the edge of the crop. The amount of spray drift is calculated using the 90th percentile estimates derived by the BBA (2000)[4] from spray-drift predictions of [redacted] (2000)[5]. The TER calculation is presented in Table 10.6- 2.

Table 10.6- 2: TER calculation for non-target plants, based on the ER_{50} = 3.0527 L product/ha

Crop	Use pattern	Distance from field edge [m]	Drift [%]	PER [L product/ha]	TER (Trigger = 5)
Cereals	1 × 1 L product/ha	1	2.77	0.0277	110

The TER is greater than the trigger of 5. Hence, there is a low risk to non-target terrestrial plants.

CP 10.6.1 Summary of screening data

Not necessary as guideline studies for terrestrial non-target plants are available.

CP 10.6.2 Testing on non-target plants

A seedling emergence study and a vegetative vigour study on Ethephon SL 480 are summarised below.

[4] BBA (2000) Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Public domain.

[5] [redacted] (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application. Public domain.

Report:	KCP 10.6.2/01; [redacted]; 2015; M-534783-01-1
Title:	Ethephon SL 480 g/L: Effects on the seedling emergence of non-target terrestrial plant species under greenhouse conditions - Final report -
Report No.:	S15-01668
Document No.:	M-534783-01-1
Guideline(s):	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; OECD 208 (2006)
Guideline deviation(s):	Deviations with no major impact occurred regarding the test conditions
GLP/GEP:	**yes**

Objective:

The objective of this study was to evaluate effects of Ethephon SL 480 on seedling emergence and early growth of non-target terrestrial plant species under defined conditions in a greenhouse.

Material and Methods:

The test item was Ethephon SL 480 (analysed: 41.0% w/w a.s. or 492.3 g a.s./L) from batch no. B3090017. Seeds of 4 monocotyledonous species (*Allium cepa* (onion), *Avena sativa* (oat), *Lolium perenne* (ryegrass), *Zea mays* (corn)) and 6 dicotyledonous species (*Brassica oleracea* (cabbage), *Cucumis sativus* (cucumber), *Daucus carota* (carrot), *Glycine max* (soybean), *Lactuca sativa* (lettuce), *Lycopersicon esculentum* (tomato)) were sown in a mixture of 90.2% sand 6.1% silt and 3.9% clay prior to application of the product on the soil surface. Twenty seeds per treatment group were sown in 15 cm diameter pots in the greenhouse. In each pot two seeds were sown except for the species *Allium cepa*, *Avena sativa* and *Lolium perenne* in which four seeds per pot were sown. There were 5 application rates and a control. Serial dilutions were sprayed on the soil surface using a spray cabin at a volume rate of 206 L/ha (at 2.5 bar; 2 km/h; from height of 41.0 cm; with nozzle 80015 VS TeeJet). Test rates were 305, 731, 1767, 4225 and 10156 mL product/ha. Control pots were sprayed with 206 L/ha of deionised water.

Following application, pots were maintained in a greenhouse under controlled conditions. Air temperature ranged from 17.8 °C to 35.6 °C with a relative air humidity between 31.1% and 94.6% and a photoperiod of 16/8 (light/dark). Assessments were made 7, 14 and 21 days after 50% of seedlings had emerged in the controls. The study was terminated 21 days after 50% of seedlings had emerged in the controls. Final assessments were made for seedling emergence, plant survival, visual phytotoxicity and shoot dry weight. Statistical analysis was performed to obtain NOER, LOER, ER_{50} values for seedling emergence, mortality and shoot dry weight. For seedling emergence and mortality Fisher`s Exact Binomial Test with Bonferroni Correction was used. Shoot dry weight was checked for normality and homogeneity of variances with the Shapiro-Wilk`s Test and Levene`s Test. Afterwards the William`s Multiple Sequential t-test was used. The study was done from May 11 to June 17, 2015.

Results:

All species met the OECD guideline validity criteria. The measured concentration of ethephon in the highest test item solution corresponded to 105% nominal. Thus, the concentration of ethephon in this representative sample was confirmed.

Seedling emergence was not statistically significantly reduced compared to the control for all species. The most sensitive species for seedling emergence was *Lolium perenne* with an inhibition of 25.0% at 1767 mL product/ha. No mortality occurred in any species except one individual of *Zea mays* was dead at day 14 at 1767 mL product/ha. No symptoms of phytotoxicity were observed for any species. There was a statistically significant effect on shoot dry weight for *Daucus carota*, *Glycine max*, *Lycopersicon esculentum* and *Zea mays* (Williams: Multiple Sequential t-test, one-sided smaller, $p \leq 0.05$). The most sensitive species was *Glycine max* with an inhibition compared to the control of 36.5%, followed by *Daucus carota* with 35.7% inhibition, both at the highest test item rate (10156 mL product/ha).

The results are summarised in the tables below.

Results for seedling emergence at 21 days after 50% of the seedlings in the control had emerged:

Emergence [mL product/ha]					
Plant Species	**ER_{50}**	**95% Confidence Limits**		**LOER**	**NOER**
		lower	**upper**		
Dicotyledonous species					
Brassica oleracea	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Cucumis sativus	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Daucus carota	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Glycine max	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Lactuca sativa	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Lycopersicon esculentum	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Monocotyledonous species					
Allium cepa	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Avena sativa	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Lolium perenne	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Zea mays	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156

[a]: calculated values were outside the range tested or not determined
n.d.: confidence limits not determined due to mathematical reasons or outside the range tested

Results for survival at 21 days after 50% of the seedlings in the control had emerged:

Survival [mL product/ha]					
Plant Species	**ER_{50}**	**95% Confidence Limits**		**LOER**	**NOER**
		lower	**upper**		
Dicotyledonous species					
Brassica oleracea	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Cucumis sativus	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Daucus carota	> 10156 [a]	n.d.	n.d.	10156 [a]	≥ 10156
Glycine max	> 10156 [a]	n.d.	n.d.	10156 [a]	≥ 10156
Lactuca sativa	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Lycopersicon esculentum	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Monocotyledonous species					
Allium cepa	> 10156 [a]	n.d.	n.d.	10156 [a]	≥ 10156
Avena sativa	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Lolium perenne	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Zea mays	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156

[a]: calculated values were outside the range tested or not determined
n.d.: confidence limits not determined due to mathematical reasons or outside the range tested

Results for shoot dry weight at 21 days after 50% of the seedlings in the control had emerged

Shoot Dry Weight [mL product/ha]					
Plant Species	**ER_{50}**	**95% Confidence Limits**		**LOER**	**NOER**
		lower	**upper**		
Dicotyledonous species					
Brassica oleracea	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Cucumis sativus	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Daucus carota	10156 [a]	n.d.	n.d.	10156	4225
Glycine max	> 10156 [a]	n.d.	n.d.	4225	1767
Lactuca sativa	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Lycopersicon esculentum	10156 [a]	n.d.	n.d.	10156	4225
Monocotyledonous species					
Allium cepa	> 10156 [a]	n.d.	n.d.	> 10156 [a]	≥ 10156
Avena sativa	>10156 [a]	n.d.	n.d.	>10156 [a]	≥10156
Lolium perenne	>10156 [a]	n.d.	n.d.	>10156 [a]	≥10156
Zea mays	10156 [a]	n.d.	n.d.	1767	731

[a]: calculated values were outside the range tested or not determined
n.d.: confidence limits not determined due to mathematical reasons or outside the range tested

Median phytotoxicity 21 days after 50 % of the seedlings in the control had emerged:

Plant species	Control	Ethephon SL 480 [mL product/ha]				
		305	731	1767	4225	10156
Dicotyledonous species						
Brassica oleracea	1	1	1	1	1	1
Cucumis sativus	1	1	1	1	1	1
Daucus carota	1	1	1	1	1	1
Glycine max	1	1	1	1	1	1
Lactuca sativa	1	1	1	1	1	1
Lycopersicon esculentum	1	1	1	1	1	1
Monocotyledonous species						
Allium cepa	1	1	1	1	1	1
Avena sativa	1	1	1	1	1	1
Lolium perenne	1	1	1	1	1	1
Zea mays	1	1	1	1	1	1

1=healthy plant; 2=slight symptoms; 3=moderate symptoms; 4 =strong symptoms; 5 =totally affected by observed symptoms

Conclusions:

For seedling emergence and mortality, no adverse effects were observed. Hence, for these parameters the NOER was 10156 mL product/ha and the ER_{50} was > 10156 mL product/ha.

For shoot dry weight, statistically significant effects were observed. For *Zea mays*, the LOER was 1767 mL product/ha and the NOER was 731 mL product/ha. For *Glycine max*, the LOER was 4225 mL product/ha and the NOER was 1767 mL product/ha. For *Daucus carota* and *Lycopersicon esculentum* the LOER was 10156 mL product/ha and the NOER was 4225 mL product/ha. For the other species tested, the NOER was 10156 mL product/ha (the highest rate tested).

An ER_{50} for shoot dry weight could not be calculated for any species due to a lack of inhibition ≥ 50%. Therefore, the ER_{50} was > 10156 mL product/ha which was the highest rate tested.

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Report: KCP 10.6.2/02 [redacted]; 2015; M-534784-01-1
Title: Ethephon SL 480 g/L: Effects on the vegetative vigour of non-target terrestrial plant species under greenhouse conditions - Final report -
Report No.: 815-01669
Document No.: M-534784-01-1
Guideline(s): EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; OECD 227 (2006)
Guideline deviation(s): Deviations with no major impact occurred regarding the test conditions
GLP/GEP: **yes**

Objective:

The objective of this study was to evaluate the effects of Ethephon SL 480 on the early growth of non-target terrestrial plant species under defined conditions in a greenhouse.

Material and Methods:

The test item was Ethephon SL 480 (analysed: 41.0% w/w a.s. or 492.3 g a.s./L) from batch no. B3090017. Six dicotyledonous species (*Brassica oleracea* (cabbage), *Cucumis sativus* (cucumber), *Daucus carota* (carrot), *Glycine max* (soybean), *Lactuca sativa* (lettuce), *Lycopersicon esculentum* (tomato)) and 4 monocotyledonous species (*Allium cepa* (onion), *Avena sativa* (oat), *Lolium perenne* (ryegrass), *Zea mays* (corn)) were sprayed at test rates of 102, 284, 792, 2194 and 6094 mL product/ha. Control plants were sprayed with deionized water.

Twenty plants per treatment group were used. Each treatment group consisted of ten pots (15 cm diameter) with two plants in each, except *Allium cepa*, *Avena sativa* and *Lolium perenne* which had four plants per pot. Soil substrate consisted of 90.2% sand, 6.1% silt and 3.9% clay. Serial dilutions were sprayed using a spray cabin at a volume rate of 212 L/ha (at 2.5 bar, 2 km/h, at height 41.0 cm, with nozzle 80015 VS TeeJet). A sample of spray solution for the highest rate was analyzed by HPLC with a Photodiode Array Detector (PDA).

Following application, plants were maintained in a greenhouse under controlled conditions. Air temperature ranged from 18.3 °C to 36.1 °C with a relative air humidity between 24.7% and 87.6% and a photoperiod of 16/8 (light/dark). Assessments for mortality and visual phytotoxicity were made 7, 14 and 21 days after application in comparison with controls. The study was terminated 21 days after application. Final assessments were made for survival, visual phytotoxicity and shoot dry weight.

Statistical analysis was performed to obtain NOER, LOER, ER_{50} values for mortality and shoot dry weight. For mortality Fisher`s Exact Binomial Test with Bonferroni Correction was used. Shoot dry weight was checked for normality and homogeneity of variances with the Shapiro-Wilk`s Test and Levene`s Test. Afterwards the William`s Multiple Sequential t-test was conducted. The study was conducted from May 29 to July 01, 2015.

Results:

All species met the OECD Guideline validity criteria. The measured concentration of ethephon in the highest test item rate solution was 80% of nominal. Thus, the concentration of ethephon in this representative sample was confirmed as this value is within 100 ± 20%.

No mortality occurred for any species, except two individuals of *Cucumis sativus* which were dead at day 21 at the highest test item rate (6094 mL product/ha). Symptoms of phytotoxicity (e.g. stunted growth, necrosis, chlorosis and leaf deformation) were observed in *Cucumis sativus*, *Daucus carota*, *Lycopersicon esculentum* and *Zea mays*. Slight symptoms were observed for *Zea mays* at the two highest test item rates (2194 and 6094 mL product/ha). Moderate symptoms were observed for *Daucus carota* at the highest test item rate. For *Cucumis sativus* and *Lycopersicon esculentum*, strong symptoms were observed at the highest test item rate.

Statistically significant effects on shoot dry weight were detected for all species (Williams Multiple Sequential t-test, one-sided smaller, $p \leq 0.05$) except *Lactuca sativa*, *Avena sativa* and *Lolium perenne*. For *Brassica oleracea* statistically significant effects were observed at 102 and 284 mL product/ha. For this species, this difference to the control was considered to be natural variability due to the fact that no significant effects were observed in the higher test item rates.

The greatest inhibition of shoot dry weight compared to the control was for *Lycopersicon esculentum* with 65.3% at 6094 mL product/ha followed by *Cucumis sativus* with 52.3% and *Allium cepa* with 48.1% inhibition at the highest test item rate, respectively.

The results are summarised in the tables below.

Results for mortality at day 21 in the vegetative vigour test:

Mortality [mL product/ha]				
Plant Species	**ER_{50}**	**± 95% CL**	**LOER**	**NOER**
Dicotyledonous species				
Brassica oleracea	> 6094[a]	n.d.	> 6094[a]	≥ 6094
Cucumis sativus	> 6094[a]	n.d.	> 6094[a]	≥ 6094
Daucus carota	> 6094[a]	n.d.	> 6094[a]	≥ 6094
Glycine max	> 6094[a]	n.d.	> 6094[a]	≥ 6094
Lactuca sativa	> 6094[a]	n.d.	> 6094[a]	≥ 6094
Lycopersicon esculentum	> 6094[a]	n.d.	> 6094[a]	≥ 6094
Monocotyledonous species				
Allium cepa	> 6094[a]	n.d.	> 6094[a]	≥ 6094
Avena sativa	> 6094[a]	n.d.	> 6094[a]	≥ 6094
Lolium perenne	> 6094[a]	n.d.	> 6094[a]	≥ 6094
Zea mays	> 6094[a]	n.d.	> 6094[a]	≥ 6094

[a]: calculated values were outside the range tested or not determined
n.d.: confidence limits not determined due to mathematical reasons or outside the range tested

Results for shoot dry weight at day 21 in the vegetative vigour test:

Shoot Dry Weight [mL product/ha]				
Plant Species	**ER_{50}**	**± 95% CL**	**LOER**	**NOER**
Dicotyledonous species				
Brassica oleracea	> 6094[a]	n.d.	> 6094[a]	≥ 6094
Cucumis sativus	5577.5	3604.7 to 11993.8	792	284
Daucus carota	> 6094[a]	n.d.	102	<102
Glycine max	> 6094[a]	n.d.	> 6094[a]	2194
Lactuca sativa	> 6094[a]	n.d.	6094	≥ 6094
Lycopersicon esculentum	3052.7	1759.2 to 7606.7	792	284
Monocotyledonous species				
Allium cepa	> 6094[a]	n.d.	102	<102
Avena sativa	> 6094[a]	n.d.	> 6094[a]	≥ 6094
Lolium perenne	> 6094[a]	n.d.	> 6094[a]	≥ 6094
Zea mays	> 6094[a]	n.d.	2194	792

[a]: calculated values were outside the range tested or not determined
n.d.: confidence limits not determined due to mathematical reasons or outside the range tested

Median phytotoxicity 21 days after application:

Plant species	Control	Phytotoxicity scores [mL product/ha] 102	284	792	2194	6094
Dicotyledonous species						
Brassica oleracea	1	1	1	1	1	1
Cucumis sativus	1	1	1.5 [a]	2 [a,b,c]	3 [a,b,c,]	4 [a,b,c]
Daucus carota	1	1	1	1	1.5 [a,b]	3 [a,b,d]
Glycine max	1	1	1	1	1	1
Lactuca sativa	1	1	1	1	1	1
Lycopersicon esculentum	1	2 [a]	2 [a]	2 [a,c]	3 [a,b,c,]	4 [a,b,c,e]
Monocotyledonous species						
Allium cepa	1	1	1	1	1	1
Avena sativa	1	1	1	1	1	1
Lolium perenne	1	1	1	1	1	1
Zea mays	1	1	1	1	2 [a]	2 [a]

[a] Stunted growth; [b] Necrosis; [c] Chlorosis; [d] Wilting, [e] Leaf deformation
1=healthy plant; 2=slight symptoms; 3=moderate symptoms; 4=strong symptoms; 5=totally affected by observed symptoms

Conclusions:

The NOER for mortality for all species was 6094 mL product/ha (the highest rate tested). Statistically significant effects on shoot dry weight occurred for all species except *Lactuca sativa*, *Avena sativa* and *Lolium perenne*. For *Daucus carota* and *Allium cepa* the LOER was 102 mL product/ha and the NOER was below the lowest rate tested. For *Cucumis sativus* and *Lycopersicon esculentum* the LOER was 792 mL product/ha, and the NOER was 284 mL product/ha. For *Zea mays* the LOER was 2194 mL product/ha and the NOER was as 792 mL product/ha. For *Glycine max* the LOER was 6094 mL product/ha and the NOER was 2194 mL product/ha. For *Brassica oleracea*, *Lactuca sativa*, *Avena sativa* and *Lolium perenne*, the NOER was 6194 mL product/ha (the highest rate tested).

For shoot dry weight, the ER_{50} (and 95% confidence limits) for *Cucumis sativus* (cucumber) was 5577.5 (3604.7 – 11993.8) mL product/ha and for *Lycopersicon esculentum* (tomato) was 3052.7 (1759.2 – 7606.7) mL product/ha. The ER_{50} for the other species was > 6094 mL product/ha (the highest rate tested).

CP 10.6.3 Extended laboratory studies on non-target plants

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

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

Please refer to Point 10.6.2.

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

No studies are required based on current data requirements.

CP 10.8 Monitoring data

No monitoring data are available and are not triggered by current data requirements.