REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: ADM.09050.H.1.A

Product name(s): **STEMPER**

Chemical active substances:

Trinexapac-ethyl, 175 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: **ADAMA**

Submission date: May 2022

Evaluation date: March 2023

Revision date: June 2023

Version history

When	What
January 2021	dRR version 1 submitted by applicant
May 2022	dRR submitted by applicant to the Polish Ministry of Agriculture and Rural Development
September 2022	Submission to the evaluation unit
March 2023	zRMS finalized dRR evaluation
June 2023	zRMS finalized RR evaluation according to the comments received

DATA PROTECTION CLAIM

Under Article 59, Regulation 1107/2009/EC, on behalf of the Sponsor Company the applicant claims data protection for these studies. The data protection status and corresponding justification as valid for the respective country will be confirmed in the respective PART A

STATEMENT FOR OWNERSHIP

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Review Comments:

This document describes the acceptable use conditions required for registration of ADM.09050.H.1.A, an emulsifiable concentrate containing 175 g/L trinexapac-ethyl for use in cereals and grass for seeds.

This Part B document only reviews data and additional information that has not previously been considered within the EU review process.

Since this document is based on the information provided by the applicant, all review comments, additions and corrections have been made using commenting boxes or highlighted in grey.

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

Critical GAP

Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn, G, Gn, Gpn or I, **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g safener/ synergist per ha
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max		
1	EU	Winter barley (HORVW)	F	Prevention of lodging	Foliar spray	BBCH 25-49	a) 1 b) 1	n/a	a) 0.8 L/ha b) 0.8 L/ha	a) 200 g/ha b) 200 g/ha	100-400	n/a	
2	EU	Spring barley (HORVS)	F	Prevention of lodging	Foliar spray	BBCH 25-37	a) 1 b) 1	n/a	a) 0.6 L/ha b) 0.6 L/ha	a) 150 g/ha b) 150 g/ha	100-400	n/a	
2	EU	Winter wheat (TRZAW)	F	Prevention of lodging	Foliar spray	BBCH 25-49	a) 1 b) 1	n/a	a) 0.5 L/ha b) 0.5 L/ha	a) 125 g/ha b) 125 g/ha	100-400	n/a	

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
7[1)]	BE	Spring barley (HORVS)	F	Growth regulator (YHALM) Lodging control (YELDU)	Foliar, spraying, overall	-/ BBCH 29-32	a) 1 b) 1	n/a	a) 0.6 L/ha b) 0.6 L/ha	a) 105 g/ha b) 105 g/ha	200-400	n/a		A	A	A	A	A	A	A
14[2)]	CZ	Winter barley (HORVW)	F	Growth regulator (YHALM)	Foliar, spraying, overall	-/ BBCH 31-35	a) 1 b) 1	n/a	a) 1.2 L/ha b) 1.2 L/ha	a) 210 g/ha b) 210 g/ha	200-400	n/a		A	A	A	A	A	A	A
25[3)]	NL	Grass for seed; festuces (FESSS) and ryegrass (LOLSS)	F	Growth regulator (YHALM) lodging control (YELDU)	foliar, spraying, overall	-/ BBCH 30-37	a) 1 b) 1	n/a	a) 0.8 L/ha b) 0.8 L/ha	a) 140 b) 140	200-400	n/a		A	A	A	A	A	A	A

[1)] supportive for uses 4, 6, 8, 9, 13, 18, ~~21~~, 22, 24, 26, 28, 29, 30, 31 complete Part B, Section 0
[2)] supportive for uses 5, 6, 9, 10, 11, 12, 15, 16, 17, 18, 19, 20, 22, 23, 26, 27, 28, 31, 32, 33, 34 complete Part B, Section 0
[3)] supportive for uses 1, 2, ~~3~~, complete Part B, Section 0

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1
** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application

Explanation for column 15 – 21 "Conclusion"

A	Acceptable, Safe use
R	Further refinement and/or risk mitigation measures required
C	To be confirmed by cMS
N	No safe use

Remarks table:

(1) Numeration necessary to allow references
(2) Use official codes/nomenclatures of EU
(3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(4) F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
(5) Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (e.g. biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
(6) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(7) Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(8) The maximum number of application possible under practical conditions of use must be provided
(9) Minimum interval (in days) between applications of the same product.
(10) For specific uses other specifications might be possible, e.g.: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
(11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
(12) If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.
(13) PHI - minimum pre-harvest interval
(14) Remarks may include: Extent of use/economic importance/restrictions

9.1.1 Overall conclusions

9.1.1.1 Effects on birds (KCP 10.1.1), Effects on terrestrial vertebrates other than birds (KCP 10.1.2), Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

The risk assessment for birds and mammals was carried out according to the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438).

The acute and reproductive risks to birds from exposure to trinexapac-ethyl following application of ADM.09050.H.1.A according to the intended uses were acceptable at tier 1.

The risks to birds from exposure to trinexapac-ethyl in drinking water from puddles did not exceed the quotient trigger value 50 in the case of less sorptive substances (Koc < 500 L/kg). The Koc of trinexapac-ethyl is 60 L/kg.

The risk assessment for effects due to secondary poisoning is not required. The log P_{ow} of trinexapac-ethyl amounts to -0.29 at pH 6.9 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The acute and reproductive risks to mammals from exposure to trinexapac-ethyl following application of ADM.09050.H.1.A according to the intended uses were acceptable at the screening step.

The risks to mammals from exposure to trinexapac-ethyl in drinking water from puddles did not exceed the quotient trigger value 50 in the case of less sorptive substances (Koc < 500 L/kg). The Koc of trinexapac-ethyl is 60 L/kg.

The risk assessment for effects due to secondary poisoning is not required. The log P_{ow} of trinexapac-ethyl amounts to -0.29 at pH 6.9 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

In conclusion, an acceptable overall risk for birds and mammals is indicated for all intended GAP uses of ADM.09050.H.1.A.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

The risk assessment for aquatic organisms was carried out according to the 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 acute and chronic risk to aquatic organisms from exposure to trinexapac-ethyl and its metabolites following application of ADM.09050.H.1.A according to the intended uses is acceptable using STEP 1 ~~2~~ PECsw values without the necessity to account for risk mitigation measures.

9.1.1.3 The acute and chronic risks to aquatic organisms from exposure to trinexapac-ethyl and its metabolites following application of ADM.09050.H.1.A all intended uses using a risk envelope approach are acceptable using STEP 1~~/2~~

PECsw values without further risk mitigation measures.

Review Comments:

The relevant predicted environmental concentrations in water (PEC_{sw}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate). The initial risk assessment was based on the worst case PEC_{sw} values and the results of laboratory toxicity testing.

For active substance and relevant metabolites PEC/RAC calculations were performed with FOCUS STEP 1. For the formulation additional calculations were performed by zRMS with FOCUS SWASH.

The calculated PEC/RAC ratios indicate an acceptable risk for all groups of aquatic organisms without the need for any mitigation measures.

9.1.1.4 Effects on bees (KCP 10.3.1)

The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).

The risk to bees from exposure to trinexapac-ethyl following application of ADM.09050.H.1.A according to the intended uses is acceptable.

9.1.1.5 Effects on arthropods other than bees (KCP 10.3.2)

The risk assessment was conducted according to the ESCORT 2 Guidance Document (2000) and the Guidance Document on Terrestrial Ecotoxicology (2002).

The in-field risk and off-crop risk to non-target arthropods other than bees from exposure to trinexapac-ethyl following application of ADM.09050.H.1.A to winter barley as a worst-case scenario is acceptable without the necessity to account for risk mitigation measures.

9.1.1.6 The risks to non-target arthropods other than bees from exposure to trinexapac-ethyl following application of ADM.09050.H.1.A to winter barley as a worst-case scenario are acceptable. Thus, no unacceptable risk is expected from the use of ADM.09050.H.1.A for all intended uses.

Review Comments:

Based on the results of the conducted risk assessment, it can be concluded that low risk for non-target arthropods is expected from the use of ADM.09050.H.1.A according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.

9.1.1.7 Effects on non-target soil meso- and macrofauna (KCP 10.4), The risks to soil meso- and macrofauna from exposure to trinexapac-ethyl and its metabolites following application of ADM.09050.H.1.A to spring barley, winter barley and

grass for seed are acceptable. Thus, no unacceptable risk is expected from the use of ADM.09050.H.1.A for all intended uses, taking a risk envelope approach into account.

Review Comments:

The long-term risks of ADM.09050.H.1.A to soil meso- and macro-organisms were assessed from toxicity exposure ratios between toxicity endpoints and maximum PEC_{soil}. The relevant predicted environmental concentration in soil (PEC_{soil}) for risk assessment covering the proposed use pattern was taken from Part B Section 8 (Environmental Fate).

Safe use of ADM.09050.H.1.A was confirmed based on TER_{LT} calculations for formulation, trinexapac-ethyl and its relevant metabolites.

9.1.1.8 Effects on soil microbial activity (KCP 10.5)

The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (2002).

The risk to soil meso- and macrofauna from exposure to trinexapac-ethyl and its metabolites following application of ADM.09050.H.1.A according to the intended uses is acceptable.

9.1.1.9 Effects on non-target terrestrial plants (KCP 10.6)

The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (2002).

Based on a deterministic approach (TER calculations) recommended for herbicides, a safe use (with respect to an acceptable risk for non-target plants) can be identified for each of the GAP uses proposed for ADM.09050.H.1.A and risk mitigation measures are not required.

9.1.1.10 The risks to non-target plants from exposure to trinexapac-ethyl following application of ADM.09050.H.1.A to spring barley, winter barley and grass for seed are acceptable. Thus, no unacceptable risk is expected from the use of ADM.09050.H.1.A for all intended uses, taking a risk envelope approach into account.

Review Comments:

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002).

Based on the risk assessment it can be concluded that the proposed use of ADM.09050.H.1.A poses no unacceptable risk to non-target plants, if applied according to the recommended use pattern. Particular precautions to reduce the environmental concentrations resulting from ADM.09050.H.1.A applications are not required.

9.1.1.11 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

No further relevant data available and considered necessary.

9.1.2 Grouping of intended uses for risk assessment

The following table documents the grouping of the intended uses to support application of the risk envelope approach (according to SANCO/11244/2011).

The risk assessment for terrestrial, aquatic and soil organisms presented in this document were performed in consideration of the risk envelope GAP use covering all other intended GAP uses for which authorisation is sought in the EU central zone. The risk envelope GAP uses are summarised in the table below.

Table 9.1-2: Critical use pattern of ADM.09050.H.1.A

Grouping according to criterion			
Group	**Intended uses (risk envelope)**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
Terrestrial vertebrates (Birds and Mammals; **9.2** and **9.3**)	according to GAP; refer to Document B0, GAP uses 4, 6, 7, 8, 9, 13, 18, 21, 22, 24, 26, 28, 29, 30, 31 1 x 105 g a.s./ha at BBCH 29-32 in spring cereals, GAP uses 5, 6, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 22, 23, 26, 27, 28, 31, 32, 33, 34 1 x 210 g a.s./ha at	Scenarios according to EFSA Birds and Mammals Guidance (2009): **Crops for bird and mammals risk assessments:** cereals and grassland	**BBCH 10-29 and BBCH 30-39**: spring cereals (post-emergence) **BBCH 30-39**: winter cereals (post-emergence) Screening assessment only for grassland, BBCH not relevant
Aquatic organisms (**9.5**)		Crops according to FOCUS surface water guidance (2015)[1]: Spring cereals, winter cereals and grass	FOCUS step 2 calculations carried out for March-May, June-Sept and Oct-Feb resulted in the same PECsw values for each critical GAP use.

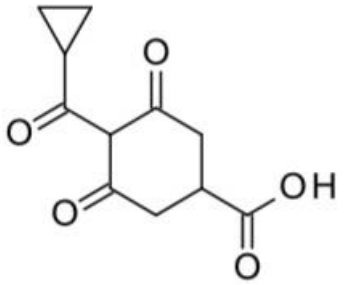
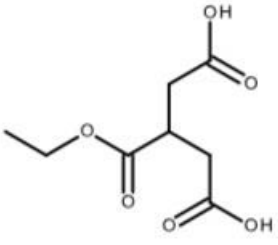
1 FOCUS (2015): Generic guidance for FOCUS surface water Scenarios. Version 1.4.

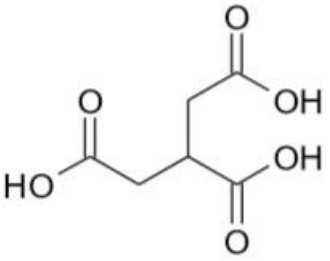
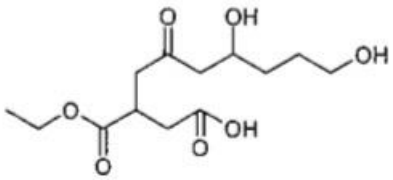
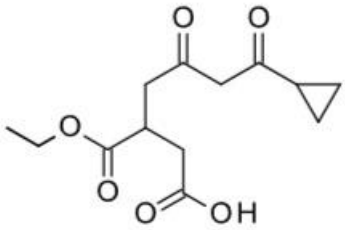
Grouping according to criterion			
Group	**Intended uses (risk envelope)**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
Bees (**9.6**)	BBCH 31-35 in winter cereals, GAP uses 1, 2, 3, 25 1 x 140 g a.s./ha at BBCH 30-37 for grass for seed	No distinction required	No distinction required
Terrestrial non-target arthropods other than bees (**9.7**)		No distinction required	No distinction required
Soil meso- and macrofauna / soil microorganisms (**9.8** and **9.9**)		Crop interception values according EFSA Journal 2014;12(5):3662	**BBCH 29:** Spring cereals corresponds to a crop interception value of 20% **BBCH 31:** winter cereals corresponds to a crop interception value of 80% **BBCH 30:** grass corresponds to a crop interception value of 60%
Non-target terrestrial plants (**9.10**)		No distinction required	No distinction required

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of ADM.09050.H.1.A is indicated in the table.

Table 9.1-3 Metabolites of trinexapac-ethyl

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
CGA179500		224.2	Soil: 93.1% AR Water: 64% AR Sediment: 6.9% AR Water/Sediment: 70.9%	Aquatic organisms Soil organisms
CGA300405		204.1	Soil (photolysis): 12.5% AR Water (photolysis): 41% AR	Aquatic organisms Soil organisms

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
CGA275537		176.1	Soil (photolysis): 10.8% AR	Aquatic organisms Soil organisms
M2		290.3	Water: 17.9% AR (photolysis in water)	Aquatic organisms
M3 (WaterM3 Photolysis)		252.3	Water: 16.9% AR (photolysis in water)	Aquatic organisms

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with the active substance trinexapac-ethyl. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of ADM.09050.H.1.A were not evaluated as part of the EU assessment of trinexapac-ethyl.

However, the provision of further data on ADM.09050.H.1.A is not considered essential, because the toxicity of the formulation to birds can be extrapolated from the data on the active substance trinexapac-ethyl.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.2-1: Endpoints and effect values relevant for the risk assessment for birds

Species	Substance	Exposure System	Results	Reference
Anas platyrhynchos	Trinexapac-ethyl	Oral 1 d Acute	**LD_{50} >2000 mg/kg bw**	EFSA Conclusion 5229/2018
Colinus virginianus	Trinexapac-ethyl	Oral 1 d Acute	LD_{50} >2250 mg/kg bw	EFSA Conclusion 5229/2018

Species	Substance	Exposure System	Results	Reference
Anas platyrhynchos	Trinexapac-ethyl	Dietary Reproductive toxicity	NOEL = 100 mg/kg bw/d	EFSA Conclusion 5229/2018
Colinus virginianus	Trinexapac-ethyl	Dietary Reproductive toxicity	**NOEL = 17.6 mg/kg bw/d**	EFSA Conclusion 5229/2018

9.2.1.1 Justification for new endpoints

No new endpoints are proposed and no justification is made.

9.2.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438). The major route of exposure is by feeding on contaminated vegetation (e.g. grass, leafy crops, and small seeds) and invertebrates (insects).

9.2.2.1 First-tier assessment (screening/generic focal species)

The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

Table 9.2-2: Screening and First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.09050.H.1.A in spring barley

Intended use	**ADM.09050.H.1.A in spring barley**				
Active substance/product	Trinexapac-ethyl				
Application rate (g/ha)	1 x 105				
Acute toxicity (mg/kg bw)	>2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
~~BBCH 29-32~~ not relevant	Small omnivorous bird	158.8	1	16.67	>120
Reprod. toxicity (mg/kg bw/d)	17.6				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
~~BBCH 29-32~~ not relevant	Small omnivorous bird	64.8	0.53	3.606	**4.881**
BBCH 10-29	Large herbivorous bird “goose” Pink-footed goose *Anser brachyrhynchus*	16.2	0.53	0.9015	19.52
BBCH 10-29	Small omnivorous bird “lark” Woodlark *Lullula arborea*	10.9	0.53	0.6066	29.01
BBCH 30-39	Small omnivorous bird “lark” Woodlark *Lullula arborea*	5.4	0.53	0.3005	58.57

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-3: Screening and First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.09050.H.1.A in winter barley

Intended use	**ADM.09050.H.1.A in winter barley**				
Active substance/product	Trinexapac-ethyl				
Application rate (g/ha)	1 x 210				
Acute toxicity (mg/kg bw)	>2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
~~BBCH 31-35~~ not relevant	Small omnivorous bird	158.8	1	33.35	>60.0

Intended use		**ADM.09050.H.1.A in winter barley**			
Active substance/product		Trinexapac-ethyl			
Application rate (g/ha)		1 x 210			
Reprod. toxicity (mg/kg bw/d)		17.6			
TER criterion		5			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
~~BBCH 31-35~~ not relevant	Small omnivorous bird	64.8	0.53	7.212	**2.440**
BBCH 30-39	Small omnivorous bird "lark" Woodlark *Lullula arborea*	5.4	0.53	0.6010	29.28

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-4: ~~First-tier~~ Screening assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.09050.H.1.A in grass for seed

Intended use		**ADM.09050.H.1.A in grass for seed**			
Active substance/product		Trinexapac-ethyl			
Application rate (g/ha)		1 x 140			
Acute toxicity (mg/kg bw)		>2000			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
~~BBCH 30-37~~ not required	Large herbivorous bird	30.5	1	4.27	>468.4
Reprod. toxicity (mg/kg bw/d)		17.6			
TER criterion		5			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
~~BBCH 30-37~~ not required	Large herbivorous bird	16.2	0.53	1.202	14.64

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

9.2.2.2 Higher-tier risk assessment

Higher tier risk assessments for the critical uses of ADM.09050.H.1.A on spring barley, winter barley and grass for seed in a risk envelope approach are not required as the first-tier risk assessments indicate acceptable risks for both acute and chronic exposure to birds.

9.2.2.3 Drinking water exposure

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is conducted for a small granivorous bird with a body weight of 15.3 g (*Carduelis cannabina*) and a drinking water uptake rate of 0.46 L/kg bw/d (*cf.* Appendix K of EFSA/2009/1438).

Leaf scenario

Since ADM.09050.H.1.A is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

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 (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).

With a K(f)oc of 60 L/kg, trinexapac-ethyl belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use winter barley also covers the risk for birds from all other intended uses in spring barley and grass for seed because the winter barley critical GAP has the highest application rate.

Effective application rate (g/ha)	=	210		
Acute toxicity (mg/kg bw)	=	>2000	quotient	=	<0.105
Reprod. toxicity (mg/kg bw/d)	=	17.6	quotient	=	11.9

No specific calculations of exposure and TER are necessary as the ratios of effective application rate of trinexapac-ethyl to acute and reprotoxic endpoints for birds are less than 50.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of trinexapac-ethyl amounts to -0.29 at pH 6.9 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating birds via secondary poisoning

Not required.

Risk assessment for fish-eating birds via secondary poisoning

Not required.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

9.2.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

9.2.4 Overall conclusions

The acute and reproductive risk to birds from exposure to trinexapac-ethyl following application of ADM.09050.H.1.A to spring barley, winter barley and grass for seed in a risk envelope approach is acceptable at tier 1.

The risks to birds from exposure to trinexapac-ethyl in drinking water from puddles did not exceed the quotient trigger value 50 in the case of less sorptive substances (Koc < 500 L/kg). The Koc of trinexapac-ethyl is 60 L/kg.

The risk assessment for effects due to secondary poisoning is not required. The log P_{ow} of trinexapac-ethyl amounts to -0.29 at pH 6.9 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

In conclusion, an acceptable overall risk for birds is indicated for all intended GAP uses of ADM.09050.H.1.A.

Review Comments:

The acute and chronic risks of ADM.09050.H.1.A to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredient and maximum residues occurring on food items.

All TER values exceed the relevant triggers indicating that ADM.09050.H.1.A does not pose an unacceptable risk to birds following applications according to recommended use pattern.

Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. The potential risk of secondary poisoning is not triggered.

9.3 Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with trinexapac-ethyl and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of ADM.09050.H.1.A were not evaluated as part of the EU assessment of trinexapac-ethyl. However, the provision of further data on the formulation ADM.09050.H.1.A is not considered essential, because because the toxicity of the formulation to mammals can be extrapolated from the data on the active substance trinexapac-ethyl.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.3-1: Endpoints and effect values relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference
Rat	Trinexapac-ethyl	Oral 1 d Acute	**LD_{50} = 4210 mg/kg bw**	EFSA Conclusion 5229/2018
Rat	Metabolite CGA275537	Oral 1 d Acute	**330 < LD_{50} < 2000 mg/kg bw**	EFSA Conclusion 5229/2018
Rat	Metabolite CGA329773	Oral 1 d Acute	**LD_{50} > 2000 mg/kg bw**	EFSA Conclusion 5229/2018

Species	Substance	Exposure System	Results	Reference
Rat	Metabolite CGA313458	Oral 1 d Acute	**LD_{50} > 2000 mg/kg bw**	EFSA Conclusion 5229/2018
Rabbit	Trinexapac-ethyl	Dietary Reproductive toxicity Teratogenicity study	**NOAEL = 60 mg/kg bw/d** (maternal)	EFSA Conclusion 5229/2018

9.3.1.1 Justification for new endpoints

No new endpoints are proposed and no justification is made.

9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Mammals and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

9.3.2.1 First-tier assessment (screening/generic focal species)

The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

Table 9.3-2: ~~First-tier~~ Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.09050.H.1.A in spring barley

Intended use	**ADM.09050.H.1.A in spring barley**				
Active substance/product	Trinexapac-ethyl				
Application rate (g/ha)	1 x 105				
Acute toxicity (mg/kg bw)	4210				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
~~BBCH 29-32~~ not required	Small herbivorous mammal	118.4	1	12.43	338.6
Reprod. toxicity (mg/kg bw/d)	60				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
~~BBCH 29-32~~ not required	Small herbivorous mammal	48.3	0.53	2.688	22.32

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-3: ~~First-tier~~ Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.09050.H.1.A in winter barley

Intended use	**ADM.09050.H.1.A in winter barley**				
Active substance/product	Trinexapac-ethyl				
Application rate (g/ha)	1 x 210				
Acute toxicity (mg/kg bw)	4210				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
~~BBCH 31-35~~ not required	Small herbivorous mammal	118.4	1	24.86	169.3
Reprod. toxicity (mg/kg bw/d)	60				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
~~BBCH 31-35~~ not required	Small herbivorous mammal	48.3	0.53	5.376	11.16

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.3-4: ~~First-tier~~ Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.09050.H.1.A in grass for seed

Intended use	**ADM.09050.H.1.A in grass for seed**				
Active substance/product	Trinexapac-ethyl				
Application rate (g/ha)	1 x 140				
Acute toxicity (mg/kg bw)	4210				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
~~BBCH 31-37~~ not required	Small herbivorous mammal	136.4	1	19.10	220.5
Reprod. toxicity (mg/kg bw/d)	60				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
~~BBCH 31-35~~ not required	Small herbivorous mammal	72.3	0.53	5.365	11.18

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

9.3.2.2 Higher-tier risk assessment

Higher tier risk assessments for the uses of ADM.09050.H.1.A on spring barley, winter barley and grass for seed in a risk envelope approach are not required as the ~~first-tier risk~~ screening assessments indicate acceptable risks for both acute and chronic exposure to mammals.

9.3.2.3 Drinking water exposure

When necessary, the assessment of the risk for mammals due to uptake of contaminated drinking water is conducted for a small omnivorous mammal with a body weight of 21.7 g (*Apodemus sylvaticus*) and a drinking water uptake rate of 0.24 L/kg bw/d (*cf.* Appendix K of EFSA/2009/1438).

Puddle scenario

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 (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).

With a K(f)oc of 60 L/kg, trinexapac-ethyl belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use winter barley also covers the risk for birds from all other intended uses in spring barley and grass for seed because the winter barley critical GAP has the highest application rate.

Effective application rate (g/ha)	=	210			
Acute toxicity (mg/kg bw)	=	4210	quotient	=	0.05
Reprod. toxicity (mg/kg bw/d)	=	60	quotient	=	3.5

No specific calculations of exposure and TER are necessary as the ratios of effective application rate of trinexapac-ethyl to acute and reprotoxic endpoints for mammals are less than 50.

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of trinexapac-ethyl amounts to -0.29 at pH 6.9 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating mammals via secondary poisoning

Not required.

Risk assessment for fish-eating mammals via secondary poisoning

Not required.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

9.3.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

9.3.4 Overall conclusions

The acute and reproductive risk to mammals from exposure to trinexapac-ethyl following application of ADM.09050.H.1.A to spring barley, winter barley and grass for seed in a risk envelope approach is acceptable at the screening step.

The risks to mammals from exposure to trinexapac-ethyl in drinking water from puddles did not exceed the quotient trigger value 50 in the case of less sorptive substances (Koc < 500 L/kg). The Koc of trinexapac-ethyl is 60 L/kg.

The risk assessment for effects due to secondary poisoning is not required. The log P_{ow} of trinexapac-ethyl amounts to -0.29 at pH 6.9 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

In conclusion, an acceptable overall risk for mammals is indicated for all intended GAP uses of ADM.09050.H.1.A.

Review Comments:

The acute and chronic risks of ADM.09050.H.1.A to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from study with active ingredient and maximum residues occurring on food items.

All TER values exceed the relevant triggers indicating that ADM.09050.H.1.A does not pose an unacceptable risk to mammals following applications according to recommended use pattern.

Evaluation of exposing to mammals through the drinking water demonstrated the acceptable risk. The potential risk of secondary poisoning is not triggered.

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

In EFSA Journal 2018;16(3):5229 reference is made to an acute toxicity study on the frog, *Xenopus laevis*. This study was conducted with the technical active substance, to fulfil data requirements in China. The 48 hour LC_{50} was >106 mg /L was greater than the existing aquatic acute vertebrate data with fish.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with trinexapac-ethyl and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of ADM.09050.H.1.A were not evaluated as part of the EU assessment of trinexapac-ethyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – trinexapac-ethyl and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Ictalurus punctatus	Trinexapac-ethyl	96 h, f	**LC_{50} = 35 mg a.s./L** $_{mm}$	EFSA Conclusion

Species	Substance	Exposure System	Results	Reference
				5229/2018
Oncorhynchus mykiss	Trinexapac-ethyl	96 h, ss	LC_{50} = 68 mg a.s./L $_{nom}$	EFSA Conclusion 5229/2018
Lepomis macrochirus	Trinexapac-ethyl	96 h, ss	LC_{50} >130 mg a.s./L $_{nom}$	EFSA Conclusion 5229/2018
Cyprinus carpio	Trinexapac-ethyl	96 h, f	LC_{50} = 57 mg a.s./L $_{nom}$	EFSA Conclusion 5229/2018
Cyprinidon variegatus	Trinexapac-ethyl	96 h, f	LC_{50} = 180 mg a.s./L $_{mm}$	EFSA Conclusion 5229/2018
Oncorhynchus mykiss	CGA179500 (Trinexapac acid)	96 h, s	**LC_{50} >100 mg a.s./L $_{nom}$**	EFSA Conclusion 5229/2018
Cyprinus carpio	CGA179500 (Trinexapac acid)	96 h, s	LC_{50} >100 mg a.s./L $_{nom}$	EFSA Conclusion 5229/2018
Pimephales promelas	Trinexapac-ethyl	35 d, (FELS), f	**NOEC = 0.41 mg a.s./L $_{mm}$** $EC_{10\ wet\ weight}$ = 0.57 mg a.s./L $EC_{10\ length}$ = 1.37 mg a.s./L $EC_{20\ wet\ weight}$ = 1.03 mg a.s./L $EC_{20\ length}$ = 3.08 mg a.s./L	EFSA Conclusion 5229/2018
Daphnia magna	Trinexapac-ethyl	48 h, ss	EC_{50} >142.5 mg a.s./L $_{nom}$	EFSA Conclusion 5229/2018
Daphnia magna	CGA179500 (Trinexapac acid)	48 h, s	**EC_{50} >111 mg a.s./L $_{nom}$**	EFSA Conclusion 5229/2018
Daphnia magna	CGA300405	48 h, s	EC_{50} >100 mg a.s./L $_{nom}$	EFSA Conclusion 5229/2018
Mysidopsis bahia	Trinexapac-ethyl	96 h, f	**EC_{50} = 6.5 mg a.s./L $_{mm}$**	EFSA Conclusion 5229/2018
Crassostrea virginica	Trinexapac-ethyl	96 h, f	EC_{50} = 89 mg a.s./L $_{mm}$	EFSA Conclusion 5229/2018
Daphnia magna	Trinexapac-ethyl	21 d, f	**NOEC = 2.4 mg a.s./L $_{mm}$**	EFSA Conclusion 5229/2018
Daphnia magna	Trinexapac-ethyl	21 d, f	NOEC = 11 mg a.s./L $_{mm}$	EFSA Conclusion 5229/2018

Species	Substance	Exposure System	Results	Reference
Pseudokirchneriella subcapitata	Trinexapac-ethyl	96 h, s	**E_rC_{50} = 24.5 mg a.s./L $_{nom}$** E_rC_{20} = 16.89 mg a.s./L $_{nom}$ E_rC_{10} = 13.91 mg a.s./L $_{nom}$ E_yC_{50} = 14.3 mg a.s./L $_{nom}$ E_yC_{20} = 11.75 mg a.s./L $_{nom}$ E_yC_{10} = 10.49 mg a.s./L $_{nom}$ E_bC_{50} = 14.3 mg a.s./L $_{nom}$ NOEC = 8 mg a.s./L	EFSA Conclusion 5229/2018
Pseudokirchneriella subcapitata	Trinexapac-ethyl	72 h, s	E_rC_{50} = 61 mg a.s./L $_{nom}$ E_rC_{20} = 28 mg a.s./L $_{nom}$ E_rC_{10} = 18 mg a.s./L $_{nom}$ E_yC_{50} = 20 mg a.s./L $_{nom}$ E_yC_{20} = 7.7 mg a.s./L $_{nom}$ E_yC_{10} = 4.7 mg a.s./L $_{nom}$ E_bC_{50} = no value provided NOEC = 10 mg a.s./L	EFSA Conclusion 5229/2018
Pseudokirchneriella subcapitata	Trinexapac-ethyl	72 h, s	E_rC_{50} = 60 mg a.s./L $_{mm}$ E_rC_{20} = 27.8 mg a.s./L $_{mm}$ E_rC_{10} = 17 mg a.s./L $_{mm}$ E_yC_{50} = no value provided E_yC_{20} = 13.2 mg a.s./L $_{mm}$ E_yC_{10} = no value provided E_bC_{50} = 27 mg a.s./L $_{mm}$ NOEC = 9.4 mg a.s./L	EFSA Conclusion 5229/2018
Pseudokirchneriella subcapitata	Trinexapac-ethyl	72 h, s	E_rC_{50} = 41.6 mg a.s./L $_{nom}$ E_rC_{20} = 26.6 mg a.s./L $_{nom}$ E_rC_{10} = 20.6 mg a.s./L $_{nom}$ E_yC_{50} = 22.8 mg a.s./L $_{nom}$ E_yC_{20} = 16.8 mg a.s./L $_{nom}$ E_yC_{10} = 14.1 mg a.s./L $_{nom}$ E_bC_{50} = no value	EFSA Conclusion 5229/2018

Species	Substance	Exposure System	Results	Reference
			provided NOEC = 10 mg a.s./L	
Anabeana flos-aquae	Trinexapac-ethyl	72 h, s	E_rC_{50} = >100 mg a.s./L $_{nom}$ E_rC_{20} = >100 mg a.s./L $_{nom}$ E_rC_{10} = >100 mg a.s./L $_{nom}$ E_yC_{50} = >100 mg a.s./L $_{nom}$ E_yC_{20} = >100 mg a.s./L $_{nom}$ E_yC_{10} = 72 mg a.s./L $_{nom}$ E_bC_{50} = no value provided NOEC = 46 mg a.s./L	EFSA Conclusion 5229/2018
Anabeana flos-aquae	Trinexapac-ethyl	72 h, s	E_rC_{50} = 295 mg a.s./L $_{nom}$ E_rC_{20} = 215 mg a.s./L $_{nom}$ E_rC_{10} = 184 mg a.s./L $_{nom}$ E_yC_{50} = 214 mg a.s./L $_{nom}$ E_yC_{20} = 165 mg a.s./L $_{nom}$ E_yC_{10} = 151 mg a.s./L $_{nom}$ E_bC_{50} = no value provided NOEC = 100 mg a.s./L	EFSA Conclusion 5229/2018
Pseudokirchneriella subcapitata	CGA179500 (Trinexapac acid)	72 h, s	E_rC_{50} = 57 mg met/L $_{nom}$ E_yC_{50} = 49.2 mg met/L $_{nom}$ NOEC = 100 mg met/L	EFSA Conclusion 5229/2018
Pseudokirchneriella subcapitata	CGA179500 (Trinexapac acid)	72 h, s	E_rC_{50} = >100 mg met/L $_{nom}$ E_bC_{50} = >100 mg met/L $_{nom}$ NOEC = >100 mg met/L	EFSA Conclusion 5229/2018
Pseudokirchneriella subcapitata	CGA179500 (Trinexapac acid)	72 h, s	E_rC_{50} = >100 mg met/L $_{nom}$ E_bC_{50} = >100 mg met/L $_{nom}$ NOEC = >100 mg met/L	EFSA Conclusion 5229/2018
Anabeana flos-aquae	CGA179500 (Trinexapac acid)	72 h, s	E_rC_{50} = 79 mg met/L $_{nom}$ E_rC_{20} = 68 mg met/L $_{nom}$ E_rC_{10} = 63 mg met/L $_{nom}$ E_yC_{50} = 73 mg met/L $_{nom}$ E_yC_{20} = 65 mg met/L $_{nom}$	EFSA Conclusion 5229/2018

Species	Substance	Exposure System	Results	Reference
			E_yC_{10} = 60 mg met/L $_{nom}$ E_bC_{50} = no value provided NOEC = 46 mg a.s./L	
Anabeana flos-aquae	CGA179500 (Trinexapac acid)	72 h, s	**E_rC_{50} = 20.1 mg met/L $_{nom}$** E_rC_{20} = no value provided E_rC_{10} = 6.18 mg met/L $_{nom}$ E_yC_{50} = 10.2 mg met/L $_{nom}$ E_yC_{20} = no value provided E_yC_{10} = no value provided E_bC_{50} = 9.74 mg met/L $_{nom}$ E_bC_{20} = no value provided E_bC_{10} = 4.66 mg met/L $_{nom}$ NOEC = 46 mg a.s./L	EFSA Conclusion 5229/2018
Microcystis aeruginosa	CGA179500 (Trinexapac acid)	96 h, s	E_rC_{50} = 72 mg met/L $_{nom}$ E_rC_{20} = 62 mg met/L $_{nom}$ E_rC_{10} = 56.3 mg met/L $_{nom}$ E_yC_{50} = no value provided E_yC_{20} = 54.8 mg met/L $_{nom}$ E_yC_{10} = 49.9 mg met/L $_{nom}$ E_bC_{50} = 62 mg met/L $_{nom}$ E_bC_{20} = no value provided E_bC_{10} = no value provided NOEC = 32 mg a.s./L	EFSA Conclusion 5229/2018
Pseudokirchneriella subcapitata	CGA300405	96h, s	**E_rC_{50} = >100 mg met/L $_{nom}$** E_rC_{20} = >100 mg met/L $_{nom}$ E_rC_{10} = 18 mg met/L $_{nom}$ E_yC_{50} = 33 mg met/L $_{nom}$ E_yC_{20} = no value provided	EFSA Conclusion 5229/2018

Species	Substance	Exposure System	Results	Reference
			E_yC_{10} = no value provided E_bC_{50} = 57 mg met/L $_{nom}$ E_bC_{20} = no value provided E_bC_{10} = no value provided NOEC = 3.2 mg a.s./L	
Lemna gibba	Trinexapac-ethyl	7 d, s	E_rC_{50} = 27.4 mg a.s./L $_{mm}$ E_rC_{20} = 5.7 mg a.s./L $_{mm}$ E_rC_{10} = 2.3 mg a.s./L $_{mm}$ E_yC_{50} = no value provided mg a.s./L $_{mm}$ E_yC_{20} = 1.4 mg a.s./L $_{mm}$ E_yC_{10} = 0.62 mg a.s./L $_{mm}$ E_bC_{50} = 8.8 mg a.s./L $_{mm}$ NOEC = 2.3 mg a.s./L	EFSA Conclusion 5229/2018
Lemna gibba	Trinexapac-ethyl	7 d, s	Frond number: E_rC_{50} = 65 mg a.s./L $_{mm}$ E_rC_{20} = 8 mg a.s./L $_{mm}$ E_rC_{10} = 2.7 mg a.s./L $_{mm}$ E_yC_{50} = 11.1 mg a.s./L $_{mm}$ E_yC_{20} = 2.2 mg a.s./L $_{mm}$ E_yC_{10} = 0.93 mg a.s./L $_{mm}$ E_bC_{50} = no value provided mg a.s./L $_{mm}$ Dry weight: E_rC_{50} = >90 mg a.s./L $_{mm}$ E_rC_{20} = 19 mg a.s./L $_{mm}$ E_rC_{10} = 8.4 mg a.s./L $_{mm}$ E_yC_{50} = 24 mg a.s./L $_{mm}$ E_yC_{20} = 8.3 mg a.s./L $_{mm}$ E_yC_{10} = 4.8 mg a.s./L $_{mm}$ E_bC_{50} = no value provided mg a.s./L $_{mm}$ NOEC = 0.95 mg a.s./L	EFSA Conclusion 5229/2018
Lemna gibba	Trinexapac-ethyl	7 d, s	Frond number: E_rC_{50} = 36.1 mg a.s./L $_{mm}$ E_rC_{20} = no value provided mg a.s./L $_{mm}$ E_rC_{10} = 2.18 mg a.s./L $_{mm}$ E_yC_{50} = 5.57 mg a.s./L $_{mm}$ E_yC_{20} = no value	EFSA Conclusion 5229/2018

Species	Substance	Exposure System	Results	Reference
			provided mg a.s./L $_{mm}$ E_yC_{10} = 1.39 mg a.s./L $_{mm}$ E_bC_{50} = no value provided mg a.s./L $_{mm}$ Dry weight: E_rC_{50} = >82.8 mg a.s./L $_{mm}$ E_rC_{20} = no value provided mg a.s./L $_{mm}$ E_rC_{10} = 4.06 mg a.s./L $_{mm}$ E_yC_{50} = 9.16 mg a.s./L $_{mm}$ E_yC_{20} = no value provided mg a.s./L $_{mm}$ E_yC_{10} = 2.79 mg a.s./L $_{mm}$ E_bC_{50} = no value provided mg a.s./L $_{mm}$ NOEC = 1.0 mg a.s./L	
Myriophyllum spicatum	Trinexapac-ethyl	14 d, ss	Shoot length: **E_rC_{50} = 1.2 mg a.s./L $_{mm}$** E_rC_{20} = 0.31 mg a.s./L $_{mm}$ E_rC_{10} = 0.22 mg a.s./L $_{mm}$ E_yC_{50} = 0.60 mg a.s./L $_{mm}$ E_yC_{20} = 0.024 mg a.s./L $_{mm}$ E_yC_{10} = 0.012 mg a.s./L $_{mm}$ E_bC_{50} = no value provided mg a.s./L $_{mm}$ Fresh weight: E_rC_{50} = 1.4 mg a.s./L $_{mm}$ E_rC_{20} = 0.022 mg a.s./L $_{mm}$ E_rC_{10} = 0.011 mg a.s./L $_{mm}$ E_yC_{50} = 0.20 mg a.s./L $_{mm}$ E_yC_{20} = 0.014 mg a.s./L $_{mm}$ E_yC_{10} = 0.0068 mg a.s./L $_{mm}$ E_bC_{50} = no value provided mg a.s./L $_{mm}$ Dry weight: E_rC_{50} = >8.8 mg a.s./L $_{mm}$ E_rC_{20} = 0.022 mg a.s./L $_{mm}$	EFSA Conclusion 5229/2018

Species	Substance	Exposure System	Results	Reference
			E_rC_{10} = 0.011 mg a.s./L $_{mm}$ E_yC_{50} = 1.9 mg a.s./L $_{mm}$ E_yC_{20} = 0.017 mg a.s./L $_{mm}$ E_yC_{10} = 0.0083 mg a.s./L $_{mm}$ E_bC_{50} = no value provided mg a.s./L $_{mm}$ NOEC = 0.025 mg a.s./L	
Lemna gibba	CGA179500 (Trinexapac acid)	7 d, s	**E_rC_{50} = 2.5 mg met/L $_{nom}$** E_rC_{20} = 0.6 mg met/L $_{nom}$ E_rC_{10} = 0.2 mg met/L $_{nom}$ E_yC_{50} = no value provided E_yC_{20} = 0.24 mg met/L $_{nom}$ E_yC_{10} = 0.12 mg met/L $_{nom}$ E_bC_{50} = 1.5 mg met/L $_{nom}$ NOEC = no value provided	EFSA Conclusion 5229/2018
Lemna gibba	CGA179500 (Trinexapac acid)	7 d, s	Frond number: E_rC_{50} = 49 mg met/L $_{nom}$ E_rC_{20} = 2.1 mg met/L $_{nom}$ E_rC_{10} = 0.40 mg met/L $_{nom}$ E_yC_{50} = 3.4 mg met/L $_{nom}$ E_yC_{20} = <0.32 mg met/L $_{nom}$ E_yC_{10} = <0.32 mg met/L $_{nom}$ E_bC_{50} = no value provided Dry weight: E_rC_{50} = >100 mg met/L $_{nom}$ E_rC_{20} = 18 mg met/L $_{nom}$ E_rC_{10} = 5.2 mg met/L $_{nom}$ E_yC_{50} = 41 mg met/L $_{nom}$ E_yC_{20} = 4.2 mg met/L $_{nom}$ E_yC_{10} = 1.3 mg met/L $_{nom}$ E_bC_{50} = no value	EFSA Conclusion 5229/2018

Species	Substance	Exposure System	Results	Reference
			provided NOEC = no value provided	
Lemna gibba	CGA179500 (Trinexapac acid)	7 d, s	Frond number: E_rC_{50} = 21.1 mg met/L $_{nom}$ E_rC_{20} = no value provided E_rC_{10} = no value provided E_yC_{50} = 1.22 mg met/L $_{nom}$ E_yC_{20} = no value provided E_yC_{10} = no value provided E_bC_{50} = no value provided Dry weight: E_rC_{50} = >206 mg met/L $_{nom}$ E_rC_{20} = no value provided E_rC_{10} = 6.37 mg met/L $_{nom}$ E_yC_{50} = 22.1 mg met/L $_{nom}$ E_yC_{20} = no value provided E_yC_{10} = 1.10 mg met/L $_{nom}$ E_bC_{50} = no value provided NOEC = 0.32mg met/L	EFSA Conclusion 5229/2018
Lemna gibba	CGA300405	7 d, s	All values **>100 mg met./L_{nom}**	EFSA Conclusion 5229/2018
Higher-tier studies (micro- or mesocosm studies)				
No studies triggered				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – ADM.09050.H.1.A

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	AG-T3-175 EC*	96 h, s	**LC_{50} = 24 mg product/L $_{nom}$**	Peither A./ 2008/ B93071
Daphnia magna	AG-T3-175 EC*	48 h, s	**EC_{50} = 15 mg product /L $_{nom}$**	Höger S./ 2008/ B93082
Anabeana flos-aquae	AG-T3-175 EC*	72 h, s	**E_rC_{50} = 93 mg product /L $_{nom}$** E_yC_{50} =60 mg product /L $_{nom}$ E_bC_{50} = no value provided NOEC= 22 mg product/L $_{nom}$	Bätscher R./ 2008/ B93093
Lemna gibba	AG-T3-175 EC*	7 d, ss	Frond number: **E_rC_{50} = 78 mg product/L $_{nom}$** E_rC_{20} = 22 mg product/L $_{nom}$ E_rC_{10} = 12 mg product/L $_{nom}$ E_yC_{50} = 27 mg product/L $_{nom}$ E_yC_{20} = 9.9 mg product/L $_{nom}$ E_yC_{10} = 5.9 mg product/L $_{nom}$ E_bC_{50} = no value provided Dry weight: E_rC_{50} = >100 mg product/L $_{nom}$ E_rC_{20} = 54 mg product/L $_{nom}$ E_rC_{10} = 33 mg product/L $_{nom}$ E_yC_{50} = 54 mg product/L $_{nom}$ E_yC_{20} = 25 mg product/L $_{nom}$ E_yC_{10} = 17 mg product/L $_{nom}$ E_bC_{50} = no value provided NOEC = 3.2 mg product/L	Höger S./ 2009/ C45577
Higher-tier studies (micro- or mesocosm studies)				
No studies triggered				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations
* compositions of ADM.09050.H.1.A and AG-T3-175 EC are provided in Part C

Review Comments:

Lemna gibba: The endpoints of ADM.09050.H.1.A expressed as geometric mean of measured concentrations of active ingredient, since analytical recoveries were not within the range 80% - 120% of the nominal values during the test period, are presented below:

EC values [mg product/L]	Parameter based on			
	frond number		dry weight of the plants	
	Growth rate r	Yield y	Growth rate r	Yield y
7-day EC_{10}	10.13	4.98	27.86	14.35
7-day EC_{20}	18.57	8.36	45.58	21.10
7-day EC_{50}	65.84	22.79	>84.41	45.58
7-day NOEC	2.70	2.70	8.44	8.44
7-day LOEC	8.44	8.44	27.01	27.01

9.5.1.1 Justification for new endpoints

No deviation from the EU agreed endpoints.

9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

The relevant global maximum FOCUS Steps 1 and 2 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.
In the following table, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group

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Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for trinexapac-ethyl for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in spring barley

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic plant**
Test species		***Ictalurus punctatus***	***Pimephales promelas***	***Mysidopsis bahia***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Myriophyllum spicatum***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}
(µg/L)		35000	410	6500	2400	24500	1200
AF		100	10	100	10	10	10
RAC (µg/L)		350	41	65	240	2450	120
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	26.698	0.0763	0.6512	0.4107	0.1112	0.0109	0.2225
Step 2							
N-Europe	0.7725	~~0.0022~~	~~0.0188~~	~~0.0119~~	~~0.0032~~	~~0.0003~~	~~0.0064~~
S-Europe	0.7725	~~0.0022~~	~~0.0188~~	~~0.0119~~	~~0.0032~~	~~0.0003~~	~~0.0064~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for trinexapac-ethyl for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in winter barley

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic plant**
Test species		***Ictalurus punctatus***	***Pimephales promelas***	***Mysidopsis bahia***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Myriophyllum spicatum***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}
(µg/L)		35000	410	6500	2400	24500	1200
AF		100	10	100	10	10	10
RAC (µg/L)		350	41	65	240	2450	120
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	13.349	0.0381	0.3256	0.2054	0.0556	0.0054	0.1112
Step 2							
N-Europe	0.3863	~~0.0011~~	~~0.0094~~	~~0.0059~~	~~0.0016~~	~~0.0002~~	~~0.0032~~
S-Europe	0.3863	~~0.0011~~	~~0.0094~~	~~0.0059~~	~~0.0016~~	~~0.0002~~	~~0.0032~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for trinexapac-ethyl for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in grass

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic plant**
Test species		***Ictalurus punctatus***	***Pimephales promelas***	***Mysidopsis bahia***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Myriophyllum spicatum***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}
(µg/L)		35000	410	6500	2400	24500	1200
AF		100	10	100	10	10	10
RAC (µg/L)		350	41	65	240	2450	120
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	17.799	0.0509	0.4341	0.2738	0.0742	0.0073	0.1483
Step 2							
N-Europe	0.515	~~0.0015~~	~~0.0126~~	~~0.0079~~	~~0.0021~~	~~0.0002~~	~~0.0043~~
S-Europe	0.515	~~0.0015~~	~~0.0126~~	~~0.0079~~	~~0.0021~~	~~0.0002~~	~~0.0043~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite CGA179500 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in spring barley

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic plant**
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Anabaena flos-aquae***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}
(µg/L)		100000	410*	111000	2400*	20100	2500
AF		100	10	100	10	10	10
RAC (µg/L)		1000	41	1110	240	2010	250
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	28.093	0.0281	0.6852	0.0253	0.1171	0.0140	0.1124
Step 2							
N-Europe	4.205	~~0.0042~~	~~0.1026~~	~~0.0038~~	~~0.0175~~	~~0.0021~~	~~0.0168~~
S-Europe	3.437	~~0.0034~~	~~0.0838~~	~~0.0031~~	~~0.0143~~	~~0.0017~~	~~0.0137~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* assumed comparable toxicity to trinexapac-ethyl due to close structual similarity

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite CGA179500 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in winter barley

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic plant**
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Anabaena flos-aquae***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}
(µg/L)		100000	410*	111000	2400*	20100	2500
AF		100	10	100	10	10	10
RAC (µg/L)		1000	41	1110	240	2010	250
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	14.046	0.0140	0.3426	0.0127	0.0585	0.0070	0.0562
Step 2							
N-Europe	2.102	~~0.0021~~	~~0.0513~~	~~0.0019~~	~~0.0088~~	~~0.0010~~	~~0.0084~~
S-Europe	1.719	~~0.0017~~	~~0.0419~~	~~0.0015~~	~~0.0072~~	~~0.0009~~	~~0.0069~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* assumed comparable toxicity to trinexapac-ethyl due to close structual similarity

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite CGA179500 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in grass

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic plant**
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Anabaena flos-aquae***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}
(µg/L)		100000	410*	111000	2400*	20100	2500
AF		100	10	100	10	10	10
RAC (µg/L)		1000	41	1110	240	2010	250
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	18.728	0.0187	0.4568	0.0169	0.0780	0.0093	0.0749
Step 2							
N-Europe	2.803	~~0.0028~~	~~0.0684~~	~~0.0025~~	~~0.0117~~	~~0.0014~~	~~0.0112~~
S-Europe	2.292	~~0.0023~~	~~0.0559~~	~~0.0021~~	~~0.0096~~	~~0.0011~~	~~0.0091~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* assumed comparable toxicity to trinexapac-ethyl due to close structual similarity

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite CGA300405 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in spring barley

Group		**Inverteb. acute**	**Algae**	**Aquatic plant**
Test species		***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Lemna gibba***
Endpoint		EC_{50}	E_rC_{50}	E_rC_{50}
(µg/L)		>100000	>100000	>100000
AF		100	10	10
RAC (µg/L)		1000	10000	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	12.358	0.0124	0.0012	0.0012
Step 2				
N-Europe	0.256	~~0.0003~~	~~<0.0001~~	~~<0.0001~~
S-Europe	0.256	~~0.0003~~	~~<0.0001~~	~~<0.0001~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite CGA300405 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in winter barley

Group		**Inverteb. acute**	**Algae**	**Aquatic plant**
Test species		***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Lemna gibba***
Endpoint		EC_{50}	E_rC_{50}	E_rC_{50}
(µg/L)		>100000	>100000	>100000
AF		100	10	10
RAC (µg/L)		1000	10000	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	6.179	0.0062	0.0006	0.0006
Step 2				
N-Europe	0.128	~~0.0001~~	~~<0.0001~~	~~<0.0001~~
S-Europe	0.128	~~0.0001~~	~~<0.0001~~	~~<0.0001~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite CGA300405 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in grass

Group		**Inverteb. acute**	**Algae**	**Aquatic plant**
Test species		***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Lemna gibba***
Endpoint		EC_{50}	E_rC_{50}	E_rC_{50}
(µg/L)		>100000	>100000	>100000
AF		100	10	10
RAC (µg/L)		1000	10000	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	8.239	0.0082	0.0008	0.0008
Step 2				
N-Europe	0.171	~~0.0002~~	~~<0.0001~~	~~<0.0001~~
S-Europe	0.171	~~0.0002~~	~~<0.0001~~	~~<0.0001~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite CGA275537 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in spring barley

Group		**Inverteb. acute**	**Aquatic plant**
Test species		***Mysidopsis bahia***	***Myriophyllum spicatum***
Endpoint		EC_{50}	E_rC_{50}
(µg/L)		650*	120*
AF		100	10
RAC (µg/L)		6.5	12
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1			
	2.097	0.3226	0.1748
Step 2			
N-Europe	<0.001	~~<0.0002~~	~~<0.0001~~
S-Europe	<0.001	~~<0.0002~~	~~<0.0001~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* acute and chronic data for CGA275537 are estimated based on to be up to 10 times more toxic than parental compound
Only risks to aquatic invertebrates and aquatic plants were calculated as the most sensitive aquatic organisms are mysid shrimp and Myriophyllum.

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite CGA275537 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in winter barley

Group		**Inverteb. acute**	**Aquatic plant**
Test species		***Mysidopsis bahia***	***Myriophyllum spicatum***
Endpoint		EC_{50}	E_rC_{50}
(µg/L)		650*	120*
AF		100	10
RAC (µg/L)		6.5	12
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1			
	1.049	0.1614	0.0874
Step 2			
N-Europe	<0.001	~~<0.0002~~	~~<0.0001~~
S-Europe	<0.001	~~<0.0002~~	~~<0.0001~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* acute and chronic data for CGA275537 are estimated based on to be up to 10 times more toxic than parental compound
Only risks to aquatic invertebrates and aquatic plants were calculated as the most sensitive aquatic organisms are mysid shrimp and Myriophyllum.

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite CGA275537 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in grass

Group		**Inverteb. acute**	**Aquatic plant**
Test species		***Mysidopsis bahia***	***Myriophyllum spicatum***
Endpoint		EC_{50}	E_rC_{50}
(µg/L)		650*	120*
AF		100	10
RAC (µg/L)		6.5	12
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1			
	1.399	0.2152	0.0001
Step 2			
N-Europe	<0.001	~~<0.0002~~	~~<0.0001~~
S-Europe	<0.001	~~<0.0002~~	~~<0.0001~~

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* acute and chronic data for CGA275537 are estimated based on to be up to 10 times more toxic than parental compound
Only risks to aquatic invertebrates and aquatic plants were calculated as the most sensitive aquatic organisms are mysid shrimp and Myriophyllum.

Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite M2 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in spring barley

Group		**Inverteb. acute**	**Aquatic plant**
Test species		***Mysidopsis bahia***	***Myriophyllum spicatum***
Endpoint		EC_{50}	E_rC_{50}
(µg/L)		650*	120*
AF		100	10
RAC (µg/L)		6.5	12
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1			
	5.499	0.8460	0.4583
Step 2			
N-Europe	0.159	0.0245	0.0133
S-Europe	0.159	0.0245	0.0133

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* acute and chronic data for M2 are estimated based on to be up to 10 times more toxic than parental compound
Only risks to aquatic invertebrates and aquatic plants were calculated as the most sensitive aquatic organisms are mysid shrimp and Myriophyllum.

Table 9.5-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite M2 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in winter barley

Group		**Inverteb. acute**	**Aquatic plant**
Test species		***Mysidopsis bahia***	***Myriophyllum spicatum***
Endpoint		EC_{50}	E_rC_{50}
(µg/L)		650*	120*
AF		100	10
RAC (µg/L)		6.5	12
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1			
	2.749	0.4229	0.2291
Step 2			
N-Europe	0.080	0.0123	0.0067
S-Europe	0.080	0.0123	0.0067

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* acute and chronic data for M2 are estimated based on to be up to 10 times more toxic than parental compound
Only risks to aquatic invertebrates and aquatic plants were calculated as the most sensitive aquatic organisms are mysid shrimp and Myriophyllum.

Table 9.5-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite M2 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in grass

Group		**Inverteb. acute**	**Aquatic plant**
Test species		***Mysidopsis bahia***	***Myriophyllum spicatum***
Endpoint		EC_{50}	E_rC_{50}
(µg/L)		650*	120*
AF		100	10
RAC (µg/L)		6.5	12
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1			
	3.666	0.5640	0.3055
Step 2			
N-Europe	0.106	0.0163	0.0088
S-Europe	0.106	0.0163	0.0088

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* acute and chronic data for M2 are estimated based on to be up to 10 times more toxic than parental compound
Only risks to aquatic invertebrates and aquatic plants were calculated as the most sensitive aquatic organisms are mysid shrimp and Myriophyllum.

Table 9.5-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite M3 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in spring barley

Group		**Inverteb. acute**	**Aquatic plant**
Test species		***Mysidopsis bahia***	***Myriophyllum spicatum***
Endpoint		EC_{50}	E_rC_{50}
(µg/L)		650*	120*
AF		100	10
RAC (µg/L)		6.5	12
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1			
	4.512	0.6942	0.3760
Step 2			
N-Europe	0.131	0.0202	0.0109
S-Europe	0.131	0.0202	0.0109

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* acute and chronic data for M3 are estimated based on to be up to 10 times more toxic than parental compound
Only risks to aquatic invertebrates and aquatic plants were calculated as the most sensitive aquatic organisms are mysid shrimp and Myriophyllum.

Table 9.5-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite M3 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in winter barley

Group		**Inverteb. acute**	**Aquatic plant**
Test species		***Mysidopsis bahia***	***Myriophyllum spicatum***
Endpoint		EC_{50}	E_rC_{50}
(µg/L)		650*	120*
AF		100	10
RAC (µg/L)		6.5	12
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1			
	2.256	0.3471	0.1880
Step 2			
N-Europe	0.065	0.0100	0.0054
S-Europe	0.065	0.0100	0.0054

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* acute and chronic data for M3 are estimated based on to be up to 10 times more toxic than parental compound
Only risks to aquatic invertebrates and aquatic plants were calculated as the most sensitive aquatic organisms are mysid shrimp and Myriophyllum.

Table 9.5-20: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite M3 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of ADM.09050.H.1.A in grass

Group		**Inverteb. acute**	**Aquatic plant**
Test species		***Mysidopsis bahia***	***Myriophyllum spicatum***
Endpoint		EC_{50}	E_rC_{50}
(µg/L)		650*	120*
AF		100	10
RAC (µg/L)		6.5	12
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**		
Step 1			
	3.008	0.4628	0.2507
Step 2			
N-Europe	0.087	0.0134	0.0073
S-Europe	0.087	0.0134	0.0073

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
* acute and chronic data for M3 are estimated based on to be up to 10 times more toxic than parental compound
Only risks to aquatic invertebrates and aquatic plants were calculated as the most sensitive aquatic organisms are mysid shrimp and Myriophyllum.

Table 9.5-21: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ADM.09050.H.1.A for each organism group based on FOCUS SWASH calculations for the use of 1.2 L product/ha in winter barley

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plant**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	***Lemna gibba***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}	E_rC_{50}
(µg/L)		24000	15000	93000	~~73000~~ 65840
AF		100	100	10	10
RAC (µg/L)		240	150	9300	~~7300~~ 6584
FOCUS Waterbody	**PECsw - drift event (µg/L)**				
Ditch	7.7096	0.0321	0.0514	0.0008	~~0.0010~~ 0.0012
Stream	5.7214	0.0238	0.0381	0.0006	~~0.0008~~ 0.0009
Pond	0.2629	0.0011	0.0018	<0.0001	<0.0001

9.5.3 Overall conclusions

The acute and chronic risks to aquatic organisms from exposure to trinexapac-ethyl and its metabolites following application of ADM.09050.H.1.A all intended uses using a risk envelope approach are acceptable using STEP 1~~/2~~ PECsw values without further risk mitigation measures.

Review Comments:

The relevant predicted environmental concentrations in water (PEC_{sw}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate). The initial risk assessment was based on the worst case PEC_{sw} values and the results of laboratory toxicity testing.

For active substance and relevant metabolites PEC/RAC calculations were performed with FOCUS STEP 1. For the formulation additional calculations were performed by zRMS with FOCUS SWASH.

The calculated PEC/RAC ratios indicate an acceptable risk for all groups of aquatic organisms without the need for any mitigation measures.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with trinexapac-ethyl. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of ADM.09050.H.1.A were not evaluated as part of the EU assessment of trinexapac-ethyl. New data submitted with this application are listed in **Błąd! Nie można odnaleźć źródła odwołania.** and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.6-1: Endpoints and effect values relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Trinexapac-ethyl	Oral	LD_{50} = >200 µg a.s./bee	EFSA Conclusion 5229/2018
Apis mellifera	Trinexapac-ethyl	Oral	**LD_{50} = >83 µg a.s./bee**	EFSA Conclusion 5229/2018
Apis mellifera	Trinexapac-ethyl	Oral	LD_{50} = >216 µg a.s./bee	EFSA Conclusion 5229/2018
Apis mellifera	Trinexapac-ethyl	Contact	LD_{50} = >200 µg a.s./bee	EFSA Conclusion 5229/2018
Apis mellifera	Trinexapac-ethyl	Contact	**LD_{50} = >100 µg a.s./bee**	EFSA Conclusion 5229/2018
Apis mellifera	Trinexapac-ethyl	Contact	LD_{50} = >200 µg a.s./bee	EFSA Conclusion 5229/2018
Apis mellifera	AG-T3-175 EC*	Oral	**LD_{50} = >86 µg a.s./bee** (= >463 µg product/bee)	Jeker L./ 2008a/ B93150
Apis mellifera	AG-T3-175 EC*	Contact	**LD_{50} = >100 µg a.s./bee** (= >539 µg product/bee)	Jeker L./ 2008a/ B93150
Apis mellifera	Trinexapac-ethyl 175 EC*	Adult chronic	10d LDD_{50} = >23 µg a.s./bee (= >0.133 mg product/bee) 10d NOEDD = 17.9 µg a.s./bee (= 0.104 mg product/bee)	Oberrauch S./ 2018a/ S18-00067
Apis mellifera	Trinexapac-ethyl 175 EC*	Larval toxicity test	22d ED_{50} = 75.0 µg a.s./larva/development period 22d NOED = 38.5 µg a.s./larva/development period	Oberrauch S./ 2018b/ S18-00066
Apis mellifera	Trinexapac-ethyl	Bee brood development	8d NOED = 12.6 µg a.s./larva/development period	EFSA Conclusion 5229/2018
Higher-tier studies (tunnel test, field studies)				
Studies not triggered				

* compositions of ADM.09050.H.1.A, Trinexapac-ethyl 175 EC and AG-T3-175 EC are provided in Part C

9.6.1.1 Justification for new endpoints

Reference is made to EFSA Journal 2018/5229.

9.6.2 Risk assessment

The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).

The EFSA Guidance on Risk Assessment on Bees, EFSA Journal 2013; 11(7): 3295, is not yet noted in the Standing Committee SCoPAFF. According to the EFSA document "Outline of the revision of the Guidance on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus spp.* And solitary bees) (EFSA,2013)" dated July 2019, EFSA Guidance 3295, 2013 continues to be reviewed and revised in a programme of work. Therefore, the EFSA Guidance document cannot be used for the risk assessment for this submission.

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of ADM.09050.H.1.A in spring barley

Intended use	ADM.09050.H.1.A in spring barley		
Active substance	Trinexapac-ethyl		
Application rate (g/ha)	1 x 105		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>83	105	1.27
Contact toxicity	>100		1.05
Product	ADM.09050.H.1.A		
Application rate (g/ha)	1 x 105		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>86	105	1.22
Contact toxicity	>100		1.05

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

Table 9.6-3: First-tier assessment of the risk for bees due to the use of ADM.09050.H.1.A in winter barley

Intended use	ADM.09050.H.1.A in winter barley		
Active substance	Trinexapac-ethyl		
Application rate (g/ha)	1 x 210		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>83	210	2.53
Contact toxicity	>100		2.1
Product	ADM.09050.H.1.A		
Application rate (g/ha)	1 x 210		

Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>86	210	2.44
Contact toxicity	>100		2.1

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

Table 9.6-4: First-tier assessment of the risk for bees due to the use of ADM.09050.H.1.A in grass

Intended use	ADM.09050.H.1.A in grass		
Active substance	Trinexapac-ethyl		
Application rate (g/ha)	1 x 140		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>83	140	1.69
Contact toxicity	>100		1.4
Product	ADM.09050.H.1.A		
Application rate (g/ha)	1 x 140		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>86	140	1.63
Contact toxicity	>100		1.40

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

The Hazard Quotients for oral (Q_{HO}) and contact exposure (Q_{HC}) are well below the trigger of 50 for the active substance as well as for the product. Therefore, an acceptable risk to bees is expected from the application of ADM.09050.H.1.A in cereals and grass.

It is noted that no chronic effects on adults or juvenile stages of bees are expected for the following reasons:

The exposure to honeybees can be caused by the application of plant protection products through direct overspray, by contact with residues on plants or by oral intake of treated food items (nectar or pollen) whilst bees are foraging on food. These sources are highly unlikely in case of the application ADM.09050.H.1.A because cereals and grass are generally considered as of low to moderate attractivity to bees. In addition, the application timing (BBCH until 37) is distinctly before flowering which is at principal growth stage 6 (BBCH Monograph, 2001). Thus, intense foraging on the crop for pollen and nectar can be excluded.

Furthermore, the results of the chronic feeding studies to adult bees and bee larvae from ADM.09050.H.1.A do not give rise to a specific concern.

In conclusion, it is reasonable to conclude that the acute and chronic risk for bees can be considered as acceptable, both from the toxicity and the exposure point of view.

9.6.2.2 Higher-tier risk assessment for bees (tunnel test, field studies)

Not relevant.

9.6.3 Effects on bumble bees

No data are considered necessary.

9.6.4 Effects on solitary bees

No data available and considered necessary.

9.6.5 Overall conclusions

The risk to bees from exposure to trinexapac-ethyl following application of ADM.09050.H.1.A to all intended uses according to a risk envelope approach is acceptable.

Review Comments:

The evaluation of the acute risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002). The submitted risk assessment, based on laboratory studies, has been accepted. It can therefore be concluded that there will be negligible acute risk associated with the exposure of *Apis mellifera* to ADM.09050.H.1.A.

The data requirements in accordance with Commission Regulation (EU) No 284/2013 for the chronic toxicity to adult honeybees and honeybee larvae are fulfilled.

The EFSA Guidance (2013) is currently under revision. As there is not harmonized approach for the chronic risk assessment for bees, therefore, Concerned Member States must decide on the acceptability of Applicant' statement regards this issue at national level.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods other than bees have been carried out with the trinexapac-ethyl representative formulation A8587B (250 ME) and another formulation A7725M (250 EC). Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target arthropods of ADM.09050.H.1.A were not evaluated as part of the EU assessment of trinexapac-ethyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri (protonymphs)	AG-T3-175 EC*	Laboratory test glass plates (2D)	LR_{50} = 0.703 L product/ha **(LR_{50} = 124 g a.s./ha)**	Jeker L./ 2008b/ B92970

Species	Substance	Exposure System	Results	Reference
Aphidius rhopalosiphi (adults)	AG-T3-175 EC*	Laboratory test glass plates (2D)	LR_{50} = 0.424 L product/ha **(LR_{50} = 74.5 g a.s./ha)**	Schmidt T./ 2009a/ B93036
Typhlodromus pyri (protonymphs)	AG-T3-175 EC*	Extended laboratory test *Phaseolus vulgaris* leaves (3 2D)	LR_{50} = 1.5 L product/ha /ha **(LR_{50} = 270 g a.s./ha)** Mortality: -1.7 % at 100 g a.s./ha 26 % at 200 g a.s./ha 76 % at 400 g a.s./ha 100% at 800 – 3200 g a.s./ha Red. of reproduction: 44 % at 100 g a.s./ha 69 % at 200 g a.s./ha	Jeker L./ 2009b/ B92968
Aphidius rhopalosiphi (adults)	AG-T3-175 EC*	Extended laboratory test barley plants (3D)	LR_{50} > 23.143 L product/ha **(LR_{50} = >4050 g a.s./ha)** ER_{50} = >4050 g a.s./ha Mortality: 34 % at 253.1 g a.s./ha 39 % at 506.3 g a.s./ha 45 % at 1012.5 g a.s./ha 40 % at 2025 g a.s./ha 43 % at 4050 g a.s./ha Red. of fecundity: 3 % at 253.1 g a.s./ha 10 % at 506.3 g a.s./ha -10 % at 1012.5 g a.s./ha 15 % at 2025 g a.s./ha -50 % at 4050 g a.s./ha	Schmidt T./ 2009c/ B93047
Aleochara bilineata	AG-T3-175 EC*	Aged-residue test quartz sand (2D/3D)	LR_{50} > 4.572 L product/ha **(LR_{50} = >800 g a.s./ha)**	Schmidt T./ 2009b/ B92913

Species	Substance	Exposure System	Results	Reference
			Mortality at 28 DAT: 15 % control 10 % at 800 g a.s./ha (highest dose tested) Red. of hatching rate at 28 DAT: 5 % at 800 g a.s./ha (highest dose tested)	
Coccinella septempunctata	AG-T3-175 EC*	Laboratory test glass plates (2D)	LR_{50} > 3.4 L product/ha **(LR_{50} = >600 g a.s./ha)** Inc. mortality (pre-imaginal larvae and pupae) at 21 DAT: 48 % at 600 g a.s./ha (highest dose tested) Mean no. eggs hatched/female/day was higher than the control validity criteria for reproduction (2 eggs hatched/female/day), therefore no test substance related effects on reproduction were observed.	Jeker L./ 2009a/ B93025
Chrysoperla carnea	AG-T3-175 EC*	Extended laboratory test *Phaseolus vulgaris* leaves (3 2D)	LR_{50} > 4.572 L product/ha **(LR_{50} = >800 g a.s./ha)** ER_{50} = >800 g a.s./ha Mortality: 17.6 % at 50 g a.s./ha 17.6 % at 100 g a.s./ha 17.6 % at 200 g a.s./ha 32.4 % at 400 g a.s./ha 47.1 % at 800 g a.s./ha Mean percentage of fertile eggs was higher than the control validity criteria (70%), therefore no test	Schmidt T./ 2009d/ B92957

Species	Substance	Exposure System	Results	Reference
			substance related effects on fertility were observed.	
Coccinella septempunctata	AG-T3-175 EC*	Extended laboratory test *Phaseolus vulgaris* leaves (3 2D)	Fungal infection of bean leaf discs was observed, which is considered to have caused mortalities in treatment groups.	Jeker L./ 2009c/ B93060
Field or semi-field tests				
Not triggered				

* compositions of ADM.09050.H.1.A and AG-T3-175 EC are provided in Part C

9.7.1.1 Justification for new endpoints

New endpoints are provided for the product trinexapac ethyl 175 g/L EC. Risk assessments are most appropriately be based on those data for the actual formulated product. The applicant provides standard laboratory data on *Typhlodromus pyri* and *Aphidius rhopalosiphi*. In addition, *Aleochara bilineata, Chrysoperla carnea* and *Coccinella septempunctata* were tested as further test species, the *A. bilineta* test contains aged residues testing.

9.7.2 Risk assessment

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2.

The risks to non-target arthropods were calculated using the winter barley GAP as a worst-case scenario. This GAP has the highest application rate and cover all other intended uses.

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.09050.H.1.A in winter barley

Intended use	ADM.09050.H.1.A in winter barley		
Active substance/product	Trinexapac-ethyl		
Application rate (g/ha)	1 x 210		
MAF	1		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	124	210	1.694
Aphidius rhopalosiphi	74.5		**2.819**
Test species Higher-tier	**Rate with ≤ 50 % effect* (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**

Intended use	ADM.09050.H.1.A in winter barley		
Active substance/product	Trinexapac-ethyl		
Application rate (g/ha)	1 x 210		
MAF	1		
Typhlodromus pyri	~~270~~ 100	210	~~Yes~~ no
Aphidius rhopalosiphi	>4050	210	yes
Aleochara bilineata	>800	210	yes
Coccinella septempunctata	>600	210	yes
Chrysoperla carnea	>800	210	yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DALT: Days after last treatment. Criteria values shown in bold breach the relevant trigger.

* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Review Comments:

Based on the results of the conducted risk assessment, the no acceptable effects were indicated for *T. pyri* based on Tier 2 study.

Taking into account the properties of trinexapac-ethyl (volatility, very low persistence in soil, used as a plant growth regulator), low toxicity of active substance and ADM.09050.H.1.A to bees, aquatic and soil invertebrates, the acceptable risk for *T. pyri* based on Tier 1 study and LR_{50}/ER_{50} values for other non-target arthropods species, in zRMS opinion, no unacceptable effects are expected in in-field habitats within one year.

9.7.2.2 Risk assessment for off-field exposure

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.09050.H.1.A in winter barley

Intended use	ADM.09050.H.1.A in winter barley				
Active substance/product	Trinexapac-ethyl				
Application rate (g/ha)	1 x 210				
MAF	1				
vdf	10 (Tier 1)				
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	124	2.77	0.582	10	0.047
Aphidius rhopalosiphi	~~84.5~~ 74.5				0.078

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

The risks to non-target arthropods other than bees from exposure to trinexapac-ethyl following application of ADM.09050.H.1.A to winter barley as a worst-case scenario are acceptable. Thus, no unacceptable risk is expected from the use of ADM.09050.H.1.A for all intended uses.

Review Comments:

Based on the results of the conducted risk assessment, it can be concluded that low risk for non-target arthropods is expected from the use of ADM.09050.H.1.A according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.

9.8 Effects on non-target soil meso- and macrofauna (KCP 10.4)

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with the trinexapac-ethyl representative formulation A8587B (250 ME) and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of ADM.09050.H.1.A were not evaluated as part of the EU assessment of trinexapac-ethyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.8-1: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance	Exposure System	Results	Reference
Eisenai andrei	AG-T3-175 EC1*	Mixed into substrate 56 d, chronic 10 % peat content	**NOEC = 181 mg product/kg dw** (31 mg a.s./kg dw) EC_{50} = 103 mg a.s./kg dw EC_{20} = 44 mg a.s./kg dw **EC_{10} = 167 mg product/kg dw** EC_{10} = 29 mg a.s./kg dw	McCormac A./ 2018/ AGAN-17-37
Eisenia fetida	CGA179500	Mixed into substrate 56 d, chronic 10 % peat content	**NOEC = 8.1 mg/kg dw**	EFSA Conclusion 5229/2018
Eisenia fetida	CGA300405	Mixed into substrate 56 d, chronic 10 % peat content	**NOEC = 1000 mg/ kg dw**	EFSA Conclusion 5229/2018

Species	Substance	Exposure System	Results	Reference
Folsomia candida	AG-T3-175 EC1*	Mixed into substrate 28 d, chronic 5 % peat content	**NOEC = 250 mg product/kg dw** (42.8 mg a.s./kg dw) EC_{50} = >171 mg a.s./kg dw	Geary N./ 2018/ AGAN-17-38
Folsomia candida	CGA300405	Mixed into substrate 21 d, chronic 5 % peat content	**NOEC = 1000 mg /kg dw**	EFSA Conclusion 5229/2018
Hypoaspis aculeifer	AG-T3-175 EC1*	Mixed into substrate 14 d, chronic 5 % peat content	**NOEC = 1000 mg product/kg dw** (171 mg a.s./kg dw) EC_{50} = >171 mg a.s./kg dw EC_{20} = >171 mg a.s./kg dw EC_{10} = >171 mg a.s./kg dw	Geary N./ 2017/ AGAN-17-39
Hypoaspis aculeifer	CGA300405	Mixed into substrate 14 d, chronic 5 % peat content	**NOEC = 1000 mg/kg dw**	EFSA Conclusion 5229/2018
Field studies				
Not triggered				
Litter bag test				
Not triggered				

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.
* Composition of AG-T3-175 EC1 is provided in the Part C

9.8.1.1 Justification for new endpoints

Risks were calculated using the endpoints derived from studies using formulation ADM.09050.H.1.A as a test substance.

9.8.2 Risk assessment

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for trinexapac-ethyl.

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.09050.H.1.A in spring barley

Intended use	ADM.09050.H.1.A in spring barley		
Chronic effects on earthworms			
Product/active substance	**NOEC/EC_{10} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Trinexapac-ethyl	~~31~~ 29	0.112	~~276~~ 259
CGA179500	8.1	0.093	87
CGA300405	1000	0.011	90909
CGA275537	~~3.1~~ 2.9*	0.008	~~388~~ 362
ADM.09050.H.1.A	~~181~~ 167	0.638	~~284~~ 262
Chronic effects on *Folsomia candida*			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Trinexapac-ethyl	42.8	0.112	382
CGA179500	4.28*	0.093	46
CGA300405	1000	0.011	90909
CGA275537	4.28*	0.008	535
ADM.09050.H.1.A	250	0.638	392
Chronic effects on *Hypoaspis aculeifer*			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Trinexapac-ethyl	171	0.112	1527
CGA179500	17.1*	0.093	184
CGA300405	1000	0.011	90909
CGA275537	17.1*	0.008	2138
ADM.09050.H.1.A	1000	0.638	1567

TER values shown in bold fall below the relevant trigger.
* Endpoints estimated to be x10 more toxic than the parent

Table 9.8-3: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.09050.H.1.A in winter barley

Intended use	ADM.09050.H.1.A in winter barley		
Chronic effects on earthworms			
Product/active substance	**NOEC/EC_{10} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Trinexapac-ethyl	~~31~~ 29	0.056	~~554~~ 518
CGA179500	8.1	0.046	176
CGA300405	1000	0.006	166667
CGA275537	~~3.1~~ 2.9*	0.004	~~775~~ 725
ADM.09050.H.1.A	~~181~~ 167	0.319	~~567~~ 524
Chronic effects on *Folsomia candida*			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Trinexapac-ethyl	42.8	0.056	764
CGA179500	4.28*	0.046	93
CGA300405	1000	0.006	166667
CGA275537	4.28*	0.004	1070
ADM.09050.H.1.A	250	0.319	784
Chronic effects on *Hypoaspis aculeifer*			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Trinexapac-ethyl	171	0.056	3054
CGA179500	17.1*	0.046	372
CGA300405	1000	0.006	166667
CGA275537	17.1*	0.004	4275
ADM.09050.H.1.A	1000	0.319	3135

TER values shown in bold fall below the relevant trigger.
* Endpoints estimated to be x10 more toxic than the parent

Table 9.8-4: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.09050.H.1.A in grass

Intended use	ADM.09050.H.1.A in grass		
Chronic effects on earthworms			
Product/active substance	**NOEC/EC_{10} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Trinexapac-ethyl	~~31~~ 29	0.075	~~413~~ 387
CGA179500	8.1	0.062	131
CGA300405	1000	0.006	166667
CGA275537	~~3.1~~ 2.9*	0.006	~~517~~ 483
ADM.09050.H.1.A	~~181~~ 167	0.425	~~426~~ 393
Chronic effects on *Folsomia candida*			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Trinexapac-ethyl	42.8	0.075	571
CGA179500	4.28*	0.062	69
CGA300405	1000	0.006	166667
CGA275537	4.28*	0.006	708
ADM.09050.H.1.A	250	0.425	588
Chronic effects on *Hypoaspis aculeifer*			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Trinexapac-ethyl	171	0.075	2280
CGA179500	17.1*	0.062	276
CGA300405	1000	0.006	166667
CGA275537	17.1*	0.006	2850
ADM.09050.H.1.A	1000	0.425	2353

TER values shown in bold fall below the relevant trigger.
* Endpoints estimated to be x10 more toxic than the parent

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The risks to soil meso- and macrofauna from exposure to trinexapac-ethyl and its metabolites following application of ADM.09050.H.1.A to spring barley, winter barley and grass for seed are acceptable. Thus, no unacceptable risk is expected from the use of ADM.09050.H.1.A for all intended uses, taking a risk envelope approach into account.

Review Comments:

The long-term risks of ADM.09050.H.1.A to soil meso- and macro-organisms were assessed from toxicity exposure ratios between toxicity endpoints and maximum PEC_{soil}. The relevant predicted environmental concentration in soil (PEC_{soil}) for risk assessment covering the proposed use pattern was taken from Part B Section 8 (Environmental Fate).

Safe use of ADM.09050.H.1.A was confirmed based on TER_{LT} calculations for formulation, trinexapac-ethyl and its relevant metabolites.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with trinexapac-ethyl and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of ADM.09050.H.1.A were not evaluated as part of the EU assessment of trinexapac-ethyl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Trinexapac-ethyl	28 d, aerobic soil type	**<25% effect at 8.6 mg a.s./kg dw**	EFSA Conclusion 5229/2018
N-mineralisation	CGA300405	28 d, aerobic soil type	**<25% effect at 200 mg met./kg dw**	EFSA Conclusion 5229/2018
N-mineralisation	CGA2755537	28 d, aerobic soil type	**<25% effect at 0.86 mg met./kg dw****	EFSA Conclusion 5229/2018
N-mineralisation	AG-T3-175 EC*	28 d, aerobic soil type	**<25% effect at 2.0 mg a.s./kg dw**	Seyfried B./ 2009/ B93227

* compositions of ADM.09050.H.1.A and AG-T3-175 EC are provided in the Part C
** Endpoint estimated to be x10 more toxic than the parent

9.9.1.1 Justification for new endpoints

Reference is made to EFSA Journal 2018/5229. Risk was calculated using the endpoints derived from study using formulation AG-T3-175 EC.

9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).
The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8

(Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 0).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of ADM.09050.H.1.A in spring barley

Intended use	ADM.09050.H.1.A in spring barley		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Trinexapac-ethyl	8.6 (at 28 d)	0.112	yes
CGA179500	0.86 (at 28 d)*	0.093	yes
CGA300405	200 (at 28 d)	0.011	yes
CGA275537	0.86 (at 28 d)*	0.008	yes
ADM.09050.H.1.A	2.0 (mg a.s./kg dw) (at 28 d)	0.112 (mg a.s./kg dw)	yes

* Endpoints estimated to be x10 more toxic than the parent

Table 9.9-3: Assessment of the risk for effects on soil micro-organisms due to the use of ADM.09050.H.1.A in winter barley

Intended use	ADM.09050.H.1.A in winter barley		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Trinexapac-ethyl	8.6 (at 28 d)	0.056	yes
CGA179500	0.86 (at 28 d)*	0.046	yes
CGA300405	200 (at 28 d)	0.006	yes
CGA275537	0.86 (at 28 d)*	0.004	yes
ADM.09050.H.1.A	2.0 (mg a.s./kg dw) (at 28 d)	0.056 (mg a.s./kg dw)	yes

* Endpoints estimated to be x10 more toxic than the parent

Table 9.9-4: Assessment of the risk for effects on soil micro-organisms due to the use of ADM.09050.H.1.A in grass

Intended use	ADM.09050.H.1.A in grass		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Trinexapac-ethyl	8.6 (at 28 d)	0.075	yes
CGA179500	0.86 (at 28 d)*	0.062	yes
CGA300405	200 (at 28 d)	0.006	yes
CGA275537	0.86 (at 28 d)*	0.006	yes
ADM.09050.H.1.A	2.0 (mg a.s./kg dw) (at 28 d)	0.075 (mg a.s./kg dw)	yes

* Endpoints estimated to be x10 more toxic than the parent

9.9.3 Overall conclusions

The risks to soil microbial activity from exposure to trinexapac-ethyl and its metabolites following application of ADM.09050.H.1.A to spring barley, winter barley and grass for seed are acceptable. Thus, no unacceptable risk is expected from the use of ADM.09050.H.1.A for all intended uses, taking a risk envelope approach into account.

Review Comments:

The use of ADM.09050.H.1.A at the proposed rates poses no unacceptable risk to soil micro-organisms.

9.10 Effects on non-target terrestrial plants (KCP 10.6)

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with trinexapac-ethyl. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of ADM.09050.H.1.A were not evaluated as part of the EU assessment of trinexapac-ethyl. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

Table 9.10-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Species	Substance	Exposure System	Results	Reference
Maize *Oat* *Ryegrass* *Onion* *Lettuce* *Oilseed rape* *Carrot* *Tomato* *Cucumber* *Pea*	AG-T3-175 EC*	21 d Seedling emergence	ER_{50} emergence = > 800 g a.s./ha	Friedrich S./ 2008b/ 08 10 48 029 S
Soya *Lettuce* *Carrot* *Tomato* *Cucumber* *Cabbage* *Oat* *Ryegrass* *Onion* *Maize*	AG-T3-175 EC*	21 d Vegetative vigour	**ER_{50} freshweight = 384 g a.s./ha (Tomato)**	Friedrich S./ 2008a/ 08 10 48 030 S

* composition of ADM.09050.H.1.A and AG-T3-175 EC are provided in Part C

9.10.1.1 Justification for new endpoints

Risks were calculated using the endpoints derived from studies using formulation ADM.09050.H.1.A .

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

9.10.2.2 Tier-2 risk assessment (based on dose-response data)

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

The risk assessment is based only on the most sensitive endpoint, obtained for tomato in the presented vigour test (**ER_{50} freshweight = 384 g a.s./ha)**. The results from the seedling emergence test show that there were no significant effect on survival of any test species was observed up to the highest application rate of 0.80 kg a.s./ha, i.e. LR_{50} > 0.80 kg a.s./ha for all tested species.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of ADM.09050.H.1.A in spring barley

Intended use	ADM.09050.H.1.A in spring barley			
Active substance/product	Trinexapac-ethyl			
Application rate (g/ha)	1 x 105			
MAF	1			
Test species	**ER_{50} (g a.s./ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g a.s./ha)**	**TER criterion: TER ≥ 5**
Tomato	384	2.77	2.91	132

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.10-3: Assessment of the risk for non-target plants due to the use of ADM.09050.H.1.A in winter barley

Intended use	ADM.09050.H.1.A in winter barley			
Active substance/product	Trinexapac-ethyl			
Application rate (g/ha)	1 x 210			
MAF	1			
Test species	**ER_{50} (g a.s./ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g a.s./ha)**	**TER criterion: TER ≥ 5**
Tomato	384	2.77	5.82	66

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.10-4: Assessment of the risk for non-target plants due to the use of ADM.09050.H.1.A in grass

Intended use	ADM.09050.H.1.A in grass			
Active substance/product	Trinexapac-ethyl			
Application rate (g/ha)	1 x 140			
MAF	1			
Test species	**ER_{50} (g a.s./ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g a.s./ha)**	**TER criterion: TER ≥ 5**
Tomato	384	2.77	3.88	99

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

The risks to non-target plants from exposure to trinexapac-ethyl following application of ADM.09050.H.1.A to spring barley, winter barley and grass for seed are acceptable. Thus, no unacceptable risk is expected from the use of ADM.09050.H.1.A for all intended uses, taking a risk envelope approach into account.

Review Comments:

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002).

Based on the risk assessment it can be concluded that the proposed use of ADM.09050.H.1.A poses no unacceptable risk to non-target plants, if applied according to the recommended use pattern. Particular precautions to reduce the environmental concentrations resulting from ADM.09050.H.1.A applications are not required.

9.11 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

No further data on effects of trinexapac-ethyl or formulation ADM.09050.H.1.A to other terrestrial organisms are available.

9.12 Monitoring data (KCP 10.8)

No further monitoring data on trinexapac-ethyl or formulation ADM.09050.H.1.A are available.

9.13 Classification and Labelling

~~Formulation ADM.05090.H.1.A is classified as H412 Harmful to aquatic life with long lasting effects.~~

~~In accordance with ECHA Guidance on the Application of the CLP Criteria v. 5.0, July 2017, ADM.05090.H.1.A is classified as aquatic environment hazard category chronic 3 because:~~

- 96h LC_{50} (for fish) >10 to ≤100 mg/L - *Oncorhynchus mykiss* 96h LC_{50} = 24 mg/L
- 48h EC_{50} (for crustacea) >10 to ≤100 mg/L - *Daphnia magna* 48h EC_{50} = 15 mg/L
- 72h or 96h E_rC_{50} (for algae or other aquatic plants) >10 to ≤100 mg/L

  – *Anabeana flos-aquae* 72h E_rC_{50} = 93 mg/L and *Lemna gibba* 7d E_rC_{50} = ~~73~~ 65.84 mg/L (mm)

~~No signal word is associated with hazard statement H412.~~

The recommended precautionary statement is P501 Dispose of contents/container in accordance with local regulations.

Review Comments:

On the May 28, 2021, the European Commission released the 17th Adaptation to Technical Progress (ATP) to the Classification, Labelling and Packaging (CLP) Regulation. The ATP is another update to the CLP Annex VI Harmonised Chemical Classification List. One of the substances listed in the 17th ATP (CLP00/ATP17) is trinexapac-ethyl which has hazard class:

- Aquatic Chronic 1 (H410) with M-factor of 1

This regulation applies from Dec. 17, 2022.

For chronic classification, the summation method in accordance with EU Regulation 1272/2008 (CLP labelling) was applied. Since the content of ingredients classified as category 1 for chronic toxicity (H410) is below the limit of 25% (being 17.5% w/w), the product ADM.09050.H.1.A should **be classified** as category 2 for chronic aquatic toxicity; **H411** according to Table 4.1.2 (copied below) in EU Regulation 1272/2008 (CLP labelling).

Table 4.1.2

Classification of a mixture for chronic (long term) hazards, based on summation of classified components

Sum of components classified as:	Mixture is classified as:
Chronic Category 1 × M (a) ≥ 25 %	Chronic Category 1
(M × 10 × Chronic Category 1) + Chronic Category 2 ≥ 25 %	Chronic Category 2
(M × 100 × Chronic Category 1) + (10 × Chronic Category 2) + Chronic Category 3 ≥ 25 %	Chronic Category 3
Chronic Category 1 + Chronic Category 2 + Chronic Category 3 + Chronic Category 4 ≥ 25 %	Chronic Category 4

(a) For explanation of the M-factor, see 4.1.3.5.5.5.

Labelling:

Hazard class(es), categories	Chronic aquatic toxicity, Category 2
Hazard pictograms or Code(s) for hazard pictogram(s)	 GHS09
Signal word	No signal word is used
Hazard statements	H411: Toxic to aquatic life with long lasting effects
Label elements for labelling	Pictogram GHS09 Signal word: No signal word is used H411: Toxic to aquatic life with long lasting effects P391: Collect spillage P501: Dispose of contents/container to an approved waste disposal plant EUH401: To avoid risks to human health and the environment, comply with the instructions for use.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/01	[redacted]	2008	AG-T3-175 EC: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) in a 96-Hour Static Test RCC Ltd., Switzerland, report no. B93071 xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx GLP Unpublished	Y	ADAMA Agan Ltd.
KCP 10.2.1/02	Höger, S.	2008	AG-T3-175 EC: Acute Toxicity to *Daphnia magna* in a 48-Hour Immobilization Test RCC Ltd., Switzerland, report no. B93082 Celsius Property B.V., report no 90018031_000081126 GLP Unpublished	N	ADAMA Agan Ltd.
KCP 10.2.1/03	Bätscher, R.	2008	AG-T3-175 EC: Toxicity to *Anabaena flos-aquae* in a 72-Hour Algal Growth Inhibition Test RCC Ltd., Switzerland, report no. B93093 Celsius Property B.V., report no 90018032_000081127 GLP Unpublished	N	ADAMA Agan Ltd.
KCP 10.2.1/04	Höger, S.	2009	AG-T3-175 EC: Toxicity of AG-T3-175 EC to the Aquatic Higher Plant *Lemna gibba* in a 7-Day Growth Inhibition Test RCC Ltd., Switzerland, report no. C45577 Celsius Property B.V., report no 90011801_000066083 GLP Unpublished	N	ADAMA Agan Ltd.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.1/01	Jeker, L.	2008a	AG-T3-175 EC: Acute Oral and Contact Toxicityto Honey Bees (*Apis mellifera* L.) RCC Ltd., Switzerland, report no. B93150 Celsius Property B.V., report no 90018033_000081128 GLP Unpublished	N	ADAMA Agan Ltd.
KCP 10.3.1.2/01	Oberrauch, S.	2018a	Trinexapac-ethyl 175 EC: Honey Bee (Apis mellifera L.) Chronic Oral Toxicity Test 10 Day Feeding Test in the Laboratory Eurofins Agroscience Services Ecotox GmbH, Germany, report no. S18-00067 ADAMA Agan Ltd., report no. 90020907 GLP Unpublished	N	ADAMA Agan Ltd.
KCP 10.3.1.3/01	Oberrauch, S.	2018b	Trinexapac-ethyl 175 EC - Honey Bee (Apis mellifera L.) 22 Day Larval Toxicity Test (Repeated Exposure) Eurofins Agroscience Services Ecotox GmbH, Germany, report no. S18-00066 ADAMA Agan Ltd., report no. 90020906 GLP Unpublished	N	ADAMA Agan Ltd.
KCP 10.3.2/01	Schmidt, T.	2009a	AG-T3-175 EC: Toxicity of AG-T3-175 EC to Adults of the Parasitoid Wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) Under Worst-case Conditions in the Laboratory RCC Ltd., Switzerland, report no. B93036 Celsius Property B.V., report no 90018034_000081129 GLP Unpublished	N	ADAMA Agan Ltd.
KCP 10.3.2/02	Jeker, L.	2008b	AG-T3-175 EC: Toxicity of AG-T3-175 EC to the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae) under Worst-Case Laboratory Conditions RCC Ltd., Switzerland, report no. B92970 Celsius Property B.V., report no 90018035_000081130 GLP Unpublished	N	ADAMA Agan Ltd.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.2/03	Schmidt, T.	2009b	AG-T3-175 EC: Toxicity to the Rove Beetle *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae) under Worst-Case Laboratory Conditions RCC Ltd., Switzerland, report no. B92913 Celsius Property B.V., report no 90018036_000081131 GLP Unpublished	N	ADAMA Agan Ltd.
KCP 10.3.2/04	Jeker, L.	2009a	AG-T3-175 EC: Toxicity to Larvae of the Seven-Spotted Ladybird *Coccinella septempunctata* (Coleoptera: Coccinellidae) under Worst-Case Laboratory Conditions RCC Ltd., Switzerland, report no. B93025 Celsius Property B.V., report no 90018037_000081132 GLP Unpublished	N	ADAMA Agan Ltd.
KCP 10.3.2/05	Schmidt, T.	2009c	AG-T3-175 EC: Toxicity of AG-T3-175 EC to Adults of the Parasitoid Wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) Under Extended Conditions in the Laboratory RCC Ltd., Switzerland, report no. B93047 Celsius Property B.V., report no 90018038_000081133 GLP Unpublished	N	ADAMA Agan Ltd.
KCP 10.3.2/06	Jeker, L.	2009b	AG-T3-175 EC: Toxicity of AG-T3-175 EC to the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae) under Extended Laboratory Conditions RCC Ltd., Switzerland, report no. B92968 Celsius Property B.V., report no 90018039_000081134 GLP Unpublished	N	ADAMA Agan Ltd.
KCP 10.3.2/07	Schmidt, T.	2009d	AG-T3-175 EC: Toxicity to Larvae of the Green Lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae) under Extended Laboratory Conditions RCC Ltd., Switzerland, report no. B92957 Celsius Property B.V., report no 90018040_000081135 GLP Unpublished	N	ADAMA Agan Ltd.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.2/08	Jeker, L.	2009c	AG-T3-175 EC: Toxicity to Larvae of the Seven-Spotted Ladybird *Coccinella septempunctata* (Coleoptera: Coccinellidae) under Extended Laboratory Conditions RCC Ltd., Switzerland, report no. B93060 Celsius Property B.V., report no 90018041_000081137 GLP Unpublished	N	ADAMA Agan Ltd.
KCP 10.4.1.1/01	McCormac, A.	2018	AG-T3-175 EC1 (Trinexapac-ethyl 175 EC) – Determination of chronic toxicity to the earthworm *Eisenia andrei* in an artificial soil substrate Mambo-Tox Ltd., UK, report no. AGAN-17-37 ADAMA Agan Ltd., report no. 90020908 GLP Unpublished	N	ADAMA Agan Ltd.
KCP 10.4.2.1/01	Geary, N.	2018	AG-T3-175 EC1 (Trinexapac-ethyl 175 EC) – A laboratory test to determine the effects of fresh residues on the springtail *Folsomia candida* (Collembola, Isotomidae) in an artificial soil substrate Mambo-Tox Ltd., UK, report no. AGAN-17-38 ADAMA Agan Ltd., report no. 90020909 GLP Unpublished	N	ADAMA Agan Ltd.
KCP 10.4.2.1/02	Geary, N.	2017	AG-T3-175 EC1 (Trinexapac-ethyl 175 EC) – A laboratory test to determine the effects of fresh residues on the predatory soil mite *Hypoaspis aculeifer* (Acari, Laelapidae) in an artificial soil substrate Mambo-Tox Ltd., UK, report no. AGAN-17-39 ADAMA Agan Ltd., report no. 90020910 GLP Unpublished	N	ADAMA Agan Ltd.
KCP 10.5/01	Seyfried, B.	2009	AG-T3-175 EC: Determinations of Effects on Soil Microflora Activity RCC Ltd., Switzerland, report no. B93227 Celsius Property B.V., report no 90018043_000081139 GLP Unpublished	N	ADAMA Agan Ltd.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.6.2/01	Friedrich, S.	2008a	Terrestrial (non-target) plant test with Trinexapac-ethyl 175 EC: Vegetative vigour test of non-target terrestrial plants BioChem agrar, Germany, report no. 08 10 48 030 S Celsius Property B.V., report no 90018044_000081140 GLP Unpublished	N	ADAMA Agan Ltd.
KCP 10.6.2/02	Friedrich, S.	2008b	Terrestrial (non-target) plant test with Trinexapac-ethyl 175 EC: Seedling emergence and seedling growth test of non-target terrestrial plants BioChem agrar, Germany, report no. 08 10 48 029 S Celsius Property B.V., report no 90018045_000081141 GLP Unpublished	N	ADAMA Agan Ltd.

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

None

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

A 2.1.3 KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

A 2.2 KCP 10.2 Effects on aquatic organisms

A 2.2.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

A 2.2.1.1.1 Study 1: Acute toxicity to fish

Comments of zRMS:	The study was conducted to OECD guideline 203 and according to the principles of GLP. No relevant deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.2.1/01
Report	AG-T3-175 EC: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) in a 96-Hour Static Test, xxxxxxxxxxxx., 2008, B93071 (report number), 90018030_000081125 (sponsor report number)
Guideline(s):	Yes, OECD 203 (1992) 92/69/EEC, C.1
Deviations:	Deviations to OECD 203 (2019)

	The light intensity of approximately 100-560 lux was lower than recommended by the guideline (540-1000 lux). The total organic carbon (TOC) of the test water was not determined. These deviations are considered minor and not affecting the quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and Methods

A. MATERIALS

1. Test material	AG-T3-175 EC (emulsifiable concentrate formulation of the active substance trinexapac-ethyl)
Description	Yellow – red brown liquid
Lot/Batch #	D-I0703
Purity	Nominal / analysed: 175 / 180 g/L trinexapac-ethyl
Density	0.97 g/mL
Stability of test material	Stable under storage conditions Expiry date: 31 Jan 2010
2. Vehicle and/or positive control	Vehicle: Test water No positive control was tested.
3. Test organism	
Species	Rainbow trout (*Oncorhynchus mykiss*)
Source	P. Hohler, trout breeding station Zeiningen, 4314 Zeiningen / Switzerland
Size	Mean body length at test start: 4.5±0.11 cm Mean body weight at test start: 0.81±0.07 g
Acclimation period	Prior to test start, the fish were acclimated for one week to the test water and temperature.
Feeding	During holding and acclimation, the fish were fed with a commercial fish diet (HOKOVIT 502, 1.2 mm, supplied by H.U. Hofmann AG, 4922 Bützberg / Switzerland). The fish were not fed 24 hours before test start and during the test.
Test units	Glass test vessels containing 15 L of test medium The loading rate was 0.38 g fish wet weight per liter test medium.
4. Environmental conditions	
Test water	Local tap water (non-chlorinated well water of drinking water quality), reduced to a total hardness of 214 mg/L (as $CaCO_3$) by ion exchange
Water temperature	13°C throughout the test period
Lighting	16-hour light (light intensity: approximately 100-560 lux) to 8-hour dark photoperiod, with a 30-minute transition period

Shaking	The test water was aerated prior to the preparation of the test media until oxygen saturation was reached. During the test, the test vessels were slightly aerated.

B. STUDY DESIGN AND METHODS

1. In life dates 09 Jun 2008 to 05 Jul 2008

2. Experimental conditions

Test design

Juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed in a static non-renewal test to aqueous test media containing the test substance at various concentrations under defined conditions. The recorded effects were mortality and visible abnormalities of the test fish.

Number of animals per treatment

Seven organisms/test substance concentration and control

Test conditions

The test was conducted in local tap water. The water temperature was maintained at 13°C and the test system was illuminated at a 16-hour light to 8-hour dark photoperiod with a 30-minute transition period. The dissolved oxygen concentration in the test media and control was at least 8.7 mg/L. The pH values in the test media and control were between 8.3 and 8.5.

Test concentrations

The following nominal concentrations of AG-T3-175 EC were tested: 2.2, 4.6, 10, 22 and 46 mg product/L. The selection of the test concentrations was based on the results of a range-finding test (non-GLP). Additionally, a control was tested in parallel (test water without test substance).

Treatment/Application

A stock solution of nominal 1000 mg product/L was freshly prepared by mixing 1501 mg of the test substance into 1.5 L of test water using stirring. Adequate volumes of this stock solution were added to the test water in the aquaria and were intensively mixed to prepare the test media with the desired test concentrations. The test media were freshly prepared just before introduction of the fish (= start of the test).

Analytics

AG-T3-175 EC concentrations in the test media were quantified by analysing the active substance trinexapac-ethyl using HPLC with UV/VIS-detection at 280 nm (column: Phenomenex Luna C18 (2); 50 mm x 4.6 mm; 3 µm; eluent: 0.4% phosphoric acid in water/acetonitrile (v/v; 7/3); flow rate 1 mL/min.; temperature: room temperature; retention time of trinexapac-ethyl: approximately 11.3 min.). Details of the analytical method validation are given in dRR Part B5.

3. Sampling and measurements

The test fish were observed after approximately 2.5, 24, 48, 72 and 96 hours test duration for mortality and visible abnormalities. Dead fish were removed at least once daily and discarded.

For analysis of the test substance concentrations, duplicate samples were taken from the test media and the control just before test start. Duplicate stability samples were taken from the test media and the control after 48 and 96 hours. However, the last samples from the highest nominal test concentration of 46 mg/L were taken after 24 hours, since at that time all fish were dead at this concentration.

The water temperature, pH and dissolved oxygen concentrations were measured at the start of the test and once every day during the test at each test concentration with surviving fish and in the control. At the same dates, the appearance of the test media was recorded.

4. Calculation of toxicity

The NOEC, LOEC, LC_0 and LC_{100} were determined directly from the raw data.

5. Statistics

The LC_{50} and the 95%-confidence interval at the observation after 96 hours were calculated by Probit Analysis.

Results and Discussion

The analytically determined concentrations of AG-T3-175 EC (calculated based on the measurement of the active substance trinexapac-ethyl) in the test media varied between 95% and 113% of the nominal values (Table A 2.2.1.1.1-2). All reported biological results were related to the nominal concentrations of the test substance.

In the control and at the test concentrations up to and including 4.6 mg product/L, all fish survived until the end of the test and no visible abnormalities were observed. At the next higher test concentration of 10 mg product /L, visible abnormalities were observed, however all fish survived until the end of the test. At the test concentration of 22 mg product/L, all test fish showed visible abnormalities and at the end of the test, two fish died at this concentration. At the highest test concentration of 46 mg product/L, all fish were dead already after 24 hours of test duration (Table A 2.2.1.1.1-1).

Thus, the 96-hour NOEC of AG-T3-175 EC to rainbow trout was determined to be 4.6 mg product/L. The 96-hour LC_0 and LOEC were both 10 mg product/L. The 96-hour LC_{50} was calculated to be 24 mg product/L with a 95% confidence interval of 18-32 mg product/L. The 96-hour LC_{100} was 46 mg product/L.

The validity criteria of the test were fulfilled since mortality in the control was 0% (required ≤ 10%), the dissolved oxygen concentration throughout the test was ≥ 8.7 mg/L with dissolved oxygen concentration at air saturation being 9.8 mg/L (required ≥ 60% of the air saturation) and analytical measurement of the test concentrations were performed.

Table A 2.2.1.1.1-1: Acute toxicity of AG-T3-175 EC to rainbow trout (96-h static test)

Nominal test substance concentration	**No. of abnormal and dead fish / No. of dead fish**					**Type of visual abnormalities**				
[mg product /L]	2.5 h	24 h	48 h	72 h	96 h	2.5 h	24 h	48 h	72 h	96 h
Control	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	-	-	-	-	-
2.2	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	-	-	-	-	-
4.6	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	-	-	-	-	-
10	0 / 0	0 / 0	0 / 0	7 / 0	7 / 0	-	-	-	-	-
22	0 / 0	7 / 0	7 / 2	7 / 2	7 / 2	-	AP	AP	AP	AP, OB
46	7 / 0	7 / 7	- / -	- / -	- / -	AP, TS	n.a.	n.a.	n.a.	n.a.
Toxicity values (95% confidence interval) [mg product/L]										
96-h LC_{50}	24 (18-32)									
96-h NOEC	4.6									
96-h LOEC	10									

- / - All fish dead
- No visual abnormalities
AP = Apathy; OB = Fish mainly at the water surface; TS = Tumbling during swimming
n.a. not applicable

Conclusion

In this test on toxicity of AG-T3-175 EC to the rainbow trout (*Oncorhynchus mykiss*), the 96-hour NOEC was determined to be 4.6 mg product/L. The 96-hour LC_0 and LOEC were both 10 mg product/L. The 96-hour LC_{50} was calculated to be 24 mg product/L (95% confidence interval: 18-32 mg product/L). The 96-hour LC_{100} was 46 mg product/L. The validity criteria were fulfilled.

Analytical data on concentrations in the test solutions

The concentrations of the active substance trinexapac-ethyl of the test substance AG-T3-175 EC were analysed in all test medium samples from the nominal test concentrations of 4.6-46 mg product/L taken at the sampling times of 0 and 96 hours or at the end of the respective exposure period when all fish were dead. The samples taken from the lowest nominal test concentration of 2.2 mg product/L were not analysed, since this test concentration was below the 96-hour NOEC, determined in this test.

The average recoveries of AG-T3-175 EC on the basis of trinexapac-ethyl found in the treatment samples ranged from 95% to 113% of the nominal concentrations (Table A 2.2.1.1.1-2).

Table A 2.2.1.1.1-2: Measured concentrations of AG-T3-175 EC in the test water

Nominal treatment	Measurements[a)] [mg product/L / % of nominal]		
[mg product/L]	0 hours	24 hours	96 hours
Control	n.d.	n.d.	n.d.
4.6	4.54 / 99	n.a.	4.37 / 95
10	11.3 / 113	n.a.	10.8 / 108
22	21.9 / 99	n.a.	21.0 / 96
46	44.1 / 96	44.8 / 97	n.a.

n.d. not detected, i.e. <LOQ of this test, i.e. 0.135 mg trinexapac-ethyl/L
n.a. not analysed
a) Mean of duplicates, except for the control (single samples)

The analytical results confirm the correct dosing of the test substance and the stability of trinexapac-ethyl in the test media over the test period of 96 hours. The biological results were related to the nominal concentrations of the test substance.

A 2.2.1.1.2 Study 2: Acute toxicity to aquatic invertebrates

Comments of zRMS:	The study was conducted to OECD guideline 202 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.2.1/02
Report	AG-T3-175 EC: Acute Toxicity to *Daphnia magna* in a 48-Hour Immobilization Test, Höger, S., 2008, B93082 (report number), 90018031_000081126 (sponsor report number)
Guideline(s):	Yes, OECD 202 (2004) 92/69/EEC, C.2
Deviations:	Deviations to OECD 202 (2004): None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and Methods

A. MATERIALS

1. Test material	AG-T3-175 EC (emulsifiable concentrate formulation of the active substance trinexapac-ethyl)
Description	Yellow – red brown liquid
Lot/Batch #	D-I0703
Purity	Nominal / analysed: 175 / 180 g/L trinexapac-ethyl

Density	0.97 g/mL
Stability of test material	Stable under storage conditions Expiry date: 31 Jan 2010

2. Vehicle and/or positive control Vehicle: Test water

The sensitivity of the *Daphnia* clone was tested twice a year using potassium dichromate. The result of the latest positive control test in March 2008 (48h-EC_{50}: 0.73 mg/L) was well within the historical range at the test facility of 0.53-1.1 mg/L (1996-2008).

3. Test organism

Species	Waterflea (*Daphnia magna* Straus)
Strain	Clone 5
Source	Originally supplied by the University of Sheffield/UK in 1992 and since then bred continuously at the test site.
Age	Neonates (not first brood progeny), 6-24 h old at the start of the test
Acclimation period	The *Daphnia* were bred in reconstituted water of identical quality (pH, main ions, total hardness) to the water used in the test.

4. Environmental conditions

Test water	Reconstituted water according to ISO 6341 (hardness: 250 mg/L as $CaCO_3$; 294 mg/L $CaCl_2 \cdot 2H_2O$; 123 mg/L $MgSO_4 \cdot 7H_2O$; 65 mg/L $NaHCO_3$; 5.8 mg/L KCl; alkalinity: 0.8 mmol/L; ratio Ca:Mg 4:1; ratio Na:K 10:1)
Water temperature	20°C
Lighting	16-hour light (light intensity: approximately 500-630 lux) to 8-hour dark photoperiod, with a 30-minute transition period
Aeration	None (prior to use, the test water was aerated until oxygen saturation was reached)

B. STUDY DESIGN AND METHODS

1. In life dates 17 Jun 2008 – 05 Jul 2008

2. Experimental conditions

Test design

Five concentrations and a blank medium control were tested under static conditions for 48 hours.

Number of animals per treatment

5 daphnids/replicate = 20 daphnids/treatment

Test conditions

The water temperature was maintained at 20°C and the test system was illuminated at a light/dark cycle of 16:8 hours with 30 minute transitional period. The dissolved oxygen concentration in the test

media was at least 8.2 mg/L during the test period. The pH values of the test media were 7.7–7.8 at test start and 7.7 at test end. Covered glass beakers (volume 100 mL) were used, filled with 50 mL test medium. The loading rate was one daphnid per 10 mL of test medium.

Test concentrations

The following nominal concentrations of AG-T3-175 EC were tested: 4.6, 10, 22 and 46 and 100 mg product/L. The selection of the test concentrations was based on the results of a range-finding test (non-GLP). Additionally, a control was tested in parallel (test water without test substance).

Treatment/Application

The test medium of the highest nominal concentration of 100 mg product/L was prepared by mixing 301.7 mg of the test substance into 3000 mL of test water using intense stirring. Adequate volumes of this test medium were diluted with test water to prepare the test media with the lower test substance concentrations. The test media were prepared just before introduction of the daphnids (i.e., start of the test).

Analytics

AG-T3-175 EC concentrations in the test media were quantified by analysing the active substance trinexapac-ethyl using HPLC with UV/VIS-detection at 280 nm (column: Phenomenex Luna C18 (2); 50 mm x 4.6 mm; 3 µm; eluent: 0.4% phosphoric acid (85%) in water/acetonitrile (v/v; 7/3); flow rate 1 mL/min.; temperature: room temperature; retention time of trinexapac-ethyl: approximately 11.2 min.). Details of the analytical method validation are given in dRR Part B5.

3. Sampling and measurements

Observations for daphnia immobilisation were made at 24 and 48 hours.

The test media were sampled at 0 h (fresh medium) and 48 h (pooled from test vessels with daphnids) for analysis of the test substance concentration.

At the start and at the end of the test, the pH values, the dissolved oxygen concentrations and the water temperature were determined at each test concentration and in the control. The appearance of the test media was visually recorded at the start of the test and after 24 and 48 hours.

4. Calculation of toxicity

The NOEC, EC_0 and EC_{100} were determined directly from the raw data.

5. Statistics

The 24-hour and 48-hour EC_{50} and the 95% confidence limits were calculated by Moving Average Interpolation.

Results and Discussion

The analytically determined concentrations of AG-T3-175 EC (calculated based on the measurement of the active substance trinexapac-ethyl) in the test media varied between 88% and 104% of the nominal values (Table A 2.2.1.1.2-2). Therefore, the biological results were based on nominal concentrations.

During the first 24 hours of the test, no immobilised test organisms were determined in the control and up to and including the test substance concentration of 10 mg product/L. At the next higher concentration of 22 mg product/L, the immobilisation was 40%. At the two highest test concentrations of 46 and 100 mg product/L, all test organisms were found to be immobile after 24 hours (Table A 2.2.1.1.2-1). The 24-hour EC_{50} of the test substance was calculated to be 23 mg product/L with 95% confidence limits of 18 and 30 mg product/L. The 24-hour EC_0 was 10 mg product/L. The 24-hour EC_{100} was 46 mg product/L.

After 48 hours of exposure, no immobilised test organisms were determined in the control and up to and including the test substance concentration of 10 mg product/L. At the concentration of 22 mg product/L, the immobilisation rate increased to 95% (Table A 2.2.1.1.2-1). The 48-hour EC_{50} was calculated to be 15 mg product/L with 95% confidence limits of 14 and 17 mg product/L. The 48-hour EC_0 and the 48-hour NOEC of AG-T3-175 EC were both 10 mg product/L, since no effect was observed up to and including this test concentration. The 48-hour EC_{100} was 46 mg product/L.

The validity criteria of the test were fulfilled since immobility in the control was 0% (required ≤ 10%) and dissolved oxygen concentrations were 8.2-8.3 mg/L in control and test vessels at the end of the test (required ≥ 3 mg/L).

Table A 2.2.1.1.2-1: Acute toxicity of AG-T3-175 EC to *Daphnia magna* (48-h static test)

Nominal test substance concentration	**Number of test**	**Immobilisation after 24 h**		**Immobilisation after 48 h**	
[mg product/L]	**organisms**	**Number**	**[%]**	**Number**	**[%]**
Control	20	0	0	0	0
4.6	20	0	0	0	0
10	20	0	0	0	0
22	20	8	40	19	95
46	20	20	100	20	100
100	20	20	100	20	100
Toxicity values (95% confidence interval) [mg product/L]					
24-hour / 48-hour EC_{50}	23 (18-30) / 15 (14-17)				
24-hour / 48-hour NOEC	10 / 10				

Conclusion

In this test on acute toxicity of AG-T3-175 EC to *Daphnia magna*, the 48-hour EC_{50} was calculated to be 15 mg product/L (95% confidence limits: 14-17 mg product/L). The 48-hour EC_0 and the 48-hour NOEC were both 10 mg product/L. The 48-hour EC_{100} was 46 mg product/L. The validity criteria were fulfilled.

Analytical data on concentrations in the test solutions

The concentrations of the active substance trinexapac-ethyl of the test substance AG-T3-175 EC were analysed in the duplicate test media samples from the nominal concentrations of 10 to 100 mg product/L from both sampling times (0 and 48 hours). The samples of the nominal test concentration of 4.6 mg product/L were not analysed since the concentration was below the 48-hour NOEC determined in this test.

The average recoveries of AG-T3-175 EC on the basis of trinexapac-ethyl found in the treatment samples ranged from 88% to 104% of the nominal concentrations (Table A 2.2.1.1.2-2).

Table A 2.2.1.1.2-2: Measured concentrations of AG-T3-175 EC in Daphnia medium

Nominal treatment [mg product/L]	Measurements[a)] [mg product/L / % of nominal]	
	0 hours	48 hours
Control	n.d.	n.d.
10	9.68 / 97	8.85 / 88
22	22.8 / 104	22.4 / 102
46	45.2 / 98	44.6 / 97
100	95.6 / 96	95.8 / 96

n.d. not detected, i.e. <LOQ of this test, i.e. 0.135 mg trinexapac-ethyl/L
a) Mean of duplicates, except for the control (single samples)

The analytical results confirm the correct dosing of the test substance and the stability of trinexapac-ethyl in the test media over the test period of 48 hours. The biological results were related to the nominal concentrations of the test substance.

A 2.2.1.1.3 Study 3: Effects on aquatic algae

Comments of zRMS:	The study was conducted to OECD guideline 201 and according to the principles of GLP. No relevant deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment

Reference:	KCP 10.2.1/03
Report	AG-T3-175 EC: Toxicity to *Anabaena flos-aquae* in a 72-Hour Algal Growth Inhibition Test, Bätscher, R., 2008, B93093 (report number), 90018032_000081127 (sponsor report number)
Guideline(s):	Yes, OECD 201 (2006) 92/69/EEC, C.3 (1992)
Deviations:	Deviations to OECD 201 (2006 with corrections 2011): Instead of constant shaking, the test solutions were stirred four times per day for 0.5 hour by magnetic stirrers. The light intensity of approximately 8000 lux was higher than recommended for *Anabaena flos-aquae*, i.e. 40-60 $\mu E \cdot m^{-2} \cdot s^{-1}$ (corresponding to 2960-4440 lux). These deviations are considered minor and not affecting the quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and Methods

A. MATERIALS

1. Test material AG-T3-175 EC (emulsifiable concentrate formulation of the active substance trinexapac-ethyl)

Description	Yellow – red brown liquid
Lot/Batch #	D-I0703
Purity	Nominal / analysed: 175 / 180 g/L trinexapac-ethyl
Density	0.97 g/mL
Stability of test material	Stable under storage conditions Expiry date: 31 Jan 2010

2. Vehicle and/or positive control Vehicle: Test water

3. Test organism

Species	Cyanobacterium ("blue-green alga") *Anabaena flos-aquae* (Lyngbye) de Brebisson
Strain	CCAP 1403/13A
Source	Culture Collection of Algae and Protozoa (CCAP, Dunstaffnage Marine Laboratory, Oban, Argyll, PA37 1QA, Scotland / UK)
Age	An inoculum culture was set up three days before the start of the test.
Acclimation period	The algae were cultivated at the test site under test conditions.

4. Environmental conditions

Test water The algae were cultivated and tested in OECD TG 201 medium. Analytical grade salts were dissolved in sterile purified water to obtain the following nominal concentrations:

Macro-nutrients:	$NaHCO_3$	50.0 mg/L
	KH_2PO_4	1.6 mg/L
	$MgSO_4$ x 7 H_2O	15.0 mg/L
	$MgCl_2$ x 6 H_2O	12.0 mg/L
	$CaCl_2$ x 2 H_2O	18.0 mg/L
	NH_4Cl	15.0 mg/L
Trace elements:	H_3BO_3	185.0 µg/L
	$MnCl_2$ x 4 H_2O	415.0 µg/L
	$ZnCl_2$	3.0 µg/L
	$CoCl_2$ x 6 H_2O	1.5 µg/L
	$CuCl_2$ x 2 H_2O	0.01 µg/L
	Na_2MoO_4 x 2 H_2O	7.0 µg/L
	$FeCl_3$ x 6 H_2O	64.0 µg/L
	Na_2EDTA x 2 H_2O	100.0 µg/L

Water hardness: 0.24 mmol/L (= 24 mg/L) as $CaCO_3$

Water temperature	23°C
Lighting	Continuous illumination at a mean light intensity (measured at the level of the test solutions) of approximately 8000 lux

	(range: 7360 to 8540 lux) using fluorescent tubes (Philips TLD 36W/840).
Shaking	The test solutions were stirred four times per day for 0.5 hour by magnetic stirrers.

B. STUDY DESIGN AND METHODS

1. In life dates 23 Jun 2008 – 08 Jul 2008

2. Experimental conditions

Test design

Five concentrations each with three replicates and six replicates of a blank test medium control group were tested under static conditions for 72 hours.

Inoculum at test start

The test was started using a nominal algal cell density of 10000 cells/mL taken from an exponentially growing pre-culture.

Test conditions

The test vessels (125 mL Erlenmeyer flasks filled with 50 mL algal suspension, covered with glass dishes) were positioned in a temperature-controlled water bath at 23°C. The test suspensions were continuously illuminated with fluorescent lighting at an intensity range between 7360 and 8540 lux. The pH at 0 and 72 hours ranged from 7.9 to 8.2 and 7.8 to 9.4, respectively.

Concentrations tested

Nominal test substance concentrations were 4.6, 10, 22, 46 and 100 mg product/L that were selected based on the results of a range-finding test (non-GLP). Additionally, a control was tested in parallel (test water without test substance).

Treatment/Application

The test medium of the highest nominal concentration of 100 mg product/L was prepared by mixing 50.4 mg of the test substance completely in 500 mL of test water using intense stirring. The test medium of the highest test concentration was diluted with test water to prepare the test media of the lower test concentrations. The test media were prepared just before the start of the test.

Analytics

AG-T3-175 EC concentrations in the test media were quantified by analysing the active substance trinexapac-ethyl using HPLC with UV/VIS-detection at 280 nm (column: Phenomenex Luna C18 (2); 50 mm x 4.6 mm; 3 µm; eluent: 0.4% phosphoric acid (85%) in water/acetonitrile (v/v; 7/3); flow rate 1 mL/min.; temperature: room temperature; retention time of trinexapac-ethyl: approximately 11.2 min.). Details of the analytical method validation are given in dRR Part B5.

3. Sampling and measurements

A small volume of the algal suspension was daily withdrawn from each test flask and the filaments of the algae in the samples were broken up to single cells by ultrasonification. The algal biomass in the samples was determined by measurement of the algal cell density using an electronic particle counter (Coulter Counter®, Model ZM). The measurements were performed at least in duplicate. In addition, at the end of the test period the shape and size of the algal cells from the control and from the test concentration with nominal 100 mg/L were visually inspected.

The test media were sampled in duplicate at the start of the test (without algae) and at the end of the test (pooled from test vessels containing algae) for analysis of the test substance concentration.

The pH was measured in each treatment at the start and at the end of the test. The water temperature and the appearance of the test media were recorded daily.

4. Calculation of toxicity

Inhibition of algal growth was determined based on the cell density (yield, Y) and the specific growth rate (r) for exponentially growing cultures using the equations recommended in the guidelines.

5. Statistics

The 72-hour EC_{10}, EC_{20} and EC_{50} values for the inhibition of average growth rate and yield and their 95% confidence intervals were calculated by Probit Analysis. For the determination of the LOEC and NOEC, average growth rate and yield at the test concentrations were compared to the control values by Dunnett's tests.

Results and Discussion

The measured test substance concentrations in the test media (based on the analysis of the active substance trinexapac-ethyl) of the test concentrations of 22 to 100 mg product/L were between 96% and 99% of the nominal values at the start and between 83% and 89% at the end of the test (Table A 2.2.1.1.3-3). Therefore, the biological results were related to the nominal concentrations of the test substance.

The biomass of algae exposed to AG-T3-175 EC and the average growth rate and yield of algae during the 72-hour test period are presented in Table A 2.2.1.1.3-1 and Table A 2.2.1.1.3-2, respectively. The test substance had a significant inhibitory effect on the average growth of the algae (growth rate and yield) after the test period of 72 hours at the concentrations of 46 and 100 mg product/L (results of Dunnett's tests, one-sided, $\alpha = 0.05$). Thus, 46 mg product/L was determined to be the 72-hour LOEC. The 72-hour NOEC was determined to be 22 mg product/L, since up to and including this test concentration the growth rate and yield of the algae after 72 hours were not significantly lower than in the control. The 72-hour EC_{50} based on growth rate was calculated to be 93 mg product/L (95% confidence interval: 88-98 mg product/L) and the 72-hour EC_{50} based on yield was calculated to be 60 mg product/L (95% confidence interval: 51-73 mg product/L).

The microscopic examination of the algal cells at the end of the test showed no difference between the algae growing at the nominal test concentration of 100 mg product/L and the algal cells in the control. The shape and size of the algal cells were obviously not affected by the test substance up to the highest test concentration.

In the control, the biomass increased by a factor of 37 over 72 hours (required ≥ 16-fold increase). The mean coefficient of variation of the daily growth rates in the control (section-by-section growth rates) during 72 hours was 6.3% (required ≤ 35%). The coefficient of variation of the average specific growth

rates in the replicates of the control after 72 hours was 1.9% (required ≤ 10%). Thus, the validity criteria were fulfilled.

Table A 2.2.1.1.3-1: Biomass of *Anabaena flos-aquae* exposed to AG-T3-175 EC

Nominal concentration	**Biomass (mean ± standard deviation) [algal cell density x 10^4 cells/mL]**		
[mg product/L]	**24 hours**	**48 hours**	**72 hours**
Control	3.4 ± 0.2	11.8 ± 0.6	36.8 ± 2.5
4.6	3.5 ± 0.4	10.5 ± 3.3	34.1 ± 7.1
10	3.7 ± 0.1	12.5 ± 0.3	41.1 ± 0.9
22	3.5 ± 0.4	11.6 ± 2.0	40.3 ± 5.9
46	3.0 ± 0.3	10.5 ± 1.2	27.2 ± 2.3
100	1.1 ± 0.3	1.5 ± 0.5	5.0 ± 0.7

Toxicity values (95% confidence interval) [mg product/L]	
E_rC_{50} (0-72h) / E_yC_{50} (0-72h)	93 (88-98) / 60 (51-73)
NOEC-72h, growth rate / yield	22 / 22
LOEC-72h, growth rate / yield	46 / 46

Table A 2.2.1.1.3-2: Average growth rate and yield of *Anabaena flos-aquae* exposed to AG-T3-175 EC

Treat-ment	**Growth rate r [day^{-1}] and inhibition I_r [%]**						**Yield Y (x 10^4) and inhibition I_Y [%]**					
	0 - 24 h		**0 - 48 h**		**0 - 72 h**		**0 - 24 h**		**0 - 48 h**		**0 - 72 h**	
[mg product /L]	**r**	**I_r**	**r**	**I_r**	**r**	**I_r**	**Y**	**I_y**	**Y**	**I_y**	**Y**	**I_y**
Control	1.22	0.0	1.23	0.0	1.20	0.0	2.4	0.0	10.7	0.0	35.8	0.0
4.6	1.25	-2.2	1.15	6.2	1.17	2.5	2.5	-4.2	9.5	11.8	33.1	7.5
10	1.30	-6.7	1.26	-2.4	1.24	-3.2	2.7	-11.8	11.4	-6.5	40.1	-12.3
22	1.24	-1.3	1.22	1.0	1.23	-2.4	2.5	-2.8	10.6	1.7	39.2	-9.8
46	1.10	10.3	1.17	4.7	1.10*	8.4	2.0	16.7	9.5	11.6	26.2*	26.8
100	0.11*	91.1	0.17*	86.1	0.53*	55.8	0.1*	94.4	0.5*	95.7	3.9*	89.0

* Mean value significantly lower than in the control (according to a Dunnett's-test, one-sided, $\alpha = 0.05$)

Conclusion

In this test on toxicity of AG-T3-175 EC to the cyanobacterium ("green alga") *Anabaena flos-aquae*, significant inhibitory effects on the growth rate and yield of the algae were observed for the test concentrations 46 mg product/L (72-hour LOEC) and 100 mg product/L. The 72-hour NOEC was determined to be 22 mg product/L. The 72-hour EC_{50} values based on growth rate and yield were calculated to be 93 and 60 mg product/L, respectively. The validity criteria were fulfilled.

Analytical data on concentrations in the test media

The concentrations of the active substance trinexapac-ethyl were determined in the duplicate test medium samples from the nominal test concentrations of 22 to 100 mg product/L. The samples from

the nominal test concentrations of 4.6 and 10 mg product/L were not analysed, since these concentrations were below the NOEC determined in this test.

The average recoveries of AG-T3-175 EC on the basis of trinexapac-ethyl found in the treatment samples at test start ranged from 96% to 99% and at test end from 83% to 89% of the nominal concentrations (Table A 2.2.1.1.3-3).

Table A 2.2.1.1.3-2: Measured concentrations of AG-T3-175 EC in algae test medium

Nominal treatment [mg product/L]	Measurements[a)] [mg product/L / % of nominal]	
	0 hours	72 hours
Control	n.d.	n.d.
22	21.0 / 96	18.2 / 83
46	44.4 / 97	39.7 / 86
100	98.5 / 99	88.7 / 89

n.d. not detected, i.e. <LOQ of this test, i.e. 0.135 mg trinexapac-ethyl/L
a) Mean of duplicates, except for the control (single samples)

The analytical results confirm the correct dosing of the test substance and the stability of trinexapac-ethyl in the test media over the test period of 72 hours. The biological results were related to the nominal concentrations of the test substance.

A 2.2.1.1.4 Study 4: Effects on aquatic macrophytes

Comments of zRMS: The study was conducted to OECD guideline 221 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met.
The study is considered to be reliable and suitable for the risk assessment.

The endpoints expressed as geometric mean of measured concentrations of active ingredient, since analytical recoveries were not within the range 80% - 120% of the nominal values during the test period, are presented below:

EC values [mg product/L]	Parameter based on			
	frond number		dry weight of the plants	
	Growth rate r	Yield y	Growth rate r	Yield y
7-day EC_{10}	10.13	4.98	27.86	14.35
7-day EC_{20}	18.57	8.36	45.58	21.10
7-day EC_{50}	65.84	22.79	>84.41	45.58
7-day NOEC	2.70	2.70	8.44	8.44
7-day LOEC	8.44	8.44	27.01	27.01

Reference:	KCP 10.2.1/04
Report	AG-T3-175 EC: Toxicity of AG-T3-175 EC to the Aquatic Higher Plant *Lemna gibba* in a 7-Day Growth Inhibition Test, Höger, S., 2009, C45577 (report number), 90011801_000066083 (sponsor report number)
Guideline(s):	Yes, OECD 221 (2006)
Deviations:	Deviations to OECD 221 (2006): None
GLP:	Yes
Acceptability:	Yes

Duplication (if vertebrate study)	-

Materials and Methods

A. MATERIALS

1. Test material

AG-T3-175 EC (emulsifiable concentrate formulation of the active substance trinexapac-ethyl)

Description	Yellow – red brown liquid
Lot/Batch #	D-I0703
Purity	Nominal / analysed: 175 / 180 g/L trinexapac-ethyl
Density	0.97 g/mL
Stability of test material	Stable under storage conditions Expiry date: 31 Jan 2010

2. Vehicle and/or positive control

Vehicle: Test water

For evaluation of the sensitivity of the test system, the reference substance 3,5-dichlorophenol was tested twice a year. The latest positive control test performed in 2008 resulted in a 7-day EC_{50} (growth rate based on frond numbers) of 10.0 mg/L, which fits with the historical range from 2003 to 2008 conducted at the test site (8.0-10.8 mg/L).

3. Test organism

Species	Duckweed *Lemna gibba*
Strain	G3
Source	Cultured at the test site since 2007 (original source: Bayer CropScience AG, 40789 Monheim, Germany)
Age	Young, rapidly growing colonies without visible lesions were taken from an exponentially growing pre-culture.
Acclimation period	The pre-culture had been maintained under the conditions of the test for more than 7 days prior to the start of the test.

4. Environmental conditions

Test water

The plants were cultivated and tested in reconstituted test water (20X AAP medium). Analytical grade salts were dissolved in sterile purified water to obtain the following nominal concentrations:

Macro-nutrients:	$NaHCO_3$	300.0 mg/L
	KH_2PO_4	20.9 mg/L
	$MgS0_4$ x 7 H_2O	294.0 mg/L
	$NaNO_3$	510.0 mg/L
	$MgCl_2$ x 6 H_2O	243.3 mg/L
	$CaCl_2$ x 2 H_2O	88.2 mg/L
Trace elements:	Na_2EDTA x 2 H_2O	6.0 mg/L
	$FeCl_3$ x 6 H_2O	3.2 mg/L
	$MnCl_2$ x 4 H_2O	8.3 mg/L
	H_3BO_3	3.7 mg/L
	Na_2MoO_4 x 2 H_2O	0.14 mg/L
	$ZnCl_2$	0.06 mg/L

	$CoCl_2$ x 6 H_2O 0.03 mg/L
	$CuCl_2$ x 2 H_2O 0.2 µg/L
Water hardness:	3.0 mmol/L (= 300 mg/L as $CaCO_3$)
pH:	Adjusted to 7.5 ± 0.1 with a hydrochloric acid solution (1 M)
Water temperature	22°C
Lighting	Continuous illumination at a mean measured light intensity of about 9100 Lux (range: 8110 to 9920 Lux) using fluorescent tubes (Philips TLD 36W-1/840), installed above the test vessels.
Shaking	None

B. STUDY DESIGN AND METHODS

1. In life dates 08 May 2009 – 25 May 2009

2. Experimental conditions

Test design

Six concentrations and a blank medium control each with three replicates were tested under semi-static conditions for seven days.

Inoculum at test start

Three *Lemna* colonies per test vessel were randomly selected from an exponentially growing pre-culture. Each colony had 4 fronds resulting in 12 fronds per vessel and 36 fronds per treatment group.

Test conditions

The pre-culture was maintained at the conditions of the test for more than 7 days prior to the start of the test. The test vessels (glass dishes, 9.5 cm diameter, filled with 150 mL test solution, approximately 21 mm water depth, covered with glass lids) were positioned in a temperature-controlled water bath. The test solutions were continuously illuminated with fluorescent lighting. The pH was 7.5-7.7 in the fresh test media and increased to 7.9-9.3 in the aged test media (increase caused by the CO_2 consumption of the plants due to their growth).

Concentrations tested

Nominal concentrations of AG-T3-175 EC: 0.32, 1.0, 3.2, 10, 32 and 100 mg product/L.
A control (test water without addition of the test substance) was tested in parallel.

Treatment/Application

The test medium of the highest nominal concentration of 100 mg product/L was prepared by mixing 101.20, 101.09 and 102.98 mg of the test substance into 1012, 1010 and 1030 mL of test water on day 0, 2 and 5, respectively, using ultrasonic treatment and intense stirring. The test medium was diluted with test water to prepare the test media of the lower test concentrations. The test media were prepared just before the start of the test and at the test medium renewals on day 2 and day 5.

Analytics

AG-T3-175 EC concentrations in the test media were quantified by analysing the active substance trinexapac-ethyl using HPLC with UV/VIS-detection at 280 nm (column: Phenomenex Luna C18 (2); 50 mm x 4.6 mm; 3 µm; eluent: 0.4% phosphoric acid in water/acetonitrile (v/v; 7/3); flow rate 1 mL/min.; temperature: room temperature; retention time of trinexapac-ethyl: approximately 10.9 min.). Details of the analytical method validation are given in dRR Part B5.

3. Sampling and measurements

On days 2, 5 and 7, the number of colonies and fronds were counted, and the plants were inspected for changes in appearance (e.g., discoloration, sinking, root length, or other abnormalities). In addition, the dry weight was determined of a sample equivalent to the inoculate at the start of the test and of all plants per test vessel at the end of the test after drying at about 60°C for 120 hours to constant weight.

Duplicate samples of the freshly prepared test media and of the aged test media (pooled replicates) of each test medium renewal period were taken from all test groups for analysis of the test substance concentration.

The pH values and the appearance of the test media were recorded at the start and end of each test medium renewal period. The water temperature was measured each working day.

4. Calculation of toxicity

The parameters average specific growth rate (r) and yield (y) were determined on basis of frond number and dry weight of plants using arithmetic mean values for each parameter per test concentration and control.

5. Statistics

The EC_{10}, EC_{20} and EC_{50} values for the inhibition of the growth rate and yield based both on frond number and on dry weight and their 95% confidence limits were calculated by Probit Analysis. The NOEC and the LOEC for the different growth parameters were determined by testing the parameters at the test concentrations for statistically significant differences to the control values using multiple Dunnett's tests.

Results and Discussion

The analytically determined concentrations of AG-T3-175 EC (calculated based on the measurement of the active substance trinexapac-ethyl) in the test media of the nominal concentrations of 3.2 to 100 mg product/L were between 87% and 92% of the nominal values at the start and between 73% and 90% of the nominal values at the end of the test medium renewal periods (Table A 2.2.1.1.4-4). As a formulation was tested, the biological results are related to the nominal concentration of the test substance.

The frond and colony counts for *Lemna gibba* and the effects of AG-T3-175 EC on the growth of *Lemna gibba* during the 7-day test are presented in Table A 2.2.1.1.4-1 and Table A 2.2.1.1.4-2, respectively. The test substance had a statistically significant inhibitory effect on the growth of *Lemna gibba* (growth rate and yield based on frond number) after the exposure period of 7 days at the test concentration of 10 mg product/L and all higher test concentrations. The growth rate and yield based on dry weight of the plants were significantly reduced first at the test concentration of 32 mg product/L. Abnormalities in growth and appearance of the test plants were recorded at the test concentration of 32 mg product/L (fronds grew upwards curved; newly formed fronds were stunted; chlorosis) and at 100 mg product/L (roots shorter than in the control; fronds grew upwards curved; newly formed fronds were stunted; chlorosis).

The 7-day NOEC was 3.2 mg product/L since up to and including this test concentration, the growth of the plants was not inhibited and no symptoms of toxicity were observed. The concentration of 10 mg product/L was determined to be the 7-day LOEC as the average growth rate and the yield based on frond numbers after the exposure period of 7 days were statistically significantly lower than in the control. The lowest 7-day EC_{50} values were calculated to be 27 mg product/L for yield (based on frond number) and 78 mg product/L for growth rate (based on frond number). Further toxicity values are presented in Table A 2.2.1.1.4-3.

The doubling time (T_d) of frond number in the control was calculated to be 1.8 days ($T_d = \ln 2 / r$), hence, clearly fulfilling the validity criterion given in the guideline ($T_d < 2.5$ d) and indicating satisfactory growth of *Lemna* under the test conditions.

Table A 2.2.1.1.4-1: Effects of AG-T3-175 EC on frond and colony counts for *Lemna gibba* during the 7-day test

Nominal test substance concentration [mg product/L]	Frond / Colony counts [mean ± standard deviation]		
	2 d	5 d	7 d
Control	29.0 ± 1.0 / 3.0 ± 0.0	87.3 ± 6.5 / 9.3 ± 0.6	188.7 ± 8.3 / 17.7 ± 0.6
0.32	28.0 ± 1.0 / 3.0 ± 0.0	92.0 ± 5.6 / 9.3 ± 0.6	204.7 ± 15.5 / 18.3 ± 0.6
1.0	27.7 ± 1.2 / 3.0 ± 0.0	91.3 ± 6.4 / 9.0 ± 0.0	189.0 ± 6.1 / 19.3 ± 0.6
3.2	27.0 ± 1.0 / 3.0 ± 0.0	85.7 ± 2.1 / 9.0 ± 0.0	185.0 ± 8.9 / 18.7 ± 2.1
10	26.3 ± 2.1 / 3.0 ± 0.0	78.7 ± 5.0 / 9.0 ± 0.0	155.0 ± 10.1 / 16.3 ± 1.2
32	22.7 ± 0.6 / 3.0 ± 0.0	52.7 ± 2.3 / 5.3 ± 0.6	85.0 ± 1.7 / 9.7 ± 0.6
100	20.3 ± 0.6 / 3.0 ± 0.0	31.3 ± 2.5 / 5.3 ± 0.6	40.3 ± 0.6 / 7.7 ± 2.1

Table A 2.2.1.1.4-2: Effects of AG-T3-175 EC on growth of *Lemna gibba* during the 7-day test

Nominal test substance concentration [mg product/L]	Based on frond number						Based on dry weight	
	Growth rate r [1/day] / %-inhibition *vs.* control			Yield y / %-inhibition *vs.* control			Growth rate r [1/day]	Yield y
							values / %-inh. *vs.* ctrl.	
	0 - 2 d	0 - 5 d	0 - 7 d	0 - 2 d	0 - 5 d	0 - 7 d	0 – 7 d	
Control	0.441 / 0.0	0.397 / 0.0	0.393 / 0.0	17.0 / 0.0	75.3 / 0.0	176.7 / 0.0	0.470 / 0.0	22.0 / 0.0
0.32	0.423 / 4.0	0.407 / -2.7	0.405 / -2.9	16.0 / 5.9	80.0 / -6.2	192.7 / -9.1	0.477 / -1.5	23.1 / -5.0
1.0	0.4171 / 5.4	0.406 / -2.3	0.394 / -0.1	15.7 / 7.8	79.3 / -5.3	177.0 / -0.2	0.475 / -1.1	22.8 / -3.6
3.2	0.405 / 8.1	0.393 / 0.9	0.391 / 0.7	15.0 / 11.8	73.7 / 2.2	173.0 / 2.1	0.482 / -2.6	24.0 / -9.1
10	0.392* / 11.1	0.376 / 5.2	0.365* / 7.2	14.3* / 15.7	66.7 / 11.5	143.0* / 19.1	0.469 / 0.04	21.9 / 0.06
32	0.318* / 27.9	0.296* / 25.4	0.280* / 28.9	10.7* / 37.3	40.7* / 46.0	73.0* / 58.7	0.422* / 10.1	15.5* / 29.4
100	0.264* / 40.2	0.192* / 51.7	0.173* / 56.0	8.3* / 51.0	19.3* / 74.3	28.3* / 84.0	0.289* / 38.4	5.6* / 74.5

Note: A negative value indicates increase in growth relative to the control

* mean value significantly lower than in the control (according to a Dunnett's test, one-sided, $\alpha = 0.05$)

Table A 2.2.1.1.4-3: Toxicity of AG-T3-175 EC for *Lemna gibba* after 7 days of exposure

EC values [mg product/L]	Parameter based on			
	frond number		dry weight of the plants	
	Growth rate r	Yield y	Growth rate r	Yield y
7-day EC_{10}	12 (9.4-14)	5.9 (4.3-7.5)	33 (30-35)	17 (14-20)
7-day EC_{20}	22 (20-25)	9.9 (7.8-12)	54 (51-57)	25 (22-29)
7-day EC_{50}	78 (71-86)	27 (23-31)	> 100 (n.d.)	54 (49-59)
7-day NOEC	3.2	3.2	10	10
7-day LOEC	10	10	32	32

( ) 95% confidence interval
n.d. could not be determined

Conclusion

In this test on toxicity of AG-T3-175 EC to the freshwater aquatic plant *Lemna gibba* (duckweed), the lowest 7-day EC_{50} values were calculated to be 27 mg product/L for yield (based on frond number) and 78 mg product/L for growth rate (based on frond number). The validity criterion was fulfilled.

Analytical data on concentrations in the test media

The concentrations of the active substance trinexapac-ethyl of the test substance AG-T3-175 EC were measured in the test medium samples taken from the nominal test concentrations of 3.2 to 100 mg product/L. The samples from the lowest nominal test concentrations of 0.32 and 1.0 mg product/L were not analysed as these concentrations were below the 7-day NOEC.

The average recoveries found in the unaged treatment samples ranged from 90% to 92% (day 0) and from 87% to 92% (day 2) of the nominal concentrations. The average recoveries found in the aged treatment samples ranged from 79% to 90% (day 2) and from 73% to 78% (day 5) of the nominal concentrations (Table A 2.2.1.1.4-4).

Table A 2.2.1.1.4-4: Measured concentrations of AG-T3-175 EC in *Lemna* test medium

Nominal treatment [mg product/L]	Measurements[a)] [mg product/L / % of nominal]			
	0 days (new)	2 days (old)	2 days (new)	5 days (old)
Control	n.d.	n.d.	n.d.	n.d.
3.2	2.95 / 92	2.51 / 79	2.91 / 91	2.35 / 73
10	9.23 / 92	8.03 / 80	8.69 / 87	7.68 / 77
32	29.0 / 90	25.8 / 81	28.2 / 88	23.8 / 74
100	90.6 / 91	89.8 / 90	92.4 / 92	78.0 / 78

n.d. not detected, i.e. <LOQ of this test, i.e. 0.103 mg trinexapac-ethyl/L
a) Mean of duplicates, except for the control (single samples)

As a formulation was tested, the biological results are related to the nominal concentration of the test

substance.

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 Study 1: Acute toxicity to the honeybee

Comments of zRMS:	The study was conducted to OECD guidelines 213 and 214 and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.1.1/01
Report	AG-T3-175 EC: Acute Oral and Contact Toxicity to Honey Bees (*Apis mellifera* L.), Jeker, L., 2008a, B93150 (report number), 90018033_000081128 (sponsor report number)
Guideline(s):	Yes, EPPO (2000), EPPO Bulletin 23: 45-55. OECD 213 (1998) OECD 214 (1998)
Deviations:	Deviations to OECD 213 (1998) and OECD 214 (1998): None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and Methods

A. MATERIALS

1. Test material	AG-T3-175 EC (emulsifiable concentrate formulation of the active substance trinexapac-ethyl)
Description	Yellow – red brown liquid
Lot/Batch #	D-I0703
Purity	Nominal / analysed: 175 / 180 g/L trinexapac-ethyl

Stability of test material	Stable under storage conditions Expiry date: 31 Jan 2010
2. Vehicle and/or positive control	Vehicle oral toxicity test: Sugar solution (50% w/v) Vehicle contact toxicity test: Deionised water with 1% Etalfix Positive control: Reference item
Reference item	Roxion (emulsifiable concentrate formulation of the active substance dimethoate)
Description	Yellow liquid
Lot/Batch #	G339A
Purity	Nominal / analysed: 400 / 375 g/L dimethoate
Stability of reference item	Stable under storage conditions Expiry date: Dec 2015
Wetting agent	Etalfix
Description	Colourless liquid
Lot/Batch #	SCI3L356
Purity	Content of a.s. (aethoxylated octylphenol): 250 g/L
3. Test organism	
Species	*Apis mellifera* L.
Source	Commercial beekeeper (Mr. Jacques Breiter, Wuhrweg 35, 4450 Sissach, Switzerland). The organisms were collected from healthy, disease-free and queen-right colonies. The bees used in the test were not treated with chemical substances, such as antibiotics etc. for at least 4 weeks before collection.
Acclimation period	Bees were acclimatised to the test conditions (i.e. temperature, humidity and light conditions) overnight.
Diet	Sugar solution (50% w/v)
Housing	After collection, the bees were held in 1 L plastic containers covered with gauze for the transport to the test facility and for acclimation.
Test units	The test units consisted of stainless steel chambers (approximately 10 x 8 x 5.5 cm, length x width x height). The front side of the unit was equipped with a removable glass plate and the back with a removable perforated stainless steel plate for air circulation. The top of the cage was fitted with two holes: One hole for bee introduction was covered with a stopper during the test and the second hole for introducing the Eppendorf vial. Through the drilled hole near the tip of the Eppendorf vial the bees had access to either the sugar solution or the solution for the oral treatment with their tongue.
4. Environmental conditions	
Temperature	23.7-25.1°C
Relative humidity	77±1.3% Due to technical reasons, the humidity was over 70% during the test. However, this deviation in humidity was not considered to

	have an influence on the biological results, as documented by the acceptable performance of the bees in the control.
Photoperiod	Normal lighting in the laboratory

B. STUDY DESIGN AND METHODS

1. In-life dates 30 Jul 2008 to 01 Aug 2008

2. Experimental conditions

Test design

The acute toxicity of the test substance AG-T3-175 EC to the honeybee, *Apis mellifera* L., after oral and contact exposure was determined under laboratory conditions. For the oral treatment, the test substance was provided via the feeding solution. For the contact treatment, the test substance was applied to the dorsal part of the thorax. Bee mortality and sub-lethal effects were assessed.

Number of animals per treatment

Ten bees/replicate; four replicates/treatment

Test doses

Oral toxicity test
2.6, 6.4, 16, 40 and 100 µg a.s./bee
The application solution of the highest dose was prepared by dissolving 1.43 mL of the test substance homogeneously in 50 mL sugar solution (50% w/v). An aliquot of this application solution was further diluted with sugar solution to obtain the application solutions for the lower test rates. The control group was fed sugar solution (50% w/v) only.

Contact toxicity test
6.25, 12.5, 25, 50 and 100 µg a.s./bee
The application solution of the highest dose was prepared by dissolving 5.71 mL of the test substance homogeneously in 10 mL deionised water (with 1% Etalfix). An aliquot of this application solution was further diluted with deionised water (with 1% Etalfix) to obtain the application solutions for the lower test rates. The control group received deionised water (with 1% Etalfix) only.

Reference item

Oral toxicity test
0.033, 0.10 and 0.35 µg a.s./bee
A stock solution was prepared by dissolving 437.5 µL of the reference substance in 100 mL sugar solution (50% w/v). An aliquot of the stock solution was further diluted with sugar solution (50% w/v) to obtain the application solutions for the test rates.

Contact toxicity test
0.033, 0.10 and 0.3 µg a.s./bee
The application solution of the highest dose was prepared by dissolving 75 µL of the reference substance in 100 mL deionised water (with 1% Etalfix). An aliquot of this application solution was further diluted with deionised water (with 1% Etalfix) to obtain the application solutions for the lower test rates.

Treatment/Application

Oral toxicity test
After starving the bees for 2 hours, a quantity of 250 µL test solution was offered to each cage of 10 bees. The food quantity consumed was recorded by weighing the test solution before and after exposure (maximum of 24 hours). Thereafter, the food was replaced with untreated sugar solution (50% w/v).

Contact toxicity test
One droplet with the volume of 1 µL of the application solution was placed on the dorsal side of the bee thorax using a calibrated micro-pipette. Bees were anaesthetised with carbon dioxide before treatment.

3. Observations and assessments

Bee mortality and symptoms of toxicity were assessed 4, 24 and 48 hours after treatment application in both the contact and the oral toxicity test.

The temperature and the relative humidity were continuously recorded during the test period.

4. Calculation of toxicity

Mortality in both the oral and contact toxicity test was corrected for mortality in the control according to Abbott (1925).

Calculations for the oral toxicity test were based on ingested doses. The oral and contact LD_{50} for the test substance were determined directly from the raw data.

5. Statistics

The oral and contact LD_{50} and their 95% confidence limits for the reference substance were calculated by Probit analysis.

Results and Discussion

Oral toxicity test

At the 48-hour assessment, mortality in the control was 2.5% (Table A 2.3.1.1.1-1). Consequently, the validity criterion was met (mortality in the control ≤ 10% at 48 hours).

In the test substance treatments, the corrected mortality at the 48-hour assessment ranged from 0.0% to 36% (Table A 2.3.1.1.1-1). Since the corrected mortality in the test substance treatments did not exceed 50%, the oral (ingested) LD_{50} was determined directly from the raw data to be > 86 µg a.s./bee.

In the reference substance treatments, the mortality at the 24-hour assessment was 0.0% at the lowest ingested dose of 0.032 µg dimethoate/bee, 28% at 0.11 µg dimethoate/bee and 100% at the highest ingested dose of 0.36 µg dimethoate/bee (Table A 2.3.1.1.1-1). The oral 24-hour LD_{50} of the reference substance was calculated to be 0.13 µg dimethoate/bee (95% confidence interval: 0.038-0.45 µg dimethoate/bee). Therefore, the performance criterion was met (oral LD_{50} at the 24-hour assessment in the range of 0.10-0.35 µg dimethoate/bee).

Contact toxicity test

Up to 48 hours after test initiation, a mortality of 5.0% occurred in the control (Table A 2.3.1.1.1-1). The validity criterion was met (mortality in the control ≤ 10% at 48 hour).

In the test substance treatments, the corrected mortality at the 48-hour assessment ranged from 0.0% to 47% (Table A 2.3.1.1.1-1). Since the corrected mortality in the test substance treatments did not exceed 50%, the contact (nominal) LD_{50} was determined directly from the raw data to be > 100 µg a.s./bee.

In the reference substance treatments, the corrected mortality at the 24-hour assessment was 16% and 24% at the two lower doses of 0.033 and 0.10 µg dimethoate/bee and was 97% at the highest dose of 0.30 µg dimethoate/bee (Table A 2.3.1.1.1-1). The contact 24-hour LD_{50} of the reference substance was calculated to be 0.12 µg dimethoate/bee (95% confidence interval: 0.027-0.51 µg dimethoate/bee). Therefore, the performance criterion was met (contact LD_{50} at the 24-hour assessment in the range of 0.10-0.30 µg dimethoate/bee).

Table A 2.3.1.1.1-1: Toxicity of AG-T3-175 EC to honey bees (Apis mellifera L.) in oral and contact toxicity tests

Oral test target dose	Oral test actual uptake	Contact test	Oral test			Contact test		
			Mortality [%]		Corr.[a] mortality [%]	Mortality [%]		Corr.[a] mortality [%]
[µg a.s./bee]	[µg a.s./bee]	[µg a.s./bee]	24 h	48 h	48 h	24 h	48 h	48 h
Control	0.0	0.0	2.5	2.5	--	5.0	5.0	--
Test substance: AG-T3-175 EC (175 g/L trinexapac-ethyl)								
2.6	2.9	6.25	2.5	2.5	0.0	5.0	5.0	0.0
6.4	7.1	12.5	0.0	2.5	0.0	5.0	7.5	2.6
16	17	25	0.0	0.0	-2.6	5.0	7.5	2.6
40	38	50	0.0	2.5	0.0	13	18	13
100	86	100	28	38	36	48	50	47
Reference substance: Roxion (400 g/L dimethoate)								
0.033	0.032	0.033	2.5	n.a.	n.a.	20	n.a.	n.a.
0.10	0.11	0.10	30	n.a.	n.a.	28	n.a.	n.a.
0.35	0.36	0.30	100	n.a.	n.a.	98	n.a.	n.a.
Endpoints								
LD_{50} oral (ingested)	> 86 µg a.s./bee (> 463 µg product/bee)[b]							
LD_{50} contact (nominal)	> 100 µg a.s./bee (> 539 µg product/bee)[b]							

a) Corrected for mortality in the control according to Abbott (1925)
b) Calculated by the applicant using the analysed content of 180 g/L trinexapac-ethyl and the density of product batch D-I0703 of 0.97 ± 0.02 g/cm³ (20°C).
n.a. not assessed

Conclusion

In this test on toxicity of AG-T3-175 EC, an emulsifiable concentrate formulation containing 175 g/L trinexapac-ethyl, to the honey bee (*Apis mellifera* L.), the oral LD_{50} (48 h) was determined to be > 86 µg a.s./bee and the contact LD_{50} (48 h) was found to be > 100 µg a.s/bee. All validity criteria were met.

A 2.3.1.1.2 KCP 10.3.1.1.1 Acute oral toxicity to bees

Please refer to A 2.3.1.1.1.

A 2.3.1.1.3 KCP 10.3.1.1.2 Acute contact toxicity to bees

Please refer to A 2.3.1.1.1.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.2.1 Study 1: Chronic toxicity to the honeybee

Comments of zRMS:	The study was conducted to OECD guideline 245 and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be reliable.

Reference:	KCP 10.3.1.2/01
Report	Trinexapac-ethyl 175 EC: Honey Bee (Apis mellifera L.) Chronic Oral Toxicity Test 10 Day Feeding Test in the Laboratory, Oberrauch, S., 2018a, S18-00067 (report number), 90020907 (sponsor report number)
Guideline(s):	Yes, OECD 245 (2017)
Deviations:	Deviations to OECD 245 (2017): None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and Methods

A. MATERIALS

1. Test material	Trinexapac-ethyl 175 EC (= AG-T3-175 EC1)
Description	Yellow-brown liquid, EC (emulsifiable concentrate)
Lot/Batch #	8227
Purity	Trinexapac-ethyl: 175 g/L nominal; 172.9 g/L analysed Density: 1.002 g/mL
Stability of test material	Stable when stored in original packaging under normal storage conditions Expiry date: February 2020
2. Vehicle and/or positive control	Vehicle: aqueous sucrose solution (50%, w/v) Positive control: reference item
Reference item	BAS 152 11 I (Dimethoate EC 400 g/L)
Description	Blue liquid
Lot/Batch #	FRE-001578
Purity	400.0 g/L dimethoate (nominal content) 429.0 g/L dimethoate (analysed content) density: 1.076 g/cm³

Stability of reference item	Stable under storage conditions (cool (+1 to +10 °C), dark, dry) Expiry date: 17 Nov 2019

3. Test organism

Species	Honey bee, *Apis mellifera* L. (Hymenoptera, Apidae)
Source	Healthy colony from test facility's own stock
Age	Adult worker bees (newly hatched; 1 to 2 days old)
Pre-treatment culturing conditions	Two days prior to start of exposure, brood combs containing capped cells which were expected to hatch on the same day were taken out of a honey bee colony and transferred into the climatic chamber. The combs were kept under test conditions. One day prior to start of exposure, the 0 – 1-day old bees were picked off the combs, transferred to the test cages and kept under test conditions until the start of exposure (acclimatisation). Non-suitable bees (affected, moribund or dead) were rejected and replaced by healthy bees before start of exposure.
Diet	During acclimatisation, the bees were fed *ad libitum* with untreated 50% (w/v) aqueous sucrose solution. During the test, the bees were fed with 50% (w/v) aqueous sucrose solution containing either the test item or the reference item or pure 50% (w/v) aqueous sucrose solution (untreated control group). The treated and untreated food was offered using syringes which were replaced daily by a new one containing fresh treated or untreated food.
Test units	Stainless steel cages (base: approximately 8 cm x 4 cm, height: approximately 6 cm) with the front side of the cages being equipped with a transparent pane to enable observation and the bottom consisting of perforated steel, which guaranteed sufficient air supply. The cages were lined with filter paper.

4. Environmental conditions

Temperature	nominal: 33 ± 2°C, actual: 31.7-33.3°C
Relative humidity	nominal: 50-70%, actual: 30.9-64.1% (deviations below 50% were short term, i.e. < 2 hours)
Photoperiod	During the test, the bees were kept in a climatic chamber in constant darkness except during application and assessments. The climatic chamber was ventilated.

B. STUDY DESIGN AND METHODS

1. In-life dates 28 May 2018 to 28 Jun 2018

2. Experimental conditions

Test design

In a 10-day chronic test, young adults of *Apis mellifera* L. were daily exposed to five doses of AG-T3-175 EC1 in 50% (w/v) aqueous sucrose solution. In parallel, an untreated control (50% (w/v) aqueous sucrose solution) and one dose of the reference item BAS 152 11 I (Dimethoate EC 400 g/L) were tested. Assessments of bee mortality and behavioural abnormalities were done daily during the study.

Number of animals per treatment

Four replicates per test and reference item treatment and untreated control were used with 10 bees per replicate.

Test doses

AG-T3-175 EC1 was tested at 62.5, 125, 250, 500 and 1000 mg a.s./kg food, corresponding to 0.362, 0.724, 1.449, 2.898 and 5.795 g product/kg food (based on analysed content of active substance and product density, calculated by the applicant).

A control group, receiving untreated 50% (w/v) aqueous sucrose solution, was tested in parallel.

Reference item

The reference item, BAS 152 11 I (Dimethoate EC 400 g/L), was tested at a single concentration of 0.9 mg a.s./kg food.

Treatment/Application

The application took place for a period of 10 consecutive days. Test item feeding solutions were freshly prepared in 50% (w/v) aqueous sucrose solution each day before administration of food. A reference item stock solution was prepared in deionised water on the day of exposure start and on two further days in the course of the test period and stored in the refrigerator. The reference item feeding solutions were prepared from the stock solution by dilution with 50% (w/v) aqueous sucrose solution at the day of use. All 50% (w/v) aqueous sucrose solutions used for the control and for dilution to obtain the test/reference item feeding solutions were prepared with deionised water and stored in the refrigerator for a maximum of 4 days.

A volume of approximately 3-4 mL feeding solution was offered to the test organisms of each test unit in feeders (plastic syringes, approximately 5 mL). The tip of each feeder was removed so that the bees had access to the feeding solution. Every morning during 10 days, the syringes of all test cages were replaced by new syringes, filled with freshly prepared feeding solutions. The weight of the syringes was determined before and after feeding on the next day in order to determine the mean food consumption of the bees per replicate. The syringes of four additional cages (without bees) were filled with approximately 3-4 mL of pure 50% (w/v) aqueous sucrose solution and weighed daily to determine evaporation.

Analytics

Analytical samples and retain samples of the feeding solutions of the control and the lowest and highest test item concentration were taken daily directly after preparation. The sample size was 5 mL for each sample. No samples of the reference item feeding solutions were taken. The samples were deep frozen within 45 min after sampling and stored in the freezer until analysis.

The analytical method was validated with regard to specificity, linearity, accuracy (recovery), precision and limit of quantification in accordance with SANCO/3029/99 rev. 4 from 11/07/2000. Please refer to dRR Part B5.

3. Observations and assessments

Mortality and behavioural abnormalities were recorded every 24 hours (± 2 hours) after application (start of feeding). In the reference item treatment group, behavioural assessments were not conducted.

The amount of feeding solution consumed was determined daily by weighing the feeders before and after feeding.

The evaporation out of the food syringes was determined by daily weighing of the syringes in the respective, additional test cages.

Temperature and humidity were recorded continuously with appropriate, calibrated equipment.

4. Calculation of toxicity

The percentage of cumulative mortality was calculated for each treatment group and assessment from the number of dead individuals in relation to the number of introduced test organisms. The cumulative mortality of the test and reference item treatments was corrected for the corresponding control mortality according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

The consumption of feeding solution per bee per day was calculated by dividing the total daily consumption per replicate by the number of living bees at the beginning of the respective feeding interval. For each treatment group, the mean consumption of feeding solution per bee per day was calculated by averaging the replicate values.

A mean value of evaporation per day was determined for the whole test period and the daily food consumption of the control and the the test/reference item treatments was corrected by the mean value of the corresponding day. When this correction led to a negative value, the food consumption of the respective replicate was considered to be “0” (no food consumption).

5. Statistics

Multiple Fisher's exact test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there were significant differences between the mortality data of the control and the test item treatment groups and to determine the NOEC and NOEDD based on mortality. The LC_{50} and LDD_{50} could not be calculated since the observed mortalities were below 50% in all test item treatment groups. Statistical calculations were made by using the statistical program ToxRat Professional 3.2.1.

Results and Discussion

The results of the study are presented in the following table.

The overall mean daily consumption of feeding solution over the entire test period was 35.9 mg/bee/day in the control and 39.1, 34.6, 38.9, 35.8 and 23.0 mg/bee/day in the test item treatments of 62.5, 125, 250, 500 and 1000 mg a.s./kg food, respectively. In the reference item treatment, the overall mean daily consumption of feeding solution was 19.9 mg/bee/day. At the end of the 10-day test period, the accumulated uptake of test item was 24.4, 43.3, 97.3, 179 and 230 µg a.s./bee at the test concentrations of 62.5, 125, 250, 500 and 1000 mg a.s./kg food, respectively. The corresponding daily mean uptake was therefore 2.44, 4.33, 9.72, 17.9 and 23.0 µg a.s./bee/day, respectively.

Cumulative mortality in the control was 5.0% after 10 days. In the test item treatments of 62.5 to 500 mg a.s./kg food, cumulative mortality ranged between 0.0% and 5.0%. At the highest test concentration of 1000 mg a.s./kg food, cumulative mortality was 32.5%, which was statistically significantly higher

than in the control (Multiple Fisher's exact test with Bonferroni-Holm adjustment, one-sided greater, $\alpha = 0.05$). Therefore, the NOEC for mortality after 10 days of continuous exposure to AG-T3-175 EC1 was determined to be 500 mg a.s./kg food. The corresponding NOEDD, based on the actual consumption of the respective feeding solutions, was 17.9 µg a.s./bee/day. The 10-day LC_{50} could not be calculated but was estimated to be above the highest tested concentration of 1000 mg a.s./kg food, corresponding to an LDD_{50} of > 23.0 µg a.s./bee/day.

No remarkable behavioural abnormalities were observed in the control group and in any of the test item treatment groups.

The actual concentrations of trinexapac-ethyl in the feeding solutions of the lowest (62.5 mg a.s./kg food) and highest (1000 mg a.s./kg food) test concentration prepared on every application day, were in the range of 88% to 105% of the nominal concentrations. No residues of trinexapac-ethyl above the limit of detection (LOD, 1.88 mg trinexapac ethyl/kg food) were found in any of the control samples.

The validity of the test was fulfilled since mean mortality in the control was below 5.0% (required ≤ 15%) and mean mortality in the reference item treatment was 100% at the end of the test (required ≥ 50%).

Table A 2.3.1.2.1-1: Mortality of bees in the chronic toxicity feeding test after 10 days exposure to AG-T3-175 EC1

Treatment group	Test concentration [mg a.s./kg food]	Overall mean consumption of feeding solution [mg/bee/day]	Dietary dose [µg a.s./bee/day]	Accumulated mean uptake of test item [µg a.s./bee]	Cumulative mortality after 10 days [%]	Cumulative corrected[a] mortality after 10 days [%]
Control	-	35.9	-	-	5.0	-
Test item AG-T3-175 EC1	62.5	39.1	2.44	24.4	2.5	-2.6
	125	34.6	4.33	43.3	2.5	-2.6
	250	38.9	9.72	97.3	0.0	-5.3
	500	35.8	17.9	179	5.0	0.0
	1000	23.0	23.0	230	32.5*	28.9
Reference item BAS 152 11 I (Dimethoate EC 400 g/L)	0.9	19.9	0.02	0.14	100	100
Endpoints after 10 days exposure						
LC_{50}	> 1000 mg a.s./kg food / > 5.795 g product/kg food[b]					
LDD_{50}	> 23.0 µg a.s./bee/day / 0.133 mg product/bee/day[b]					
NOEC	500 mg a.s./kg food / 2.898 g product/kg food[b]					
NOEDD	17.9 µg a.s./bee/day / 0.104 mg product/bee/day[b]					

* Significantly different compared to control (multiple Fisher's exact test with Bonferroni-Holm adjustment, one sided greater, $\alpha = 0.05$)

a) Corrected for control mortality according to the formula of Abbott (1925), modified by Schneider-Orelli (1947)

b) Calculated by the applicant using the analysed content of 172.9 g/L trinexapac-ethyl and the density of 1.002 g/cm³.

Conclusion

In this 10-day chronic toxicity feeding study with AG-T3-175 EC1 in the honey bee, the 10-day LC_{50} could not be calculated but was estimated to be above the highest tested concentration of 1000 mg a.s./kg food (> 5.795 g product/kg food), corresponding to an LDD_{50} of > 23.0 µg a.s./bee/day (> 0.133 mg product/bee/day). The 10-day NOEC for mortality was determined to be 500 mg a.s./kg food (2.898 g product/kg food), corresponding to an NOEDD of 17.9 µg a.s./bee/day (0.104 mg product/bee/day). The validity criteria were fulfilled.

A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

A 2.3.1.3.1 Study 1: Toxicity to honeybee larvae

Comments of zRMS:	The study was conducted to OECD guideline 239 and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be reliable.

Reference:	KCP 10.3.1.3/01
Report	Trinexapac-ethyl 175 EC - Honey Bee (Apis mellifera L.) 22 Day Larval Toxicity Test (Repeated Exposure), Oberrauch, S., 2018b, S18-00066 (report number), 90020906 (sponsor report number)
Guideline(s):	Yes, OECD (2016): Series on Testing and Assessment Number 239: Guidance Document on Honey Bee (*Apis mellifera*) Larval Toxicity Test, Repeated Exposure, Paris 2016.
Deviations:	Deviations to OECD (2016): None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and Methods

A. MATERIALS

1. Test material	Trinexapac-ethyl 175 EC (emulsifiable concentrate formulation of the active substance trinexapac-ethyl)
Description	Yellow-brown liquid, EC (emulsifiable concentrate)
Lot/Batch #	8227
Purity	Trinexapac-ethyl: 175 g/L nominal; 172.9 g/L analysed Density: 1.002 g/mL

Stability of test material	Stable when stored in original packaging under normal storage conditions Expiry date: February 2020
2. Vehicle and/or positive control	Vehicle: untreated diet (50% weight of fresh royal jelly + 50% weight of an aqueous solution containing yeast extract, glucose and fructose) Positive control: reference item
Reference item	Dimethoate (BAS 152 I)
Description	White to grey solid
Lot/Batch #	COD-002332
Purity	99.0% (w/w)
Stability of reference item	Stable under storage conditions (ambient (15-25°C), dark and dry) Expiry date: 31 Dec 2018
3. Test organism	
Species	Honeybee, *Apis mellifera carnica* POLLMANN (Hymenoptera, Apidae)
Source	Three different bee hives located and maintained at the test facility
Age	Synchronised first instar (L1) larvae (one day old)
Pre-treatment culturing conditions	The hives were adequately fed, healthy, queen-right and as far as possible parasite-free. No chemical substances (such as antibiotics, anti-Varroa treatments, pesticides, etc.) were used in the hive within the four weeks preceding the start of the test.
Method of producing L1 larvae:	Four days prior to the grafting of larvae, queens of several colonies were confined in their own colony in an excluder cage containing a comb with empty cells. Three days prior to the grafting, maximum 30 hours after encaging, the queens were released from the cages. The combs containing eggs were left in the excluder cages during the incubation stage until hatching on day 1. On day 1, the combs were transferred to the laboratory using an insulated container in order to avoid temperature variation. In the laboratory, three combs were selected for grafting, containing the highest number of synchronised larvae.
Diet	The food was composed of three different artificial diets which were adapted to the needs of the larvae at different stages of development: - Diet A: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose - Diet B: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose - Diet C: 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose
Test units	Crystal polystyrene grafting cells (NICOTPLAST, diameter 9 mm, depth 8 mm) were sterilised with ethanol and placed into

a well of a sterile 48-well cellular culture plate (Greiner Bio One). The open plates were placed into a hermetically sealed desiccator with a water saturated atmosphere from day 1 until day 8. On day 8, the plates were transferred into a second desiccator containing a dish filled with a saturated NaCl solution. The desiccators were placed in an incubator with forced air circulation. On day 15, each plate was covered by its lid and transferred from the desiccator into an incubator with automated humidity control.

4. Environmental conditions

Temperature	Target: 34-35°C, but not below 23°C or above 40°C Actual from grafting on day 1 until transfer of plates into new incubator on day 8: 32.9-34.2°C Actual from day 8 until transfer of plates into new incubator on day 15: 32.8-33.9°C Actual from day 15 until last assessment on day 22: 33.4-34.5°C Deviations (≥ 2 hours) from the preferred temperature range on day 1 and 4 were considered unlikely to have made any discernible impact on the test performance as the validity criteria were fulfilled.
Relative humidity	From grafting on day 1 until transfer of plates into new incubator on day 8: target: 95% ± 5%, actual: 95.5 ± 5.8%, 99.1 ± 3.7% and 95.3 ± 5.9% (reserve plates, control group and test/reference item groups, respectively) From day 8 until transfer of plates into new incubator on day 15: target 80% ± 5%, actual: 75.9% ± 2.5% From day 15 until last assessment on day 22: target: 50-80%, actual: 64.1% ± 2.7%
Photoperiod	During the test, the bees were kept in constant darkness except during grafting, feeding and assessments.

B. STUDY DESIGN AND METHODS

1. In-life dates 11 May 2018 to 23 Jul 2018

2. Experimental conditions

Test design

The effects of the test item AG-T3-175 EC1 on the emergence of adult honey bees (*Apis mellifera* L.) from repeated feeding exposure were assessed in a 22-day laboratory test. Honey bee larvae were either treated with the test item at five concentrations, the reference item dimethoate (BAS 152 I) at a single concentration or remained untreated (control). Cumulative mortalities during the larval and pupation phase as well as the adult emergence rate were assessed.

Number of animals per treatment

16 larvae/replicate; 3 replicates/test and reference item treatment and control; each replicate comprised larvae from one of the three different hives.

Test doses

The toxicity of AG-T3-175 EC1 was determined at 31.3, 62.5, 125, 250 and 500 mg a.s./kg food (0.181, 0.362, 0.724, 1.449 and 2.898 g product/kg food, based on analysed content of active substance and product density, calculated by the applicant), equivalent to cumulative doses of 4.82, 9.63, 19.3, 38.5 and 77.0 µg a.s./larva per developmental period.

A control group, receiving untreated artificial diet, was tested in parallel.

Reference item

The reference item, dimethoate was tested at 48.0 mg a.s./kg food, equivalent to a cumulative dose of 7.39 µg a.s./larva per developmental period.

Treatment/Application

The stock solution of the test item was prepared by diluting 637 mg to 20 mL with autoclaved, deionised water. Test item solutions were prepared by serial dilution of the stock solution with autoclaved, deionised water. The reference item stock solution was prepared by diluting 107 mg to 20 mL with autoclaved, deionised water. A ten-fold dilution of this stock solution with autoclaved, deionised water served as reference item solution. The treated diets for feeding from day 3 until day 6 were prepared daily. Since the larval diet was prepared in adjusted concentration considering an absence of 10% (v/v) water, the lacking volume of water was added in form of 10% (v/v) test item solution. For the control group, 10% (v/v) of the final diet volume was added in form of autoclaved, deionised water. For the dimethoate reference item group, 10% (v/v) of the final diet volume was added in form of reference item solution.

At test start (day 1), 20 µL of untreated diet A was dropped into each grafting cell of the well-plate, then one larva was grafted from the comb to the cell, onto the surface of the diet, using a grafting tool. All larvae were fed once a day (except at day 2). At day 3, 20 µL of treated diet B were administered to each larva. At day 4, 5 and 6, larvae were fed with 30, 40 and 50 µL of treated diet C, respectively.

Analytics

Samples of the lowest and highest test item solutions and of the respective solvent were taken directly after preparation. After stabilisation by addition of 1% (v/v) formic acid, the samples were analysed for trinexapac-ethyl by high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) detection.

The analytical method was validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection in accordance with SANCO/3029/99 rev. 4 from 11/07/2000. Please refer to dRR Part B5.

3. Observations and assessments

Assessment of mortality during the larval phase was conducted before feeding from day 4 until day 6 and on day 7 and 8. Mortality during the pupation phase was assessed on day 15 and day 22. A stereo microscope was used to assist mortality assessment, if necessary. At each assessment time dead larvae and pupae were removed.

On day 8, the presence of uneaten food was qualitatively recorded. Other observations and any other adverse effects were qualitatively recorded to aid in the interpretation of mortality in comparison to the control group.

Assessment of adult emergence was conducted on day 22. Bees were counted as successfully emerged if they showed signs of adult eclosion.

Air temperature and relative air humidity throughout the study were recorded in intervals of 15 minutes.

4. Calculation of toxicity

The cumulative larval mortality [%] for each treatment group on day 8 was calculated from the number of dead larvae in relation to the total number of larvae per treatment group after re-grafting on day 3.

Mortality during the pupation phase was evaluated on day 15 and on day 22. The cumulative larval and pupal mortality [%] on day 15 was calculated from the number of dead larvae/pupae divided by the total number of larvae per treatment group after re-grafting on day 3. The cumulative pupal mortality [%] on day 22 was calculated from the number of larvae and pupae that failed to emerge until day 22 in relation to the total number of larvae that entered pupation phase on day 8.

Adult emergence was calculated as the number of successfully emerged bees in relation to the total number of larvae per treatment group after re-grafting on day 3.

The cumulative mortalities were corrected for control mortality according to the formula of Abbott (1925), modified by Schneider-Orelli (1947).

5. Statistics

Multiple Fisher's exact test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a significant difference between the test item groups and the control group for larval mortality on day 8 and pupal mortality from day 8 through 22. Cochran-Armitage test (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a significant difference between the test item groups and the control group for larval and pupal mortality on day 15 and for adult emergence on day 22.

The EC_{10}/ED_{10} and EC_{20}/ED_{20} for adult emergence on day 22 could not be calculated since there was no clear concentration/dose-response relationship. The EC_{50} with 95% confidence limits was calculated using Trimmed Spearman-Karber procedure. The calculation was performed using control corrected percentage of non-emerged bees.

For the statistical evaluation, the statistics program ToxRat professional, Version 3.2.1 was used.

Results and Discussion

The results of the study are presented in the following table.

Cumulative larval mortality on day 8 was 0.0% in the control, 0.0-20.8% in the test item treatments from 31.3 to 500 mg a.s./kg food and 100% in the reference item treatment. For the highest test item concentration of 500 mg a.s./kg food, cumulative larval mortality was statistically significantly higher than in the control (Multiple Fisher's exact test with Bonferroni-Holm adjustment, one sided greater, $\alpha = 0.05$). Cumulative larval and pupal mortality on day 15 was 14.6% in the control and 10.4-50.0% in the in the test item treatments from 31.3 to 500 mg a.s./kg food. Furthermore, cumulative pupal mortality from day 8 to 22 was 14.6% in the control and 12.5-39.5% in the in the test item treatments from 31.3 to 500 mg a.s./kg food. Both for cumulative larval and pupal mortality on day 15 and cumulative pupal mortality from day 8 to 22, a statistically significant increase was determined for the highest test item concentration of 500 mg a.s./kg food (larval and pupal mortality on day 15: Cochran-Armitage test, one sided greater, $\alpha = 0.05$, pupal mortality from day 8 to 22: Multiple Fisher's exact test with Bonferroni-Holm adjustment, one sided greater, $\alpha = 0.05$).

Adult emergence in the control was 85.4%. In the test item treatments from 31.3-250 mg a.s./kg food, adult emergence ranged from 79.2% to 87.5%. At the highest test concentration of 500 mg a.s./kg

food, adult emergence was 47.9%, which was statistically significantly lower than in the control (Cochran-Armitage test, one sided greater, $\alpha = 0.05$).

Based on the results for adult emergence at day 22, the EC_{50} was determined to be 487 mg a.s./kg food (95% confidence limits: 434-548 mg a.s./kg food), corresponding to an ED_{50} of 75.0 µg a.s./larva per developmental period (95% confidence limits: 66.8-84.4 µg a.s./larva per developmental period). EC_{10} and EC_{20} values and their corresponding ED_{10} and ED_{20} values could not be determined due to the lack of a clear dose-response relationship. The NOEC was determined at 250 mg a.s./kg food, equivalent to an NOED of 38.5 µg a.s./larva per developmental period. The LOEC corresponded with the highest test concentration of 500 mg a.s./kg food, equivalent to an NOED of 77.0 µg a.s./larva per developmental period.

During the assessments of mortality and emergence, no other test item related observations such as deviating sizes, appearances and malformations of the test organisms were made.

On day 8, uneaten food was observed in the three highest test item groups of 125, 250 and 500 mg a.s./kg food.

The actual concentrations of trinexapac-ethyl in the lowest and highest test item solutions were equivalent to recoveries between 97% and 108% of nominal. Therefore, the concentrations of the test item solutions were confirmed and the endpoints were based on nominal concentrations. No residues of trinexapac-ethyl were found in the solvent samples, i.e. they were below the limit of detection (LOD, 10.4 mg trinexapac ethyl/L).

The validity of the test was fulfilled since cumulative larval mortality from day 4 to day 8 in the control was 0.0% (required ≤ 15%), adult emergence on day 22 in the control was 85.4% (required ≥ 70%) and larval mortality on day 8 for the reference item was 100% (required ≥ 50%).

Table A 2.3.1.3.1-1: Effects of AG-T3-175 EC1 on honey bee larval mortality, pupal mortality and adult emergence

Treatment group	Test concentration [mg a.s./kg food]	Cumulative dose [µg a.s./larva per developmental period][a)]	Cumulative larval mortality on day 8	Cumulative larval and pupal mortality on day 15		Cumulative pupal mortality from day 8 to 22		Adult emergence on day 22[c)]	
			Actual [%]	Actual [%]	Corrected[b)] [%]	Actual [%]	Corrected[b)] [%]	Actual [%]	Inhibition *vs.* control[d)] [%]
Control	-	-	0.0	14.6	-	14.6	-	85.4	-
Test item AG-T3-175 EC1	31.3	4.82	2.1	10.4	-4.9	12.8	-2.1	85.4	0.0
	62.5	9.63	0.0	10.4	-4.9	12.5	-2.5	87.5	-2.5
	125	19.3	2.1	16.7	2.5	19.1	5.3	79.2	7.3
	250	38.5	0.0	16.7	2.5	16.7	2.5	83.3	2.5
	500	77.0	20.8*	50.0*	41.5	39.5*	29.2	47.9*	43.9
Reference item dimethoate (BAS 152 I)	48.0	7.39	100	-	-	-	-	-	-

Endpoints for day 22	
EC_{10} / ED_{10}	Not determinable due to the lack of a clear dose-response relationship
EC_{20} / ED_{20}	Not determinable due to the lack of a clear dose-response relationship
EC_{50} / ED_{50} (95% confidence limits)	487 mg a.s./kg food (434-548 mg a.s./kg food) / 75.0 µg a.s./larva per developmental period (66.8-84.4 µg a.s./larva per developmental period)
NOEC / NOED	250 mg a.s./kg food / 38.5 µg a.s./larva per developmental period
LOEC / LOED	500 mg a.s./kg food / 77.0 µg a.s./larva per developmental period

* Significantly different compared to control (cumulative larval mortality on day 8: Multiple Fisher's exact test with Bonferroni-Holm adjustment, one sided greater, $\alpha = 0.05$, cumulative larval and pupal mortality on day 15: Cochran-Armitage test, one sided greater, $\alpha = 0.05$, cumulative pupal mortality from day 8 to 22: Multiple Fisher's exact test with Bonferroni-Holm adjustment, one sided greater, $\alpha = 0.05$, adult emergence on day 22: Cochran-Armitage test, one sided greater, $\alpha = 0.05$)

a) Based on the cumulative feeding volume from day 3 until day 6 of 140 µL diet/larva and a density of the diet of 1.1 g/cm³

b) Corrected for control mortality according to the formula of Abbott (1925), modified by Schneider-Orelli (1947)

c) Statistical evaluation was performed for non-emergence.

d) Negative values indicate a higher emergence compared to the control group.

Conclusion

Based on adult emergence on day 22 in this honey bee chronic larval toxicity study with AG-T3-175 EC1, the EC_{50} was determined to be 487 mg a.s./kg food (95% confidence limits: 434-548 mg a.s./kg food), corresponding to an ED_{50} of 75.0 µg a.s./larva per developmental period (95% confidence limits: 66.8-84.4 µg a.s./larva per developmental period). EC_{10} and EC_{20} values and their corresponding ED_{10} and ED_{20} values could not be determined due to the lack of a clear dose-response relationship. The NOEC was determined at 250 mg a.s./kg food, equivalent to an NOED of 38.5 µg a.s./larva per developmental period. The validity criteria were fulfilled.

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.3.2 KCP 10.3.2 Effects on arthropods other than bees

A 2.3.2.1 Study 1: Standard Laboratory Test - Effects on *Aphidius rhopalosiphi*

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment..

Reference:	KCP 10.3.2/01
Report	AG-T3-175 EC: Toxicity of AG-T3-175 EC to Adults of the Parasitoid Wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) Under Worst-case Conditions in the Laboratory, Schmidt, T., 2009a, B93036 (report number), 90018034_000081129 (sponsor report number)
Guideline(s):	Yes, Mead-Briggs, M. A. et al. (2000): A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, Aphidius rhopalosiphi (De Stephani-Perez) (Hymenoptera: Braconidae). In: Guidelines to evaluate side-effects of plant protection products to non-target arthropods (eds. Candolfi et al., 2000), IOBC/WPRS, Gent, p. 13-25.
Deviations:	Deviations to guideline stated above: None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and Methods

A. MATERIALS

1. Test material	AG-T3-175 EC (emulsifiable concentrate formulation of the active substance trinexapac-ethyl)
Description	Liquid
Lot/Batch #	D-I0703
Purity	Nominal / analysed: 175 / 180 g/L trinexapac-ethyl
Stability of test material	Stable under storage conditions Expiry date: 31 Jan 2010
2. Vehicle and/or positive control	Vehicle/negative control: Deionised water

	Positive control: Reference item
Reference item	Roxion (containing 400 g/L dimethoate)
Description	Yellow liquid
Lot/Batch #	G339A
Purity	Nominal / analysed: 400 / 375 g/L dimethoate
Stability of reference item	Stable under storage conditions Expiry date: Dec 2015

3. Test organism

Species	Hymenopteran parasitoid wasp (*Aphidius rhopalosiphi* De Stefani-Perez)
Source	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth, Germany
Acclimation period	A cohort of wasp pupae was placed in a suitable emergence container to collect the adult wasps within 48 hours of emergence. The adult wasps were kept under the same environmental conditions as in the study.
Diet	During the acclimation period, the wasps were fed with honey. During the exposure phase, the wasps were provided with a honey-water solution (e.g. 1:3 v/v). During the 24 hours of the parasitisation phase, no food was provided.
Test units	Mortality test Two glass plates (approximately 9.9 cm in square length) were held apart by a shallow untreated squared frame (internal dimensions: approximately 9.5 x 9.5 cm and approximately 2 cm high). Three holes on each of the four sides provided sufficient air ventilation. The holes (except two) were covered by a fine-gauge mesh. One hole was left uncovered for the introduction of the parasitoids, and was later sealed with a stopper. The other hole on the opposite of the cage was connected to a water-bath using a cotton wick. The test units were ventilated with a vacuum pump, which was connected to one of the mesh-covered holes in the stainless steel casing by plastic tubing. Reproduction test Barley seedlings planted in pots (approximately 10-40 plants per pot; height *ca.* 10 cm), infested with >100 adult and/or nymphal cereal aphids (i.e., *Rhopalosiphum padi* (L.)). The soil in the pots was covered with a layer of dry sand to create a uniform surface. The barley plants were enclosed within a ventilated clear acrylic, cylindrical cage (approximately 9 cm in diameter and approximately 20 cm high). Top and side walls of the cylinder were covered with fine nylon mesh.

4. Environmental conditions	The study was performed in a temperature/humidity-controlled cabinet.
Temperature	Mortality and parasitisation phase: 18.8-21.3°C Reproduction phase: 18.8 to 20.3°C
Relative humidity	Mortality and parasitisation phase: 61.5-82.9% Reproduction phase: no regulation
Photoperiod	Mortality phase: 16:8 hours light:dark (light intensity ranged from 1200–1600 lux) Reproduction phase: 16:8 hours light:dark (light intensity ranged from 7300–9300 lux)

B. STUDY DESIGN AND METHODS

1. In-life dates 19 Oct 2008 to 03 Nov 2008

2. Experimental conditions

Test design

Mortality test
The glass plates were sprayed with the reference substance, with deoinised water (control) and with the test substance, respectively, and left to dry for approximately one hour. Afterwards, the parts of the test unit were joined together and ten adult wasps not older than 48 hours were transferred into each test unit (test start). The exposure time was 48 hours.

Reproduction test
After test substance exposure, 15 surviving female wasps were removed from each test unit and transferred individually into the reproduction test units for the assessment of fecundity. This reproduction phase was carried out with the control and the rates of the test substance in which a minimum number of 15 surviving healthy wasps (corrected mortality rate ≤ 50%) were found at the end of the exposure phase. Therefore, no reproduction phase was performed for 1143 and 2286 mL AG-T3-175 EC/ha. The reproduction phase was carried out for the treatment with 572 mL AG-T3-175 EC/ha since mortality was marginally higher than 50%. The wasps were confined with the host aphids for 24 h and removed afterwards. After 11 days, the number of parasitised aphids (mummies) was assessed.

Number of animals per treatment

Mortality test
Ten wasps (≥ 5 females)/replicate; 4 replicates/treatment

Reproduction test
One female wasps/replicate; 15 replicates/treatment (only treatment groups 143, 286 and 572 mL AG-T3-175 EC/ha)

Rates tested

The following nominal test substance rates were tested:
143, 286, 572, 1143 and 2286 mL AG-T3-175 EC/ha (corresponding to 25, 50, 100, 200 and 400 g a.s./ha.

The test included a control treatment sprayed with deionised water only.

The reference substance was tested at 20 mL Roxion/ha (corresponding to 8 g dimethoate/ha).

Treatment/Application

Prior to test start, the highest test rate of AG-T3-175 EC was prepared by mixing 5.72 mL of the test substance in 500 mL deionised water. An aliquot of this application solution was further diluted with deionised water to obtain the application solutions for the lower test rates. The application solution of the reference substance was prepared by mixing 50 µL of Roxion in 500 mL deionised water.

Adequate volumes of application solutions were sprayed onto the glass plates of each replicate by means of a track sprayer (Spray Lab from Schachtner, Germany). The sprayer was calibrated to deliver a target of 2.0 ± 0.2 mg spray solution/cm^2, corresponding to 200 L/ha, by weighing the amount of water delivered.

3. Observations and assessments

Mortality, symptoms of toxicity and reproduction were evaluated.

After approximately 2, 24 and 48 hours of exposure, mortality and symptoms of toxicity of the wasps were assessed.

The number of mummies (i.e. parasitised aphids) developed after 11 days was recorded for each individual replicate.

The temperature and the relative humidity were continuously recorded during the mortality and parasitisation phase. For the reproduction phase, only the temperature was continuously recorded. The light intensity was recorded once for each the mortality and the reproduction phase.

4. Calculation of toxicity

The mortality of wasps was calculated for each treatment as number of moribund and dead wasps combined relative to the number of wasps at test start. The mortality in the treatments was not corrected by the mortality of the control group, since no mortality occurred in the control group after 48 hours of exposure.

The mean number of mummies produced per individual wasp for each treatment and the percentage change relative to the control was calculated.

5. Statistics

The LR_{50} and its 95% confidence limits were calculated by Probit Analysis using simple linear regression.

Results and Discussion

After 48 hours of exposure (endpoint of the mortality assessment), mean overall mortality in the control and in the reference substance treatment was 0% and 100%, respectively. In the test substance treatments, mean mortality varied between 2.5% and 100% (Table A 2.3.2.1-1). The 48-hour LR_{50} was determined to be 424 mL AG-T3-175 EC/ha (95% confidence interval: 362-497 mL AG-T3-175

EC/ha). In terms of active substance trinexapac-ethyl, this corresponds to 74.5 g a.s./ha (95% confidence interval: 63.4-87.0 g a.s./ha).

The mean parasitisation success (reproduction after 11 days) of the wasps in the control was 20 mummies per female. In the test substance treatments up to 571 mL AG-T3-175 EC/ha, the mean parasitisation success of the wasps ranged between 10 and 12 mummies per female without a rate-response relationship (Table A 2.3.2.1-1). The NOER was calculated to be < 143 mL AG-T3-175 EC/ha (< 25 g a.s./ha).

The test is considered to be valid since the mortality in the control and toxic reference group was 0.0% (required ≤ 13%) and 100%, respectively, and, in addition, the cumulative number of mummies was 20 per female in the control group (required: ≥ 5 mummies per female). Furthermore, in the control treatment there was no wasp producing zero value (required: no more than two wasps producing zero values).

Table A 2.3.2.1-1: Effects of AG-T3-175 EC on mortality and reproduction of *A. rhopalosiphi* in a standard laboratory test

Treatment		Mortality after 48 hours of exposure [%]	Reproduction after 11 days [number of mummies per female]	
		Mean ± SD	Mean ± SD	% of control
Control		0.0 ± 0.0	20 ± 11.7	-
Test substance				
[mL product/ha]	[g a.s./ha]			
143	25	2.5 ± 5.0	10 ± 8.4	52.4
286	50	38 ± 5.0	12 ± 9.0	59.1
572	100	58 ± 15	12 ± 4.6	62.5
1143	200	95 ± 5.8	n.a.	n.a.
2286	400	100 ± 0.0	n.a.	n.a.
Reference substance				
[mL product/ha]	[g a.s./ha]			
20	8	100 ± 0.0	n.a.	

Note Reproduction was not performed for 1143 and 2286 mL AG-T3-175 EC/ha since mortality after 48 hours of exposure was > 90%.
SD Standard deviation
n.a. Not applicable

Conclusion

Under worst-case laboratory conditions (artificial substrate), the LR_{50} of AG-T3-175 EC for the parasitoid wasp, *Aphidius rhopalosiphi*, was 424 mL product/ha (74.5 g a.s./ha). The NOER for reproduction was calculated to be < 143 mL product/ha (< 25 g a.s./ha). All validity criteria were met.

A2.3.2.2 Study 2: Standard Laboratory Test - Effects on *Typhlodromus pyri*

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.2/02
Report	AG-T3-175 EC: Toxicity of AG-T3-175 EC to the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae) under Worst-Case Laboratory Conditions, Jeker, L., 2008b, B92970 (report number), 90018035_000081130 (sponsor report number)
Guideline(s):	Blümel, S. *et al*. (2000): Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products. In: Guidelines to evaluate side-effects of plant protection products to non-target arthropods (eds. Candolfi, M. P. *et al*.). IOBC/WPRS, Gent, p. 121-143. The reproductive efficiency of the mites was not investigated since this parameter is not required for the Tier 1 risk assessment following the ESCORT 2 evaluation scheme.
Deviations:	Deviations to guideline stated above: None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and Methods

A. MATERIALS

1. Test material	AG-T3-175 EC (emulsifiable concentrate formulation of the active substance trinexapac-ethyl)
Description	Yellow – red brown liquid
Lot/Batch #	D-I0703
Purity	Nominal / analysed: 175 / 180 g/L trinexapac-ethyl
Stability of test material	Stable under storage conditions Expiry date: 31 Jan 2010
2. Vehicle and/or positive control	Vehicle/negative control: Deionised water Positive control: Reference item
Reference item	Roxion (containing 400 g/L dimethoate)
Description	Yellow liquid
Lot/Batch #	G339A
Purity	Nominal / analysed: 400 / 375 g/L dimethoate
Stability of reference item	Stable under storage conditions Expiry date: Dec 2015
3. Test organism	
Species	Predatory mite (*Typhlodromus pyri* SCHEUTEN), protonymphs ≤ 24 hours old

Source	Breeding stock at the test site; originally supplied by Syngenta Crop Protection AG, 4002 Basel, Switzerland
Acclimation period	A synchronised cohort of protonymphs was produced starting with eggs from the culture that had been produced during a 24-hour period. The eggs were maintained under equivalent conditions as used for the test until hatch of protonymphs (approximately four days). The test was started not later than 24 hours after moulting of the larvae.
Diet	The mites were fed with a 1:1 mixture of walnut (*Juglans regia*) and apple (*Malus vulgaris*) pollen at day 0 (after application) and day 3.
Test units	Test units consisted of two glass cover slides (approximately 24 x 50 mm) fixed longitudinally together with the gap small enough to prevent mites from escaping. A barrier of non-drying glue gel formed the boundary of the actual test arena (approximately 10-13 cm^2) on the slides. After the spray deposits had dried, each test unit was placed on a wet filter paper that laid on top of a foam fitting into the perforated lower part of a plastic Petri dish. The Petri dish was closed with a lid containing a fine gaze for ventilation, and was placed in a plastic dish filled partly with water to keep the foam soaked.
4. Environmental conditions	The study was performed in a temperature/humidity-controlled cabinet.
Temperature	24.3-25.6°C
Relative humidity	76 ± 3.6% The humidity dropped twice below 60% during assessment. However, this short-term deviations in humidity are not considered to have any influence on biological results, as documented by the acceptable performance of mites in the control.
Photoperiod	16:8 hours light:dark (light intensity ranged from 1120-1320 lux)

B. STUDY DESIGN AND METHODS

1. In-life dates	03 Nov 2008 to 25 Nov 2008

2. Experimental conditions

Test design

Lethal effects of the test substance on the predatory mite *Typhlodromus pyri* were assessed in a multiple rate test under worst-case laboratory conditions. The test organisms were exposure via contact to dry residues on glass plates (artificial substrate) for 7 days. Mortality was assessed after 3 and 7 days.

Number of animals per treatment

Twenty protonymphs/replicate; four replicates/treatment

Rates tested

The tested rates were 0.2125, 0.425, 0.85, 1.7, and 3.4 L AG-T3-175 EC/ha corresponding to 0.0375, 0.075, 0.15, 0.3 and 0.6 kg a.s./ha.

A control group was exposed to residues of deionised water.

The reference substance Roxion (400 g/L dimethoate) was sprayed at a rate equivalent to 16 mL product/ha (6.4 g a.s./ha).

Treatment/Application

Prior to the start of the test, the highest test rate of AG-T3-175 EC was prepared by mixing 8.5 mL of test substance in 500 mL deionised water. An aliquot of this application solution was further diluted with deionised water to obtain the application solutions of the lower test rates.

The application solution of the reference substance was prepared by making up 40µL in 500 mL deionised water.

The control test units were sprayed with deionised water only.

Appropriate volumes of the application solutions were sprayed onto the glass plates of each replicate by means of an adequate spraying apparatus (i.e. Spray Lab from Schlachtner, Germany). Prior to the application, the sprayer had been calibrated to deliver 2.0 ± 0.2 mg spray solution/cm^2 (equivalent to 200 L/ha), and the spray pattern had been visually checked. After treatment, the test units were left to air-dry and then 20 protonymphs were transferred to each test arena using a fine pointed brush (start of the test). The transfer of the organisms to the test units was completed within approximately 100 minutes after the application when the spray deposits had dried.

3. Observations and assessments

The number of surviving mites and mite mortality was assessed on day 3 and 7 after application.

The temperature and the relative humidity were continuously recorded. The light intensity was recorded once during the test.

4. Calculation of toxicity

Mortality was calculated by adding the number of mites which had escaped (i.e. “escapees”) to the number of those which had died. Escapees were calculated as the sum of those mites which could not be found on the test arena, those which were stuck in the glue barrier and those which were found to be drowned in the water supply.
Mortality was corrected using the formula of Abbott (1925) with improvements by Schneider-Orelli (1947).

5. Statistics

The LR_{50} and its 95% confidence interval were calculated by Probit analysis.

Results and Discussion

After 7 days of exposure (endpoint of the mortality assessment), the mean mortality values in the control and in the reference substance treatment were 6.3% and 100%, respectively. The mean corrected mortality in the test substance treatments ranged from 1.3% to 100% (Table A 2.3.2.2-1). The 7-day LR_{50} was determined to be 0.703 L AG-T3-175 EC/ha (95% confidence interval: 0.210-2.36 L AG-T3-175 EC/ha). This corresponds to 0.124 kg a.s./ha (95% confidence interval: 0.037-0.417 kg a.s./ha).

The test is considered to be valid since mean mortality in the control and toxic reference group was 6.3% (required ≤ 20%) and 100% (required 50 - 100%), respectively.

Table A 2.3.2.2-1: Mortality of *Typhlodromus pyri* exposed to AG-T3-175 EC on glass plates after 7 days of exposure

Application rate		Total mortality	Corrected mortality
[L product/ha]	[kg a.s./ha]	[% ± SD]	[%]
Control	-	6.3 ± 2.5	n.a.
0.2125	0.0375	7.5 ± 5.0	1.3
0.425	0.075	35 ± 4.1	31
0.85	0.15	60 ± 14	57
1.7	0.3	88 ± 13	87
3.4	0.6	100 ± 0.0	100
Toxic reference		100 ± 0.0	100

SD Standard deviation
n.a. Not applicable

Conclusion

Under worst-case laboratory conditions (artificial substrate), the 7-day LR_{50} of AG-T3-175 EC for the predatory mite, *Typhlodromus pyri*, was determined to be 0.703 L product/ha (0.124 kg a.s./ha). All validity criteria were met.

A 2.3.2.3 Study 3: Standard Laboratory Test - Effects on *Aleochara bilineata*

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.2/03
Report	AG-T3-175 EC: Toxicity to the Rove Beetle *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae) under Worst-Case Laboratory Conditions, Schmidt, T., 2009b, B92913 (report number), 90018036_000081131 (sponsor report number)
Guideline(s):	Grimm, C. et al. (2000): A test for evaluating the chronic effects of plant protection products on the rove beetle *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae) under laboratory and extended laboratory conditions. In: Candolfi M.P. et al. (eds): Guidelines to evaluate side-effects of plant protection products to non-target arthropods. pp 1-12, IOBC/WPRS, Gent.
Deviations:	Deviations to guideline stated above: None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and Methods

A. MATERIALS

1. Test material	AG-T3-175 EC (emulsifiable concentrate formulation of the active substance trinexapac-ethyl)
Description	Liquid
Lot/Batch #	D-I0703
Purity	Nominal / analysed: 175 / 180 g/L trinexapac-ethyl
Stability of test material	Stable under storage conditions Expiry date: 31 Jan 2010
2. Vehicle and/or positive control	Vehicle/negative control: Deionised water Positive control: Reference item
Reference item	Roxion (containing 400 g/L dimethoate)
Description	Yellow liquid
Lot/Batch #	G339A
Purity	Nominal / analysed: 400 / 375 g/L dimethoate
Stability of reference item	Stable under storage conditions Expiry date: Dec 2015
3. Test organism	
Species	Parasitoid rove beetle *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae)
Source	Onion fly pupae parasitised by *A. bilineata* were obtained from a commercial supplier (De groene vlieg, Duivenwaardsedijk 1, NL-3244 Lg Nieuwe Tonge, Netherlands).

Acclimation period	The beetles were acclimatised to the test conditions from their emergence on until test start. Adults within 8 days of their emergence were used for the test.
Diet	Defrosted yellow mealworm larvae and red mosquito larvae were fed during the test and food was replaced twice a week when water was added. One, two and three weeks after application, approximately 500 onion fly pupae per test unit were carefully mixed into the substrate as hosts for the beetle larvae.
Test units	Exposure phase The exposure test units consisted of a plastic container (16 cm x 11 cm x 6 cm) covered with a lid that supported a rough nylon gauze (mesh size: 0.18 mm). Per replicate, 1000 g of wetted substrate (quartz sand) were filled into each test unit resulting in a substrate depth of approximately 4 cm. Hatching phase The hatching test units consisted of two plastic containers (16 cm x 12 cm x 5 cm) placed into one another. A gauze (mesh size: 1.8 mm) was fitted into the bottom of the inner container, onto which the parasitised pupae from the exposure phase were placed. Consequently, hatching beetles fell through the gauze into the outer container where they could be easily counted and discarded afterwards. The hatching test units were covered with lids with the same fine nylon gauze as used in the exposure phase.
4. Environmental conditions	The study was performed in a temperature/humidity-controlled cabinet.
Temperature	18.9-20.3°C (mean: 19.6°C)
Relative humidity	49.3-85.1% (mean: 72.6%) The humidity dropped at seven short-term occasions below 60%. However, these deviations are not considered to have an influence on the biological results, as documented by the acceptable performance of the beetles in the control.
Photoperiod	16:8 hours light:dark (light intensity ranged from 990 to 1370 lux, measured at test unit level)

B. STUDY DESIGN AND METHODS

1. In-life dates	25 Jul 2008 to 09 Oct 2008

2. Experimental conditions

Test design

The test consisted of an exposure phase of 28 days for the adults and, after the removal of the adults, a hatching phase of additional 48 days for the new generation of beetles emerging from the parasitised fly pupae.

The substrate (quartz sand) was sprayed with the test substance, the reference substance or deionised water (control). After the spray deposits had dried, the pre-selected beetles were transferred into each test unit (start of the test).

After 28 days of exposure, surviving beetles were removed from the test units. Afterwards, the test units were left to dry for one week. After 35 days, parasitised fly pupae were regained from the substrate by sieving of the substrate and transferring into the hatching test units.

Number of animals per treatment

Exposure phase
Twenty beetles (ten males and ten females)/replicate; 4 replicates/treatment

Hatching phase
All beetles emerging from the parasitised fly pupae/replicate; 4 replicates/treatment

Rates tested

The tested rates were 285.75, 571.5, 1143, 2286 and 4572 mL AG-T3-175 EC/ha (corresponding to 50, 100, 200, 400 and 800 g a.s./ha)

A control group was exposed to deionised water only.

Roxion (400 g/L dimethoate) was sprayed at a rate equivalent to 2200 mL product/ha (880 g a.s./ha).

Treatment/Application

Prior to test start, the highest test rate was prepared by mixing 5.715 mL of test substance in 500 mL deionised water. An aliquot of this stock solution was further diluted with deionised water to obtain the application solutions for the lower test rates.

The application solution of the toxic reference standard was prepared by mixing 2.75 mL of Roxion in 500 mL deionised water.

The control test units were sprayed with deionised water only.

Appropriate volumes of the application solutions were sprayed onto the substrate of each replicate by means of an adequate spraying apparatus (i.e. Spray Lab from Schachtner, Germany). The sprayer was calibrated to deliver a target of 4.0 ± 0.4 mg spray solution/cm^2, corresponding to 400 L/ha, by weighing the amount of water delivered.

3. Observations and assessments

After 28 days, the adult beetles were removed and the number of alive and dead beetles was assessed. Missing beetles were recorded as dead.

The number of hatching beetles (i.e. parasitised fly pupae) was recorded for each replicate. Hatching of the beetles was monitored every 1 to 3 days until less than two beetles hatched per replicate in the control treatment per day.

The temperature and the relative humidity were continuously recorded. The light intensity was recorded once during the test (at test start).

4. Calculation of toxicity

The ER_{50} and its 95% confidence limits could not be calculated since reproduction was not reduced > 50% in all test substance treatments. Therefore, the EC_{50} for reproduction was determined directly from the raw data.

5. Statistics

Differences between the test substance treatments and the control were tested with the Student-t test with Bonferroni-correction.

Results and Discussion

After 28 days of exposure, mean mortality was 15% in the control and 99% in the reference substance treatment. Mean mortality in the test substance treatments ranged from 20% to 33% without a rate-response relationship (Table A 2.3.2.3-1).

The mean hatching rate was 676 beetles per replicate in the control and 2.3 beetles per replicate in the reference substance treatment. The values of mean hatching rate in the test substance treatments ranged from 558 to 667 beetles per replicate (Table A 2.3.2.3-1). The hatching rates in the test substance treatments represented between 83% and 99% of the hatching rate in the control and were not statistically significantly lower. Therefore, the ER_{50} was determined to be > 4572 mL AG-T3-175 EC/ha (> 800 g a.s./ha).

The test is considered to be valid since the mean hatching rate in the control was 676 beetles (required ≥ 400 beetles) and the mean hatching rate in the reference substance treatment was reduced by 99.7% relative to the control (required ≥ 50%).

Table A 2.3.2.3-1: Effects of AG-T3-175 EC on mortality and hatching rate of *Aleochara bilineata* Gyll. in a standard laboratory test

Treatment		Mortality [%]	Hatching rate [number of beetles emerging]	
		Mean ± SD	Mean ± SD	% of control
control		15 ± 9.1	676 ± 309	-
test substance				
[mL product/ha]	[g a.s./ha]			
286	50	33 ± 18	558 ± 109	83
572	100	31 ± 11	667 ± 77	99
1143	200	20 ± 7.1	592 ± 83	87
2286	400	26 ± 8.5	632 ± 70	93
4572	800	30 ± 10	643 ± 73	95
reference substance				
[mL product/ha]	[g a.s./ha]			
2200	880	99 ± 2.5	2.3 ± 3.2	0.3

SD Standard deviation

Conclusion

Under worst-case laboratory conditions, the ER_{50} for reproduction (measured as hatching rate) of *Aleochara bilineata* treated with AG-T3-175 EC was determined to be > 4572 mL product/ha (> 800 g a.s./ha). All validity criteria were met.

A 2.3.2.4 Study 4: Standard Laboratory Test - Effects on *Coccinella septempunctata*

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.2/04
Report	AG-T3-175 EC: Toxicity to Larvae of the Seven-Spotted Ladybird *Coccinella septempunctata* (Coleoptera: Coccinellidae) under Worst-Case Laboratory Conditions, Jeker, L., 2009a, B93025 (report number), 90018037_000081132 (sponsor report number)
Guideline(s):	Schmuck, R. et al. (2000): A laboratory test system for assessing effects of plant protection products on the plant-dwelling insect Coccinella septempunctata L. (Coleoptera: Coccinellidae). In: Guidelines to evaluate side-effects of plant protection products to non-target arthropods (eds. Candolfi et al. 2000), IOBC/WRPS, Gent, p. 45-56.
Deviations:	Deviations to guideline stated above: None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and Methods

A. MATERIALS

1. Test material	AG-T3-175 EC (emulsifiable concentrate formulation of the active substance trinexapac-ethyl)
Description	Yellow – red brown liquid
Lot/Batch #	D-I0703
Purity	Nominal / analysed: 175 / 180 g/L trinexapac-ethyl
Stability of test material	Stable under storage conditions Expiry date: 31 Jan 2010
2. Vehicle and/or positive control	Vehicle/negative control: Deionised water Positive control: Reference item
Reference item	Roxion (containing 400 g/L dimethoate)
Description	Yellow liquid
Lot/Batch #	G339A
Purity	Nominal / analysed: 400 / 375 g/L dimethoate

Stability of reference item	Stable under storage conditions Expiry date: Dec 2015

3. Test organism

Species	Seven-spotted ladybird *Coccinella septempunctata* L. Second instars larvae were used for testing.
Source	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth, Germany
Acclimation period	Eggs from a synchronized cohort were left undisturbed until the larvae had developed to second instars. Larvae were then transferred to small plastic vessels, where they were kept individually under test conditions until start of the test.
Diet	Green peach aphids *Myzus persicae* and pea aphids *Acyrthosiphon pisum, ad libitum* Reproduction phase: Food supply for adult beetles: Pea seedlings infested with *M. persicae*, and with *A. Pisum*; Additional feeding with pollen was carried out regularly in order to increase egg production.
Test units	Mortality test Each test unit consisted of a glass plate (40 × 18 × 0.6 cm) with a second plate of the same size with 10 holes (diameter 5.5 cm) placed on top. Hollow glass cylinders (outside diameter 5.2 cm, inside diameter 4.7 cm, height 4 cm, inner walls coated with talcum) were placed into each hole to confine the *C. septempunctata* larvae. On top, the glass cylinders were sealed with fine mesh gauze (0.2 to 0.4 mm netting). Reproduction test The test units consisted of transparent plastic containers (approx. 15.5 cm high and 11 cm diameter, volume approx. 1 L) sealed on top with a fine mesh. Two paper tissues (approx. 20 cm × 20 cm) and one plastic tube were placed inside the units as egg laying substrate. Egg clutches collected from the different treatments were held in separate Petri dishes to assess the hatching rate.

4. Environmental conditions

Temperature	24.8±0.46°C The temperature dropped below 23°C once during assessment. However, this short-term deviation in temperature was not considered to have an influence on biological results, as documented by the acceptable performance of beetles in the control.
Relative humidity	77±3.2% The humidity dropped below 60% on three occasions during assessment. However, these short-term deviations in humidity were not considered to have an influence on biological results, as documented by the acceptable performance of beetles in the control.
Photoperiod	16:8 hours light:dark (light intensity ranged from 1750 to 3400 lux, measured at test unit level)

B. STUDY DESIGN AND METHODS

1. In-life dates 27 Oct 2008 (start of the first test which was repeated since the test substance rates were wrongly calculated) to 22 Jan 2009

2. Experimental conditions

Test design

Mortality test
Second instars larvae of *C. septempunctata* were exposed to dried residues of the test substance, reference substance or deionised water (control) sprayed onto glass plates. The duration of the mortality phase was 21 days.

Reproduction test
On day 16 after application, at least 90% of the viable pupae had hatched in the control and the test substance treatment groups and beetles in the control started to lay eggs 10 days later. Seven days later, the assessment of the reproduction performance was initiated. All beetles were sexed and pooled within their respective treatment groups and placed into the reproduction test units.

The number of reproduction units depended on the number of hatched adults and the sex ratio. For the control and the test substance treatments up to and including 0.218 L/ha, four reproduction units were set up. For the test substance treatments of 0.544 L/ha and 1.36 L/ha, only two reproduction units, and for the highest test substance treatment of 3.4 L/ha, only one reproduction unit was set up. The reproduction phase lasted 14 days.

Number of animals per treatment

Mortality test
40 individual larvae (4 glass plates with 10 larvae each)/treatment

Reproduction test
Up to 4 replicates/treatment, containing all hatched pupae of the treatment group (a similar sex ratio in all reproduction units was aimed for)

Rates tested

The following nominal test substance rates were tested:
0.087, 0.218, 0.544, 1.36 and 3.4 L AG-T3-175 EC/ha (corresponding to 0.0154, 0.0384, 0.096, 0.24 and 0.6 kg a.s./ha).

A control group was exposed to residues of deionised water.

The reference substance (positive control) Roxion was tested at 45 mL product/ha.

Treatment/Application

The test substance was dissolved in aqueous application solutions. Prior to test start, the highest test rate of the test substance AG-T3-175 EC was prepared by mixing 8.5 mL of the test substance in 500 mL deionised water. An aliquot of this application solution was further diluted with deionised water to obtain the application solutions for the lower test rates.

The application solution of the reference substance was prepared by making up 113 µL in 500 mL with deionised water.

The control test units were sprayed with deionised water only.

Appropriate volumes of the application solutions were sprayed onto the glass plates of each replicate by means of an adequate spraying apparatus (i.e. Spray Lab from Schlachtner, Germany). Prior to the application, the sprayer had been calibrated to deliver 2.0 ± 0.2 mg spray solution/cm^2 (equivalent to 200 L/ha), and the spray pattern had been visually checked.

3. Observations and assessments

Total pre-imaginal mortality of *C. septempunctata* during the mortality phase was assessed three times per week and larval mortality, pupation as well as adult hatching were recorded. Additionally, any behavioural abnormalities of the larvae and abnormal appearance of the larvae, pupae or adults were noted.

The assessment of the reproductive performance started one week after the control beetles started to lay eggs. Over a period of two weeks, all eggs deposited on the tissue papers and plastic tubes were collected daily (except on weekends) and checked for fertility (larvae hatch). Additionally, sex-specific development of the adults was assessed.

4. Calculation of toxicity

Pre-imaginal mortality was calculated for each treatment as sum of dead larvae, dead pupae and adults dying during emergence. Mortality in the treatments was corrected for any losses in the control group according to the method of Abbott (1925) with improvements by Schneider-Orelli (1947).

The mean number of eggs laid per female beetle per day was determined by dividing the total number of eggs laid within each treatment group by the mean number of viable females in that treatment group (corrected for mortality during egg laying). In addition, the number of fertile eggs was assessed from the larval hatch.

5. Statistics

Due to the over all low mortality, the LR_{50} could not be calculated and was thus determined directly from the raw data.

Because of to the species inherent variability in egg-laying performance, fertility was evaluated only qualitatively, according to the test guideline.

Results and Discussion

After 21 days of exposure (endpoint of the mortality assessment), the mean pre-imaginal mortality values in the control and in the reference substance treatment were 23% and 100%, respectively. The mean corrected pre-imaginal mortality in the test substance treatments ranged between 13% and 48% (Table A 2.3.2.4-1). Due to the overall low mortality, the LR_{50} (pre-imaginal and imaginal) could not be calculated and was thus determined directly from the raw data to be > 3.4 L AG-T3-175 EC/ha (> 0.6 kg a.s./ha).

The mean egg production of females in the control was 14 fertile eggs per female per day. The mean egg production of females in the test substance treatments ranged from 3.9 to 13 fertile eggs per female per day (Table A 2.3.2.4-1). All test substance treatment rates were within the control validity criteria for reproduction (i.e. 2 fertile eggs per female per day). Hence, no test substance related effect on reproduction was observed.

The test is considered to be valid since the mean pre-imaginal mortality in the control and toxic reference group was 23% (required ≤ 30%) and 100% (required > 40%), respectively. Additionally in

the control group, fertility was 14 fertile eggs per female per day (required ≥ 2 fertile eggs per female per day).

Table A 2.3.2.4-1: Toxicity of AG-T3-175 EC to the seven-spotted ladybird *C. septempunctata* in a standard laboratory test

Treatment		Larvae mortality [%]		Pre-imaginal mortality (larvae and pupae) [%]		Fertility (number of hatched eggs per female per day)
		Mean	Corrected	Mean	Corrected	Mean ± SD
Control		10	-	23	-	14 ± 11
Test substance						
[L product/ha]	[kg a.s./ha]					
0.087	0.0154	25	17	33	13	11 ± 8.8
0.218	0.0384	25	17	33	13	10 ± 8.8
0.544	0.096	43	37	60	48	6.3 ± 5.5
1.36	0.24	35	28	48	32	13 ± 11
3.4	0.6	40	33	60	48	3.9 ± 3.5
Reference substance						
[mL product/ha]	[kg a.s./ha]					
0.045	0.018	100	n.a.	100	n.a.	n.a.

n.a. Not applicable
SD Standard deviation

Conclusion

Under worst-case laboratory conditions, the LR_{50} (pre-imaginal and imaginal) of AG-T3-175 EC for the seven-spotted ladybird *C. septempunctata* was determined to be > 3.4 L product/ha (> 0.6 kg a.s./ha). No test substance related effect on reproduction was observed. All validity criteria were met.

A 2.3.2.5 Study 5: Extended Laboratory Test - Effects on *Aphidius rhopalosiphi*

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.2/05
Report	AG-T3-175 EC: Toxicity of AG-T3-175 EC to Adults of the Parasitoid Wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) Under Extended Conditions in the Laboratory, Schmidt, T., 2009c, B93047 (report number), 90018038_000081133 (sponsor report number)
Guideline(s):	Mead-Briggs, M. A. et al. (2000): A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, Aphidius rhopalosiphi (De Stephani-Perez) (Hymenoptera: Braconidae). In: Guidelines to evaluate side-effects of plant protection products to non-target arthropods (eds. Candolfi et al., 2000), IOBC/WPRS, Gent, p. 13-25.
Deviations:	Deviations to guideline "Mead-Brigs, M.A. et al. (2009): An extended laboratory test for evaluating the effects of plant protection products on the eparasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae), BioControl, DOI 10.1007/s10526-009-926-7": None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and Methods

A. MATERIALS

1. Test material	AG-T3-175 EC (emulsifiable concentrate formulation of the active substance trinexapac-ethyl)
Description	Liquid
Lot/Batch #	D-I0703
Purity	Nominal / analysed: 175 / 180 g/L trinexapac-ethyl
Stability of test material	Stable under storage conditions Expiry date: 31 Jan 2010
2. Vehicle and/or positive control	Vehicle/negative control: Deionised water Positive control: Reference item
Reference item	Roxion (containing 400 g/L dimethoate)
Description	Yellow liquid
Lot/Batch #	G339A
Purity	Nominal / analysed: 400 / 375 g/L dimethoate
Stability of reference item	Stable under storage conditions Expiry date: Dec 2015
3. Test organism	
Species	Hymenopteran parasitoid wasp (*Aphidius rhopalosiphi* De Stefani-Perez)

Source	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth, Germany
Acclimation period	A cohort of wasp pupae was placed in a suitable emergence container to collect the adult wasps within 48 hours of emergence. The adult wasps were kept under the same environmental conditions as in the study.
Diet	During the acclimation period, the wasps were fed with honey. During the exposure phase (48 hours), the wasps were provided with a fructose solution (10% w/v in water), sprayed on the barley plants. During the 24 hours of the parasitisation phase, no food was provided.
Test units	Mortality test The exposure test units for mortality assessment consisted of planted barley seedlings (approx. 8-10 plants per pot, at the 2nd leaf growth stage), trimmed to a uniform height of 10 cm before spraying. Before treatment, plants were sprayed with a fructose solution. Afterwards, the soil in the plots was covered with a layer of dry sand. After application, the treated plants were enclosed within a ventilated clear acrylic cylinder (approximately 9 cm in diameter and 20 cm high) as soon as the spray residue had dried on the plants. Top and side walls of the cylinder were covered with fine nylon mesh. Reproduction test The reproduction phase was carried out in identical test units as for the mortality phase except that plants were untreated (no fructose solution, no test substance). Per treatment, 15 pots of 10-40 barley seedlings, infested with >100 adult and/or nymphal cereal aphids (i.e., *Rhopalosiphum padi* (L.)) were prepared.
4. Environmental conditions	The study was performed in a temperature/humidity-controlled cabinet.
Temperature	Mortality and parasitisation phase: 19.3-20.3°C Reproduction phase: 18.9 to 20.6°C
Relative humidity	Mortality and parasitisation phase: 68-83% Reproduction phase: no regulation
Photoperiod	Mortality phase: 16:8 hours light:dark (light intensity ranged from 1500–2100 lux) Reproduction phase: 16:8 hours light:dark (light intensity ranged from 7300–9100 lux)

B. STUDY DESIGN AND METHODS

1. In-life dates	28 Oct 2008 to 10 Nov 2008
2. Experimental conditions	

Test design

Mortality test
The barley plants were sprayed with the reference substance, with deoinised water (control) and with the test substance at five doses. Female wasps were exposed to the treated barley plants for 48 hours.

Reproduction test
After test substance exposure, 15 surviving female wasps were removed from each test unit and transferred individually into the reproduction test units for the assessment of fecundity. This reproduction phase was carried out with the control and the rates of the test substance in which a minimum number of 15 surviving healthy wasps (corrected mortality rate ≤ 50%) were found at the end of the exposure phase, i.e. with all test substance rates. The wasps were confined with the host aphids for 24 h and removed afterwards. After 12 days, the number of parasitised aphids (mummies) was assessed.

Number of animals per treatment

Mortality test
Five female wasps/replicate; six replicates/treatment

Reproduction test
One female wasps/replicate; 15 replicates/treatment

Rates tested

The following nominal test substance rates were tested:
1446, 2893, 5786, 11571 and 23143 mL AG-T3-175 EC/ha (corresponding to 253.1, 506.3, 1012.5, 2025 and 4050 g a.s./ha based on a content of 175 g a.s./L product)

The test included a control treatment sprayed with deionised water only.

The reference substance Roxion was tested at 20 mL product/ha (corresponding to 8 g dimethoate/ha).

Treatment/Application

Prior to test start, the highest test rate of AG-T3-175 EC was prepared by mixing 28.93 mL of the test substance in 500 mL deionised water. An aliquot of this application solution was further diluted with deionised water to obtain the application solutions for the lower test rates. The application solution of the reference substance was prepared by mixing 50 µL of Roxion in 500 mL deionised water.

Adequate volumes of application solutions were sprayed onto the barley plants of each replicate by means of a track sprayer (Spray Lab from Schachtner, Germany). The sprayer was calibrated to deliver a target of 4.0 ± 0.4 mg spray solution/cm^2, corresponding to 400 L/ha, by weighing the amount of water delivered.

3. Observations and assessments

During the initial 3 hours of exposure, the number of wasps settling on treated plants was assessed to evaluate potential repellent effects of the test substance.

After approximately 2, 24 and 48 hours of exposure, mortality and symptoms of toxicity of the wasps were assessed.

The number of mummies (i.e. parasitised aphids) developed after 12 days was recorded for each individual replicate.

The temperature and the relative humidity were continuously recorded during the mortality and parasitisation phase. For the reproduction phase, only the temperature was continuously recorded. The light intensity was recorded once for each the mortality and the reproduction phase.

4. Calculation of toxicity

The mortality of wasps was calculated for each treatment as number of moribund and dead wasps combined relative to the number of wasps at test start. The mortality in the treatments was not corrected by the mortality of the control group, since no mortality occurred in the control group after 48 hours of exposure.

The mean number of mummies produced per individual wasp for each treatment and the percentage change relative to the control was calculated.

5. Statistics

Due to the low mortality in the test substance treatments and due to the low effects of the test substance on reproduction, the LR_{50} and ER_{50} were determined directly from the raw data.

Results and Discussion

During the initial 3 hours of exposure, an average of 76% and 42% of the wasps in the control and reference substance treatment, respectively, was found on the plants. In the test substance treatments, 43% to 50% of the wasps were observed on the plants indicating to a repellent effect of the test substance (Table A 2.3.2.5-1).

After 48 hours of exposure (endpoint of the mortality assessment), mean overall mortality in the control and in the reference substance treatment was 0% and 100%, respectively. In the test substance treatments, mean mortality varied between 0% and 10% (Table A 2.3.2.5-1). Therefore, the 48-hour LR_{50} was determined to be > 23143 mL AG-T3-175 EC/ha (> 4050 g a.s./ha).

The mean parasitisation success (reproduction after 12 days) of the wasps in the control was 8 mummies per female. In the test substance treatments, the mean parasitisation success of the wasps ranged between 7 and 12 mummies per female without a rate-response relationship (Table A 2.3.2.5-1). Therefore, the 48-hour ER_{50} for reproduction was determined to be > 23143 mL AG-T3-175 EC/ha (> 4050 g a.s./ha).

The test is considered to be valid since (corrected) mortality in the control and toxic reference group was 0.0% (required ≤ 10%) and 100% (required > 50%), respectively, and, in addition, the cumulative number of mummies was 8 mummies per female in the control group (required ≥ 5 mummies per female). Furthermore, in the control treatment there was one wasp producing zero value (required: no more than two wasps producing zero values).

Table A 2.3.2.5-1: Toxicity of AG-T3-175 EC to the parasitoid wasp *Aphidius rhopalosiphi* in an extended laboratory test

Treatment		Residence of females on treated barley plants [%]		Mortality after 48 hours of exposure [%]	Reproduction after 12 days [number of mummies per female]	
		Mean ± SD	% of control	Mean ± SD	Mean ± SD	% of control
control		76 ± 8.4	-	0.0 ± 0.0	8 ± 3.8	-
test substance						
[mL product/ha]	[g a.s./ha]					
1446	253.1	50 ± 8.7	66	0.0 ± 0.0	8 ± 8.8	97
2893	506.3	46 ± 7.5	61	0.0 ± 0.0	7 ± 4.3	90
5786	1012.5	42 ± 9.7	55	0.0 ± 0.0	9 ± 3.8	110
11571	2025	45 ± 4.8	60	3.3 ± 8.2	7 ± 4.4	85
23143	4050	43 ± 8.9	57	10 ± 11	12 ± 8.6	150
reference substance						
[mL product/ha]	[g a.s./ha]					
20	8	42 ± 7.9	55	100 ± 0.0	n.a.	

n.a. Not applicable
SD Standard deviation

Conclusion

Under extended laboratory conditions (i.e. treated barley plants as substrate), the LR_{50} for mortality after 48 hours and the ER_{50} for reproduction at day 12 of AG-T3-175 EC for the parasitoid wasp, *Aphidius rhopalosiphi*, were determined to be > 23143 mL product/ha (> 4050 g a.s./ha).

A 2.3.2.6 Study 6: Extended Laboratory Test - Effects on *Typhlodromus pyri*

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable for the risk assessment.

Reference:	KCP 10.3.2/06
Report	AG-T3-175 EC: Toxicity of AG-T3-175 EC to the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae) under Extended Laboratory Conditions, Jeker, L., 2009b, B92968 (report number), 90018039_000081134 (sponsor report number)
Guideline(s):	Blümel, S. *et al*. (2000): Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products. In: Guidelines to evaluate side-effects of plant protection products to non-target arthropods (eds. Candolfi, M. P. *et al*.). IOBC/WPRS, Gent, p. 121-143. Oomen, P. A. (1988): Guideline for the evaluation of side-effects of pesticides on *Phytoseiulus persimilis* A.-H.IOBC/wprs Bulletin XI/4: 51-63.
Deviations:	Deviations to Blümel, S. *et al*. (2000): None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and Methods

A. MATERIALS

1. Test material	AG-T3-175 EC (emulsifiable concentrate formulation of the active substance trinexapac-ethyl)
Description	Yellow – red brown liquid
Lot/Batch #	D-I0703
Purity	Nominal / analysed: 175 / 180 g/L trinexapac-ethyl
Stability of test material	Stable under storage conditions Expiry date: 31 Jan 2010
2. Vehicle and/or positive control	Vehicle/negative control: Deionised water Positive control: Reference item
Reference item	Roxion (containing 400 g/L dimethoate)
Description	Yellow liquid
Lot/Batch #	G339A
Purity	Nominal / analysed: 400 / 375 g/L dimethoate
Stability of reference item	Stable under storage conditions Expiry date: Dec 2015

3. Test organism

Species	Predatory mite (*Typhlodromus pyri* SCHEUTEN), protonymphs ≤ 24 h old
Source	Breeding stock at the test site
Acclimation period	A synchronised cohort of protonymphs was produced starting with eggs from the culture that had been produced during a 24-hour period. The eggs were maintained under equivalent conditions as used for the test until hatch of protonymphs (approximately four days). The test was started not later than 24 hours after moulting of the larvae.
Diet	The mites were fed with a 1:1 mixture of walnut (*Juglans regia*) and apple (*Malus vulgaris*) pollen at day 0 (after application) and days 3, 7, 10, and 13.
Test units	Per test unit, a French bean leaf disk (*Phaseolus vulgaris* L., Fabaceae; diameter approx. 5 cm) was positioned on top of a wet cotton pad in a petri dish. The outer rim of the leaf disk was coated with a barrier of non-drying glue gel. A fine forceps was used to pierce a hole into the leaf disk and a small piece of the cotton pad was pulled through, to provide a moisture source for the mites. The treatment applications were sprayed onto the upper side of the leaf disks in the assembled test units with the insect glue barrier in place. To prevent excessive loss of humidity and to protect the leaf disk, the test units (petri dishes) were covered with a lid of fine mesh.
4. Environmental conditions	The study was performed in a temperature/humidity-controlled cabinet.
Temperature	24 ± 3.6°C The temperature dropped only once below 20°C during assessment. However this deviation is not considered to have an influence on the biological results, as documented by the acceptable performance of mites in the control.
Relative humidity	72 ± 6.3% The humidity dropped only once below 60% during assessment. However this deviation is not considered to have an influence on the biological results, as documented by the acceptable performance of mites in the control.
Photoperiod	16:8 hours light:dark (light intensity ranged from 1100-1300 lux)

B. STUDY DESIGN AND METHODS

1. In-life dates	21 Nov 2008 to 09 Dec 2008

2. Experimental conditions

Test design

Lethal and sub-lethal effects of the test substance on the predatory mite *Typhlodromus pyri* were assessed in a multiple rate test under extended laboratory conditions. The test organisms were exposure via contact to dry residues on bean leaf disks for 14 days. Cumulative mortality was assessed after 7 days.

The reproduction phase was carried out with the following treatments: 0.57 and 1.14 L AG-T3-175 EC/ha. Since mortality in the treatments above 1.14 L AG-T3-175 EC/ha exceeded 50%, these treatments were ecxluded from the reproduction phase. The cumulative reproduction per female from day 7 to day 14 was assessed.

Number of animals per treatment

Ten protonymphs/replicate; six replicates/treatment

Rates tested

0.57, 1.14, 2.29, 4.57, 9.15 and 18.3 L AG-T3-175 EC/ha, corresponding to 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 kg a.s./ha

A control group was exposed to residues of deionised water.

The reference substance Roxion was tested at 20 mL product/ha (corresponding to 8 g dimethoate/ha).

Treatment/Application

Prior to the start of the test, the highest test rate of AG-T3-175 EC was prepared by mixing 45.75 mL of test substance in 500 mL deionised water. An aliquot of this application solution was further diluted with deionised water to obtain the application solutions of the lower test rates. Only the lowest test rate of 0.1 kg a.s./ha was prepared by mixing 1.43 mL of test substance into 500 mL of deionised water.

The application solution of the reference substance was prepared by making up 0.05 mL in 500 mL deionised water.

The control test units were sprayed with deionised water only.

Appropriate volumes of the application solutions were sprayed onto the test units by means of an adequate spraying apparatus (i.e. Spray Lab from Schlachtner, Germany). The sprayer was calibrated to deliver 2.0 ± 0.2 mg spray solution/cm^2 (equivalent to 200 L/ha) by weighing the amount of water delivered.

3. Observations and assessments

The number of surviving mites and mite mortality was assessed on days 3 and 7 after application.

On day 7, 10, 13 and 14, the number of females and males was recorded: On day 10, 13 and 14 additionally the number of larvae and eggs was recorded and both larvae and eggs were removed.

The temperature and the relative humidity were continuously recorded. The light intensity was recorded once during the test.

4. Calculation of toxicity

Mortality was calculated by adding the number of mites which had escaped (i.e. “escapees”) to the number of those which had died. Escapees were calculated as the sum of those mites which could not be found on the test arena, those which were stuck in the glue barrier and those which were found to be drowned in the water supply (cotton wick). Mortality was corrected using the formula of Abbott (1925) with improvements by Schneider-Orelli (1947).

For each treatment (control, 0.57 and 1.14 L AG-T3-175 EC/ha), the mean cumulative number of eggs per female during the reproduction period was calculated. Additionally, the percent reduction as compared to the control was calculated.

5. Statistics

The LR_{50} and its 95% confidence interval were calculated by Probit analysis. The NOER for reproduction were determined by means of a Williams t-test.

Results and Discussion

After 7 days of exposure (endpoint of the mortality assessment), the mean mortality values in the control and in the reference substance treatment were 3.3% and 100%, respectively. The mean corrected mortality in the test substance treatments ranged from 0.0% to 100% (Table A 2.3.2.6-1). The LR_{50} was determined to be 1.5 L AG-T3-175 EC/ha (95% confidence interval: 0.6-4.1 L AG-T3-175 EC/ha). In terms of active substance trinexapac-ethyl, this corresponds to 0.27 kg a.s./ha (95% confidence interval: 0.1-0.71 kg a.s./ha).

The mean egg production from day 7 to day 14 in the control was 4.0 eggs per female. In the test substance treatments of 0.57 and 1.14 L AG-T3-175 EC/ha, the mean egg production from day 7 to day 14 was 2.3 and 1.3 eggs per female, respectively (Table A 2.3.2.6-1). Both were statistically significantly lower than in the control. Therefore, the NOER and LOER for reproduction were determined to be < 0.57 and 0.57 L AG-T3-175 EC/ha, respectively (corresponding to < 0.10 and 0.10 kg a.s./ha, respectively).

The test is considered to be valid since mortality in the control and toxic reference group was 3.3% (required ≤ 20%) and 100% (required 50 - 100%), respectively, and in addition, mean egg production was 4.0 eggs per female (required ≥ 4 eggs per female) in the control group.

Table A 2.3.2.6-1: Toxicity of AG-T3-175 EC to the predatory mite *Typhlodromus pyri* in an extended laboratory test

Treatment		Mortality after 7 days of exposure [%]		Reproduction from day 7 to day 14 [number of eggs per female]	
		Mean ± SD	Corrected	Mean ± SD	% of control
control		3.3 ± 5.2	-	4.0 ± 1.1	-
test substance					
[L product/ha]	[kg a.s./ha]				
0.57	0.1	2 ± 4	-1.7	2.3* ± 2.4	56
1.14	0.2	28 ± 15	26	1.3* ± 1.0	31
2.29	0.4	77 ± 15	76	n.a.	n.a.
4.57	0.8	100 ± 0.0	100	n.a.	n.a.
9.15	1.6	100 ± 0.0	100	n.a.	n.a.
18.3	3.2	100 ± 0.0	100	n.a.	n.a.
reference substance					
[mL product/ha]	[g a.s./ha]				
20	8	100 ± 0.0	100	n.a.	n.a.

n.a. Not applicable.
SD Standard deviation
* Statistically significantly different to the control, results of a Williams t-test, α=0.05, one-sided smaller

Conclusion

Under extended laboratory conditions (i.e. treated bean leaf disks as substrate), the 7-day LR_{50} of AG-T3-175 EC for the predatory mite, *Typhlodromus pyri*, was determined to be 1.5 L productc/ha (0.27 kg a.s./ha). There was a 44% reduction in reproduction relative to control at 0.57 L product/ha (100 g a.s./ha) and a 69% reduction in reproduction at 1.14 L product/ha (200 g a.s./ha). The statistical NOER and LOER for reproduction were determined to be < 0.57 and 0.57 L product/ha, respectively (corresponding to < 0.10 and 0.10 kg a.s./ha, respectively).

A 2.3.2.7 Study 7: Extended Laboratory Test - Effects on *Chrysoperla carnea*

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.2/07
Report	AG-T3-175 EC: Toxicity to Larvae of the Green Lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae) under Extended Laboratory Conditions, Schmidt, T., 2009d, B92957 (report number), 90018040_000081135 (sponsor report number)
Guideline(s):	Vogt, H. et al. (2000): Laboratory test method to test effects of plant protection products on larvae of Chrysoperla carnea (Neuroptera: Chrysopidae). In: Guidelines to evaluate side-effects of plant protection products to non-target arthropods (eds. Candolfi, M. P. et al.).
Deviations:	Deviations to guideline stated above: None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and Methods

A. MATERIALS

1. Test material	AG-T3-175 EC (emulsifiable concentrate formulation of the active substance trinexapac-ethyl)
Description	Liquid
Lot/Batch #	D-I0703
Purity	Nominal / analysed: 175 / 180 g/L trinexapac-ethyl
Stability of test material	Stable under storage conditions Expiry date: 31 Jan 2010
2. Vehicle and/or positive control	Vehicle/negative control: Deionised water Positive control: Reference item
Reference item	Roxion (containing 400 g/L dimethoate)

Description	Yellow liquid
Lot/Batch #	G339A
Purity	Nominal / analysed: 400 / 375 g/L dimethoate
Stability of reference item	Stable under storage conditions Expiry date: Dec 2015

3. Test organism

Species	Neuropteran predator *Chrysoperla carnea*
Source	Sautter & Stepper GmbH, Biologischer Pflanzenschutz, Rosenstr. 19, D-72119 Ammerbuch, Germany
Acclimation period	Prior to the start of the exposure phase, *C. carnea* eggs and larvae were held in approximately 1 L plastic containers covered with a cotton cloth, at a temperature set at 25±2°C, a relative humidity set at 75±15% and a photoperiod set at 16 hours light (> 1000 Lux)/8 hours dark.
Diet	Mortality test The larvae were fed with fresh eggs of the lepidopteran species *Ephestia kuehniella* Z. (supplier: Landi REBA, Lyonstr. 18, 4053 Basel / Switzerland). Food was replaced every 1 to 3 days or whenever necessary. Reproduction test The adults were fed with a mixture of 15 mL condensed milk, 1 egg, 1 egg yolk, 30 g honey, 20 g fructose, 30 g dried brewer's yeast, 50 g wheat germ and 45 mL water. The food was thinly spread onto paper strips and positioned on the inside walls of the reproduction containers. Damp cotton wool provided extra water. Food was replaced every 1 to 3 days or whenever necessary.
Test units	Mortality test The exposure test units consisted of a glass plate (40 × 18 × 0.6 cm (length × width × height)) with a second plate of the same size with 10 holes (diameter 5.5 cm) placed on top. The bottom glass plate was covered by a piece of filter paper, reaching into a water bath. Ten treated bean leaves (*Phaseolus vulgaris* L. var. Autan, Fabaceae; BBCH code 12) were placed onto the filter paper in such a way, that one leaf filled one hole of the top plate. The treated side (upper leaf side) of each leaf faced upwards. Hollow glass cylinders (outside diameter 5.2 cm, inside diameter 4.7 cm, height 4 cm) were then placed into each hole to confine the *C. carnea* larvae. The glass cylinders were covered with fine mesh gauze (0.2 to 0.4 mm netting) to prevent emerging *C. carnea* adults from escaping. The inner walls of the cylinders were coated with talcum to prevent the larvae from climbing. Reproduction test Transparent plastic containers (approximately 15.5 cm high and 11 cm diameter) were used for the reproduction phase of the test, i.e. assessment of oviposition. The containers were covered on the top with fine mesh gauze lids. The gauze prevented the adults from escaping and was also the preferred substrate for egg laying.

4. Environmental conditions	The study was performed in a temperature/humidity-controlled room.
Temperature	19.3 to 25.9°C (mean: 24.8°C) The temperature dropped occasionally below 20°C during assessment. However, this short-term deviation in temperature was not considered to have an influence on the biological results, as documented by the acceptable performance of larvae and adults in the control.
Relative humidity	31.3 to 88.3% (mean: 76.7%) The humidity dropped occasionally below 60% during assessment. However, this short-term deviation in humidity was not considered to have an influence on the biological results, as documented by the acceptable performance of larvae and adults in the control.
Photoperiod	Mortality test 16:8 hours light:dark (light intensity ranged from 3700–5300 lux) Reproduction test 16:8 hours light:dark (light intensity ranged from 3600–4400 lux)

B. STUDY DESIGN AND METHODS

1. In-life dates 11 Nov 2008 to 29 Dec 2008

2. Experimental conditions

Test design

Mortality test
First instar larvae of *C. carnea* (2 days old) were exposed to dried residues of the test substance, reference substance or deionised water (control) sprayed onto bean leaves. The duration of the exposure phase was 35 days.

Reproduction test
All test organisms that developed to the adult stage were pooled within their respective treatment groups and placed into reproduction test units. For all test substance treatments, the reproduction phase was set up. Emerging adults from the same treatment group were placed into one plastic container up to the density of 20 adult lacewings. Two containers were established for the control and the test groups of 286-1143 mL AG-T3-175 EC/ha and one container was used for test groups of the two highest test rates. The sex ratio of the insects was maintained as similar as possible in all containers. The duration of the reproduction phase was 12 days.

Number of animals per treatment

Mortality test
Fourty individual larvae (replicates) per treatment (4 glass plates with 10 larvae each)

Reproduction test
Up to 2 replicates with a maximum of 20 adult lacewings per treatment

Rates tested

The following nominal test substance rates were tested:
285.8, 571.5, 1143, 2286 and 4572 mL AG-T3-175 EC/ha (corresponding to 50, 100, 200, 400 and 800 g a.s./ha based on a content of 175 g trinexapac-ethyl/L product)

The test included a control treatment sprayed with deionised water only.

The reference substance Roxion was tested at 45 mL product/ha (corresponding to 18 g dimethoate/ha).

Treatment/Application

Prior to test start, the highest test rate of AG-T3-175 EC was prepared by mixing 11.430 mL of test substance in 500 mL of deionised water. An aliquot of this application solution was further diluted with deionised water to obtain the application solutions for the lower test rates.

Adequate volumes of application solutions were sprayed onto the upper sides of the leaf disks of each replicate by means of a track sprayer (Spray Lab from Schachtner, Germany). The sprayer was calibrated to deliver a target of 2.0 ± 0.2 mg spray solution/cm^2, corresponding to 200 L/ha, by weighing the amount of water delivered.

3. Observations and assessments

Total pre-imaginal mortality of *C. carnea* during the mortality phase was assessed three times per week and larval mortality, pupation as well as adult hatching were recorded. Additionally, any behavioural abnormalities of the larvae and abnormal appearance of the larvae, pupae or adults were noted.

After the end of the pre-oviposition period and approximately one week after first egg laying all eggs deposited in the reproduction test units during a 24-hour period were counted. The reproduction phase comprised a total of two 24-hour egg laying periods. The eggs attached to the gauze by the females were incubated for determination of viability in additional transparent plastic containers. Since the females in the treatments of 285.8 and 2286 mL AG-T3-175 EC/ha oviposited only on the walls of the reproduction containers and not on the nets, no fertility of eggs could be determined for these treatments.

The temperature and the relative humidity were continuously recorded during the test. The light intensity was recorded once for each the mortality and the reproduction phase.

4. Calculation of toxicity

Pre-imaginal mortality was calculated for each treatment as sum of dead larvae, dead pupae and adults dying during emergence or pupation. Pre-imaginal mortality was corrected using the formula of Abbott (1925) with improvements by Schneider-Orelli (1947).

5. Statistics

The LR_{50} was extrapolated by Probit analysis. However, due to scattering of data, no confidence intervals could be achieved. Since corrected mortality values were below 50%, the LR_{50} was determined directly from the raw data.

Results and Discussion

Mean values of pre-imaginal mortality in the control and in the reference treatment were 15% and 85%, respectively. In the test substance treatments, corrected pre-imaginal mortality varied between 17.6% and 47.1% (Table A 2.3.2.7-1). Since corrected mortality values of all test substance treatments

were below 50%, the LR_{50} after 35 days of exposure was determined to be > 4572 mL AG-T3-175 EC/ha (> 800 g a.s./ha).

The mean egg production per female and per day in the control was 21.2. The mean egg production in the test substance treatments ranged between 5.4 and 30.6 eggs per female per day without a rate-response relationship (Table A 2.3.2.7-1). In the treatment with the lowest test rate of 285.8 mL AG-T3-175 EC/ha, a low mean number of 5.4 eggs per female and day was counted but was not considered to be a treatment-related effect since the mean number of eggs per female in the higher test substance treatments were in the range of the control value. The largest number of eggs per female and day was found in the treatment with the highest test substance rate of 4572 mL AG-T3-175 EC/ha. Therefore, no indication was found for a negative impact of AG-T3-175 EC on fecundity up to the highest test rate of 4572 mL/ha (800 g a.s./ha).

The mean percentage of fertile eggs per female and day was 86% in the control. In the test substance treatments, the mean percentage of fertile eggs varied between 81% and 90% (Table A 2.3.2.7-1). The mean values of all test substance treatments were above the validity criterion for control reproduction. Therefore, no indication was found for a negative impact of AG-T3-175 EC on fertility up to the highest test rate of 4572 mL product/ha (800 g a.s./ha).

The test is considered to be valid since the mean pre-imaginal mortality in the control and toxic reference group was 15.0% (required ≤ 20%) and 85% (required ≥ 50%), respectively. Additionally in the control group, fecundity was 21.2 eggs per female per day (required ≥ 15 eggs per female per day) and the percentage of fertile eggs per female and day was 86% (required ≥ 70%).

Table A 2.3.2.7-1: Toxicity of AG-T3-175 EC to the green lacewing *Chrysoperla carnea* in an extended laboratory test

Treatment		Pre-imaginal mortality [%]		Fecundity [number of eggs/female/day]	Fertility [number of fertile eggs/female/ day]	Percentage of hatched eggs per female per day [%]
		Mean	Corr.	Mean ± SD	Mean ± SD	Mean ± SD
control		15.0	-	21.2 ± 5.7	18 ± 4.6	86 ± 1.2
test substance						
[mL product/ha]	[g a.s./ha]					
285.8[a)]	50	30.0	17.6	5.4 ± 1.6	n.a.	n.a.
571.5	100	30.0	17.6	18.6 ± 5.0	17 ± 4.1	90 ± 2.6
1043	200	30.0	17.6	20.7 ± 15	16 ± 10	76 ± 5.8
2286[a)]	400	42.5	32.4	22.7 ± 4.7	20 ± n.a.	90 ± n.a.
4572	800	55.0	47.1	30.6 ± 1.5	25 ± 3.9	81 ± 8.8
reference substance						
[mL product/ha]	[g a.s./ha]					
45	18	85.0	n.a.	n.a.	n.a.	

n.a. Not applicable

SD Standard deviation

a) No fertility data were available since laid eggs could not be sampled quantitatively.

Conclusion

Under extended laboratory conditions (i.e. bean leaves as substrate), the LR_{50} for pre-imaginal mortality of AG-T3-175 EC for the the green lacewing, *Chrysoperla carnea*, was determined to be >

4572 mL product/ha (> 800 g a.s./ha). No indication was found for a negative impact of AG-T3-175 EC on fecundity and fertility up to the highest test rate of 4572 mL product/ha (800 g a.s./ha).

A 2.3.2.8 Study 8: Extended Laboratory Test - Effects on *Coccinella septempunctata*

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.2/08
Report	AG-T3-175 EC: Toxicity to Larvae of the Seven-Spotted Ladybird *Coccinella septempunctata* (Coleoptera: Coccinellidae) under Extended Laboratory Conditions, Jeker, L., 2009c, B93060 (report number), 90018041_000081137 (sponsor report number)
Guideline(s):	Schmuck, R. et al. (2000): A laboratory test system for assessing effects of plant protection products on the plant-dwelling insect Coccinella septempunctata L. (Coleoptera: Coccinellidae). In: Guidelines to evaluate side-effects of plant protection products to non-target arthropods (eds. Candolfi et al. 2000), IOBC/WRPS, Gent, p. 45-56. Oomen, P. A. (1988): Guideline for the evaluation of side-effects of pesticides on Phytoseiulus persimilis A.-H.IOBC/wprs Bulletin XI/4: 51-63.
Deviations:	Deviations to Schmuck, R. et al. (2000): None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and Methods

A. MATERIALS

1. Test material	AG-T3-175 EC (emulsifiable concentrate formulation of the active substance trinexapac-ethyl)
Description	Yellow – red brown liquid
Lot/Batch #	D-I0703
Purity	Nominal / analysed: 175 / 180 g/L trinexapac-ethyl
Stability of test material	Stable under storage conditions Expiry date: 31 Jan 2010
2. Vehicle and/or positive control	Vehicle/negative control: Deionised water Positive control: Reference item
Reference item	Roxion (containing 400 g/L dimethoate)

Description	Yellow liquid
Lot/Batch #	G339A
Purity	Nominal / analysed: 400 / 375 g/L dimethoate
Stability of reference item	Stable under storage conditions Expiry date: Dec 2015

3. Test organism

Species	Seven-spotted ladybird *Coccinella septempunctata* L. Second instars larvae (three days old) were used for testing.
Source	Katz Biotech AG, An der Birkenpfuhlheide 10, D-15837 Baruth, Germany
Acclimation period	Eggs from a synchronized cohort were left undisturbed until the larvae had developed to second instars. Larvae were then transferred to small plastic vessels, where they were kept individually under test conditions until start of the test.
Diet	Mortality test and acclimation Green peach aphids *Myzus persicae* and pea aphids *Acyrthosiphon pisum, ad libitum* Reproduction test Food supply for adult beetles: Pea seedlings infested with *M. persicae*, and with *A. pisum*; additional feeding with pollen was carried out regularly in order to increase egg production.
Test units	Mortality test Each test unit consisted of a glass plate (40 × 18 × 0.6 cm) with a second plate of the same size with 10 holes (diameter 5.5 cm) placed on top. The bottom glass plate was covered by a piece of filter paper, reaching into a water bath. Ten treated bean leaves (*Phaseolus vulgaris* L. var. Autan, Fabaceae; BBCH code 12) were placed onto the filter paper in such a way, that one leaf filled one hole of the top plate. The treated side (upper leaf side) of the leaves faced upwards. Hollow glass cylinders (outside diameter 5.2 cm, inside diameter 4.7 cm, height 4 cm) were then placed into each hole to confine the *C. septempunctata* larvae. The glass cylinders were covered with fine mesh gauze (0.2 to 0.4 mm netting). The inner walls of the cylinders were coated with talcum to prevent the larvae from climbing. Reproduction test The test units consisted of transparent plastic containers (approx. 15.5 cm high and 11 cm diameter, volume approx. 1 L) sealed on top with a fine mesh. Two paper tissues (approx. 20 cm × 20 cm) and one plastic tube were placed inside the units as egg laying substrate. Egg clutches collected from the different treatments were held in separate Petri dishes to assess the hatching rate.

4. Environmental conditions	The study was performed in a temperature/humidity-controlled room.
Temperature	25±0.45°C The temperature dropped occasionally below 20°C during assessment. However, this short-term deviation in temperature was not considered to have an influence on the biological

	results, as documented by the acceptable performance of beetles in the control.
Relative humidity	77±3.2% The humidity dropped occasionally below 60% during assessment. However, this short-term deviation in humidity was not considered to have an influence on the biological results, as documented by the acceptable performance of beetles in the control.
Photoperiod	16:8 hours light:dark (light intensity ranged from 1100 - 3400 lux, measured at test unit level)

B. STUDY DESIGN AND METHODS

1. In-life dates — 14 Oct 2008 (start of the first test which was repeated since the test substance rates were wrongly calculated) to 12 Jan 2009

2. Experimental conditions

Test design

Mortality test
Second instars larvae of *C. septempunctata* were exposed to dried residues of the test substance, reference substance or deionised water (control) sprayed onto bean leaves. The duration of the mortality phase was 21 days.

Reproduction test
On day 21 after application, at least 90% of the viable pupae had hatched in the control and the test substance treatment groups and beetles in the control started to lay eggs on the same day. Seven days later, the assessment of the reproduction performance was initiated. All beetles were sexed and pooled within their respective treatment groups and placed into the reproduction test units.

The number of reproduction units depended on the number of hatched adults and the sex ratio. For the control, four reproduction units were set up while for the test substance treatment with 0.1 kg a.s./ha one reproduction unit was feasible. No reproduction was assessed for the reference substance and the test substance rates of 0.2, 0.4, 0.8 and 1.6 kg a.s./ha, since mortality was above 50%. The reproduction phase lasted 14 days.

Number of animals per treatment

Mortality test
40 larvae (4 glass plates with 10 larvae each)/treatment

Reproduction test
Up to 4 replicates/treatment, containing all hatched pupae of the treatment group (a similar sex ratio in all reproduction units was aimed for)

Rates tested

The following nominal test substance rates were tested:
0.575, 1.15, 2.3, 4.6, and 9.2 L/ha (corresponding to 0.1, 0.2, 0.4, 0.8 and 1.6 kg a.s./ha).

A control group was exposed to residues of deionised water.

The reference substance (positive control) Roxion was tested at 45 mL product/ha.

Treatment/Application

The test substance was dissolved in aqueous application solutions. Prior to test start, the highest test rate of the test substance AG-T3-175 EC was prepared by mixing 22.85 mL of test substance in 500 mL deionised water. An aliquot of this application solution was further diluted with deionised water to obtain the application solutions for the lower test rates.

The application solution of the reference substance was prepared by making up 112.5 µL in 500 mL with deionised water.

The control test units were sprayed with deionised water only.

Appropriate volumes of the application solutions were sprayed onto the upper sides of the leaf disks of each replicate by means of an adequate spraying apparatus (i.e. Spray Lab from Schlachtner, Germany). Prior to the application, the sprayer had been calibrated to deliver 2.0 ± 0.2 mg spray solution/cm^2 (equivalent to 200 L/ha), and the spray pattern had been visually checked.

3. Observations and assessments

Total pre-imaginal mortality of *C. septempunctata* during the mortality phase was assessed three times per week and larval mortality, pupation as well as adult hatching were recorded. Additionally, any behavioural abnormalities of the larvae and abnormal appearance of the larvae, pupae or adults were noted.

The assessment of the reproductive performance started one week after the control beetles started to lay eggs. Over a period of two weeks, all eggs deposited on the tissue papers and plastic tubes were collected daily (except on weekends) and checked for fertility (larvae hatch). Additionally, sex-specific development of the adults was assessed.

4. Calculation of toxicity

Pre-imaginal mortality was calculated for each treatment as sum of dead larvae, dead pupae and adults dying during emergence or pupation. Mortality in the treatments was corrected for any losses in the control group according to the method of Abbott (1925) with improvements by Schneider-Orelli (1947).

The mean number of eggs laid per female beetle per day was determined by dividing the total number of eggs laid within each treatment group by the mean number of viable females in that treatment group (corrected for mortality during egg laying). In addition, the number of fertile eggs was assessed from the larval hatch.

5. Statistics

The LR_{50} for larvae mortality was calculated by Logit analysis using simple linear regression. The LR_{50} for pre-imaginal mortality (larvae and pupae) could not be calculated and was thus determined directly from the raw data.

Due to the species inherent variability in egg-laying performance, fertility was evaluated only qualitatively, according to the test guideline.

Results and Discussion

After 21 days of exposure (endpoint of the mortality assessment), the mean pre-imaginal mortality values in the control and in the reference substance treatment were 27% and 100%, respectively (Table A 2.3.2.8-1).

At all test substance rates, the bean leaf discs showed phytotoxicity. It was assumed that this damage was caused by the test substance. It was further assumed that as a result of this damage, growth of mould was enhanced (no mould in the untreated control).

The corrected mortality increased with the treatment rates and ranged from 38% to 91% for the larvae and from 76% to 97% for the larvae and pupae. The study director claimed that the unusually high number of dead pupae and dead hatching adults was most likely caused by the mould and not treatment related. Therefore, the LR_{50} for both, the larvae and pre-imaginal (larvae and pupae) mortality was reported (Table A 2.3.2.8-1). The LR_{50} for larvae mortality was determined to be 0.196 kg a.s./ha (95% confidence interval: 0.06-0.66 kg a.s./ha). Due to the overall high mortality (> 50% in all test substance treatment rates) the LR_{50} for pre-imaginal mortality (larvae and pupae) could not be calculated and was determined directly from the raw data to be < 0.1 kg a.s./ha.

The mean egg production from day 21 onwards in the control was 14 fertile eggs per female per day. The mean egg production of females in the test substance treatment of 0.1 kg a.s./ha was 6.3 fertile eggs per female per day (Table A 2.3.2.8-1) which is above the validity criterium of ≥ 2 fertile eggs per female per day.

The test is considered to be valid since the mean pre-imaginal mortality in the control and toxic reference group was 27% (required ≤ 30%) and 100% (required > 40%), respectively. Additionally in the control group, fertility was 14 fertile eggs per female per day (required ≥ 2 fertile eggs per female per day).

Table A 2.3.2.8-1: Toxicity of AG-T3-175 EC to the seven-spotted ladybird *C. septempunctata* in an extended laboratory test

Treatment		Larvae mortality [%]		Pre-imaginal mortality (larvae and pupae) [%]		Fertility (number of hatched eggs per female per day)
		Mean	Corrected	Mean	Corrected	Mean ± SD
Control[a)]		19	-	27	-	14 ± 13
Test substance						
[L product/ha]	[kg a.s./ha]					
0.575	0.1	50	38	83	76	6.3 ± 7.3
1.15	0.2	63	54	88	83	n.a.
2.3	0.4	68	60	93	90	n.a.
4.6	0.8	75	69	88	83	n.a.
9.2	1.6	93	91	98	97	n.a.
Reference substance						
[mL product/ha]	[kg a.s./ha]					
0.045	0.018	100	n.a.	100	n.a.	n.a.

a) Three dead larvae killed due to technical mishandling, therefore excluded for mortality calculation
n.a. Not applicable
SD Standard deviation

Conclusion

In this extended laboratory test on toxicity of AG-T3-175 EC to *C. septempunctata*, high numbers of dead pupae and dead hatching adult were observed which were not treatment related but were assumed to be a secondary effect due to mould infestation. The LR_{50} for pre-imaginal mortality (larvae and pupae) could not be calculated and was determined directly from the raw data to be < 0.1 kg a.s./ha (< 0.575 L product/ha). The LR_{50} for larvae mortality was determined to be 0.196 kg a.s./ha (1.12 L product/ha).

As mortality was higher than 50%, no reproduction phase was carried out, except for the lowest treatment (0.1 kg a.s./ha = 0.575 L product/ha). The fertility in the lowest treatment group was 6.3 fertile eggs per female per day which was above the validity criterium of ≥ 2 fertile eggs per female per day.

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

A 2.4.1.1.1 Study 1: Sub-lethal effects on earthworms

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.4.1.1/01
Report	AG-T3-175 EC1 (Trinexapac-ethyl 175 EC) – Determination of chronic toxicity to the earthworm *Eisenia andrei* in an artificial soil substrate, McCormac, A., 2018, AGAN-17-37 (report number), 90020908 (sponsor report number)
Guideline(s):	Yes, OECD 222 (2016)
Deviations:	Deviations to OECD 222 (2016): In a combined approach allowing for determination of both the NOEC and EC_x, eight treatment concentrations (in a geometric series) should have been used instead of five treatment concentrations. However, since the NOEC, LOEC, EC_{10}, EC_{20} and EC_{50} values for reproduction were covered by the tested concentration range, this deviation is not considered to affect the quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and Methods

A. MATERIALS

1. Test material	AG-T3-175 EC1
Description	Light-amber fluid, EC (emulsifiable concentrate)
Lot/Batch #	8162
Purity	Trinexapac-ethyl: 175 g/L nominal; 170.5 g/L analysed Density: 0.996 g/mL
Stability of test material	Stable when stored in original packaging under normal storage conditions Expiry date: March 2019
2. Vehicle and/or positive control	Vehicle control: Purified water Reference item: The chronic (sub-lethal) toxicity of the reference item carbendazim to worms from the same culture as used in the study was evaluated in a separate bioassay run within 12 months of the study. The EC_{50} was calculated to be 2.8 mg a.s./kg dry soil (with 95% confidence limits of 2.4 and 3.3 mg a.s./kg dry soil). This is in the range of 1-5 mg a.s./kg dry soil as stated in OECD 222 (2016).
3. Test organism	
Species	Earthworm *Eisenia andrei*
Source	In-house culture maintained at the test facility (original source: Bias Laboratories Ldt., Kirkcaldy, Fife, UK)
Age	Adults approximately 7 months old with clitellum; body weight at test start: 250 - 600 mg/worm (nominal), 316-455 mg/worm (actual, 20 worms randomly selected at test start)
Acclimatisation	Approximately three days in artificial soil substrate with food (both as used in the test) and at ambient room temperature of 21°C
Diet	Finely-ground rolled oat flakes (Mornflake Ltd., Crewe, UK) were fed to the worms during culturing, acclimatisation and the test. One day after application, 5 g of food were added to the test chambers and were moistened with 1 mL of purified water. Additional food was supplied on a weekly basis by adding 5 g of finely-ground rolled oat flakes moistened with 1 mL purified water. As a final feed, 10 g of the finely-ground rolled oat flakes were added to each test chamber at day 28 after adult earthworms had been removed and the soil had been returned to the original arenas.
Test units	Polystyrene boxes (17.1 cm x 11.3 cm in area, by 6 cm deep) with ventilated lids and containing 500 g soil (dry weight basis, layer of approximately 5 cm deep) were used for the test.
4. Environmental conditions	
Soil	Artificial soil was prepared with the following constituents (percentage distribution on dry weight basis):
	Sphagnum peat 10% w/w

	Kaolinite clay	20% w/w
	Sand (> 50% of particle size 0.05-0.2 mm)	69.6% w/w
	Calcium carbonate ($CaCO_3$)	0.4% w/w (for adjustment to pH 6 ± 0.5)
Temperature	20-22°C	
Photoperiod	16-hour light (light intensity: 480-570 lux) to 8-hour dark photoperiod	

B. STUDY DESIGN AND METHODS

1. In-life dates 09 Nov 2017 to 08 Jan 2018

2. Experimental conditions

Test design

Adult earthworms were exposed to soil treated with the test item at five concentrations or remaining untreated (control) for a period of 28 days. After this period, the adults were removed from the test vessels and mortality, behavioural effects and biomass development (body weight change) were determined. The reproduction rate was determined after an additional period of 28 days (on day 56) based on the number of juveniles.

Number of animals per treatment

Ten earthworms/replicate; four replicates/test item treatment and eight replicates/control

Test conditions

By application, the soil moisture content in each test vessel was adjusted to 50% of WHC. The soil moisture content at study end (day 56) was 48-55% of WHC. The pH value in the test item treatments and control was 6.2 at the start of the test and 5.4-6.6 at the end of the test. Throughout the bioassay, the test arenas were stored in a controlled-environment room maintained at 20-22ºC and the test arenas were kept under a 16-hour light (light intensity: 480-570 lux) to 8-hour dark photoperiod.

Test concentrations

AG-T3-175 EC1 was tested at 181, 327, 584, 1051 and 1893 mg product/kg dry soil corresponding to 31, 56, 100, 180 and 324 mg a.s./kg dry soil trinexapac-ethyl (based on analysed content of active substance and product density). Test item solutions were prepared in purified water. A control (receiving purified water only) was tested in parallel. The reference item carbendazim was tested in a separate study.

Treatment/Application

The replicate test arenas were treated individually. To make up each replicate batch of treated soil, the equivalent of 500 g dry soil (actually corresponding to 503.58 g of the initial soil mix as this already held a small moisture content) was partially moistened with 89 mL purified water, followed by 50 mL of the final test item solution. This resulted in bringing the total soil moisture content to 50% of WHC. The treated soil was mixed well using an electrical mixer. The test worms were placed onto the soil immediately after the treatment of each arena.

3. Sampling and measurements

Immediately following treatment incorporation at day 0, groups of ten worms were weighed, placed on the surface of the soil in each of the test chambers and their behaviour observed. After 28 days of

exposure, the soil was removed from the test chambers and any adult worms present were recovered. The living worms were gently cleaned before being re-weighed to determine the mean weight of individuals in each replicate. The worms were also examined for any other harmful effects (e.g. behavioural abnormalities or open wounds). The test soil, along with any egg cocoons and juvenile worms (but excluding the original adult test worms), was then returned to the original arenas. After a further 28 days (i.e. 56 days after treatment), the number of juvenile worms and unhatched cocoons in each replicate arena was recorded.

The room temperature was recorded hourly throughout the bioassay. Light intensity was measured at the start of the test. At the beginning (day 0) and end (day 56) of the bioassay, samples of soil were taken from replicate 1 of the control and of each test item concentration treatment in order to measure the soil pH.

The moisture content of the test soil was brought to 50% WHC at day 0 and the moisture was replenished at 7, 14, 21, 28, 35, 42 and 49 days after application, as necessary.

4. Calculation of toxicity

The parameters used for endpoint determination were mortality, biomass development (body weight change) and reproduction. Mean percentage mortality and mean percentage change in worm fresh weight (including standard deviation) were calculated for each test group. Furthermore, the mean number of juveniles at day 56 (including coefficient of variation) in each test group and the percentage change in number relative to the control were calculated for the test item treatments.

5. Statistics

The 28-day mortality in each test item treatment was compared to that in the control using Fisher's Exact Test ($\alpha = 0.05$) (Sokal and Rohlf, 1981). Following a check for normality of distribution (Shapiro-Wilk test, $\alpha = 0.05$) and for equality of variances (Levene's test, $\alpha = 0.05$), mean percentage body weight change in the test item treatments were compared to the control using either t-test for independent samples ($\alpha = 0.05$) or Mann-Whitney U-test ($\alpha = 0.05$) (Fowler et al., 1998; Levene, 1960). These tests were applied to identify the LOEC and NOEC values for mortality and body weight change.

For reproduction at day 56, both the normality of the data (Shapiro-Wilk test, $\alpha = 0.05$) and the equality of variance (Levene's test, $\alpha = 0.05$) were checked, prior to comparison of the test item results to the control either by one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$) or by Mann-Whitney U-test ($\alpha = 0.05$). The results were used to determine both the LOEC and the NOEC with respect to effects on reproduction. Probit regression analysis (Finney, 1952) was performed on the data for the numbers of progeny, to determine key effect concentrations (EC_{50} , EC_{20} and EC_{10}). The 95% confidence intervals for the EC_x values were also calculated. A Chi-square test for goodness of fit ($\alpha = 0.05$) was performed on the Probit line.

Results and Discussion

The results of the study are presented in the following table.

Mortality during the first 28 days of the test was 1% in the control and 0-3% in the test item treatments. There were no significant differences in mortality (Fisher's Exact Test, $\alpha = 0.05$) for any test item treatment in comparison with the control. Thus, the survival rate of adult earthworms after 28 days of exposure to AG-T3-175 EC1 was not affected up to and including the highest test concentration of 324 mg a.s./kg dry soil (1893 mg product/kg dry soil, NOEC for mortality). All surviving adult worms at day 28 appeared healthy with no abnormal behaviour being observed.

The mean body weight change of adult worms from day 0 to day 28 was 30% in the control and ranged between 34 and 38% in the test item treatments. There were no significant differences in body weight change (either t-test for independent samples, $\alpha = 0.05$, or Mann-Whitney U-test, $\alpha = 0.05$) for any test item treatment in comparison with the control. Thus, the biomass development (body weight change) of adult earthworms after 28 days of exposure to AG-T3-175 EC1 was not affected up to and including the highest test concentration of 324 mg a.s./kg dry soil (1893 mg product/kg dry soil, NOEC for body weight change).

The mean number of juveniles per replicate at the end of the study (day 56) was 95 in the control group. In the test item treatments, the mean number of juveniles per replicate ranged between 88 at the lowest test concentration of 31 mg a.s./kg dry soil (181 mg product/kg dry soil) and 4 at the highest test concentration of 324 mg a.s./kg dry soil (1893 mg product/kg dry soil). The mean number of juveniles per replicate was statistically significantly reduced at the test concentrations of 56-324 mg a.s./kg dry soil (327-1893 mg product/kg dry soil; one-way ANOVA and Dunnett's t-test, $\alpha = 0.05$, or Mann-Whitney U-test, $\alpha = 0.05$). Therefore, the NOEC and LOEC for reproduction were determined as 31 mg a.s./kg dry soil (181 mg product/kg dry soil) and 56 mg a.s./kg dry soil (327 mg product/kg dry soil), respectively. The EC_{10}, EC_{20} and EC_{50} values for reproduction were calculated to be 29, 44 and 103 mg a.s./kg dry soil (167, 260 and 604 mg product/kg dry weight), respectively.

The validity of the test was fulfilled since each control replicate produced ≥ 68 juveniles (required ≥ 30 juveniles) and the coefficient of variance of the reproduction rate per test vessel in the control was 21.2% (required ≤ 30%). Furthermore, mean mortality of adults in the control was 1% (required ≤ 10%) after 28 days.

Table A 2.4.1.1.1-1: Effects of AG-T3-175 EC1 on mortality, body weight change and reproduction of *Eisenia andrei*

Treatment		Mortality after 28 days of exposure [%]	Mean % change in worm fresh weight (day 0-28) [% ± SD]	Reproduction after 56 days	
[mg a.s./kg dry soil]	[mg product/kg dry soil]			[mean number of juveniles/replicate ± CV in %]	Reduction compared to control [%]
0 (control)	0 (control)	1	30 ± 9.8	95 ± 21.2	-
31	181	0	37 ± 8.0	88 ± 31.7	7
56	327	0	34 ± 4.5	63* ± 10.3	34
100	584	0	34 ± 7.4	65* ± 34.8	32
180	1051	3	38 ± 8.3	32* ± 43.1	66
324	1893	0	38 ± 2.9	4* ± 86.6	96

Endpoints [mg a.s./kg dry soil] (95% confidence limits) / [mg product/kg dry soil] (95% confidence limits)	
EC_{10} (reproduction)[a)]	29 (13-43) / 167 (79-249)
EC_{20} (reproduction)[a)]	44 (26-61) / 260 (151-356)
EC_{50} (reproduction)[a)]	103 (78-138) / 604 (458-808)
NOEC (reproduction)	31 / 181
LOEC (reproduction)	56 / 327
NOEC (mortality, body weight change)	324 / 1893
LOEC (mortality, body weight change)	> 324 / > 1893

SD: standard deviation; CV: coefficient of variance

Note: There were no significant differences in mortality (Fisher's Exact Test, $\alpha = 0.05$) and body weight change (either t-test for independent samples, $\alpha = 0.05$, or Mann-Whitney U-test, $\alpha = 0.05$) for any test item treatment in comparison with the control.
All surviving adult worms at day 28 appeared healthy with no abnormal behaviour being observed.
The number of unhatched cocoons was 2-11 in the control and 1-9, 3-13, 2-4, 3-6 and 0-3 in the test item treatments of 31, 56, 100, 180 and 324 mg a.s./kg dry soil, respectively, at the end of the study (day 56).

* Statistically significantly different from the control, according to one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$) or Mann-Whitney U-test ($\alpha = 0.05$)

a) EC_x values and their 95% confidence limits were derived by Probit regression analysis.

Conclusion

In this test on sub-lethal effects of AG-T3-175 EC1 on the earthworm *Eisenia andrei*, the EC_{50}, EC_{20}, and EC_{10} for reproduction were determined to be 29, 44 and 103 mg a.s./kg dry soil (167, 260 and 604 mg product/kg dry weight), respectively. The NOEC for reproduction was determined as 31 mg a.s./kg dry soil (181 mg product/kg dry soil). The NOEC for mortality and biomass development (body weight change) was 324 mg a.s./kg dry soil (1893 mg product/kg dry soil). All validity criteria were fulfilled.

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A 2.4.2.1 KCP 10.4.2.1 Species level testing

A 2.4.2.1.1 Study 1: Effects on *Folsomia candida*

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.4.2.1/01
Report	AG-T3-175 EC1 (Trinexapac-ethyl 175 EC) – A laboratory test to determine the effects of fresh residues on the springtail *Folsomia candida* (Collembola, Isotomidae) in an artificial soil substrate, Geary, N., 2018, AGAN-17-38 (report number), 90020909 (sponsor report number)
Guideline(s):	Yes, OECD 232 (2016)
Deviations:	Deviations to OECD 232 (2016): In a combined approach allowing for determination of both the NOEC and EC_x, eight treatment concentrations (in a geometric series) should have been used instead of five treatment concentrations. However, the tested concentrations were sufficient to show that EC_{50} was higher than the highest concentration tested. The water content of the soil at the end of the test is not reported. This deviation to guideline and reporting deficiency is not considered to have affected the quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Material and Methods

A. MATERIALS

1. Test material	AG-T3-175 EC1
Description	Light-amber fluid, EC (emulsifiable concentrate)
Lot/Batch #	8162
Purity	Trinexapac-ethyl: 175 g/L nominal; 170.5 g/L analysed Density: 0.996 g/mL
Stability of test material	Stable when stored in original packaging under normal storage conditions Expiry date: March 2019
2. Vehicle and/or positive control	Vehicle control: Purified water Reference item: The effects on reproduction of the reference item boric acid to springtails from the same culture as used in the study was evaluated in a separate bioassay run within 12 months of the study. The EC_{50} was calculated to be 111 mg a.s./kg dry soil (with 95% confidence limits of 106 and 117 mg a.s./kg dry soil). This is around 100 mg a.s./kg dry soil given as an approximate value in OECD 232 (2016).
3. Test organism	
Species	Collembolan *Folsomia candida*
Source	In-house culture maintained at the test facility since 2015 (original source: Bias Laboratories Ldt., Kirkcaldy, Fife, UK)
Age	Juvenile collembolans (11 days old)
Acclimatisation	Prior to the range-finding and definite test, the culture was maintained at test temperature and a 12-hour light (light intensity: 480-560 lux) to 12-hour dark photoperiod.
Diet	Approximately 30 mg of dried granulated baker's yeast ('Easy Bake Yeast'; Allinson, Peterborough, UK) were added to the soil surface of each test arena at the beginning of the test, and was also replenished at day 14.
Test units	The test arenas were glass jars (approximately 125 mL capacity and 4.5 cm in diameter), secured with a close-fitting lid. Each test unit was filled with 30 g of artificial soil. To allow ventilation, the arena lids were opened for brief periods, every 1-4 days.
4. Environmental conditions	
Soil	Artificial soil was prepared with the following constituents (percentage distribution on dry weight basis):

Sphagnum peat	5% w/w
Kaolinite clay	20% w/w
Sand (> 50% of particle size 0.05-0.2 mm)	74.80% w/w
(range-finding test)	74.79% w/w
(definite test)	

	Calcium carbonate ($CaCO_3$) 0.20% w/w (range-finding test, for adjustment to pH 6 ± 0.5) 0.21% w/w (definite test, for adjustment to pH 6 ± 0.5) Two days (range-finding test) and four days (definitive test) prior to treatment, the dry artificial soil was partially pre-moistened by adding purified water (reaching approximately 25% of the maximum water holding capacity (WHC)) and then during treatment, the artificial soil was made up to 50% WHC.
Temperature	19.4–20.6ºC (range-finding test), 19.4-20.3ºC (definite test)
Photoperiod	16 hour light (light intensity: 550-750 lux in the range-finding test and 610-750 lux in the definite test) to 8 hour dark photoperiod

B. STUDY DESIGN AND METHODS

1. In-life dates 16 Oct 2017 to 12 Dec 2017

2. Experimental conditions

Test design

Juvenile collembolans were exposed to soil treated with the test item at five concentrations for a period of 28 days. A water control (deionised water) was tested in parallel. The reference item boric acid was tested in a separate study. After four weeks of exposure, the number of adults was counted and mortality was determined. The reproduction output was determined by counting the number of juveniles.

Number of animals per treatment

For the range-finding bioassay, two replicates per test item treatment and control were used, each set up with 10 collembolans. In the definite test, four replicates per test item treatment and eight replicates for the control were used with 10 collembolans per replicate.

Test conditions

By application, the soil moisture content in each test vessel was adjusted to 50% of WHC. The pH value in the test item treatments and control was 6.03-6.20 at the start of the test and 5.60-5.77 at the end of the test for the definite test. Throughout the bioassay, the test arenas were stored in a controlled-environment room maintained at 19.4–20.6ºC in the range-finding test and 19.4-20.3ºC in the definite test and the test arenas were kept under a 16-hour light (light intensity: 550-750 lux in the range-finding test and 610-750 lux in the definite test) to 8-hour dark photoperiod.

Test concentrations

AG-T3-175 EC1 was tested at 62.5, 125, 250, 500 and 1000 mg product/kg dry soil corresponding to 10.7, 21.4, 42.8, 85.6 and 171 mg a.s./kg dry soil trinexpac-ethyl (based on analysed content of active substance and product density, calculated by the applicant). The test concentrations were based on the results of a range-finding test performed at 0.1, 1, 10, 100 and 1000 mg product/kg dry soil. Test item solutions were prepared in purified water. A control (receiving purified water only) was tested in parallel. The reference item boric acid was tested in a separate study.

Treatment/Application

For preparation of the application solutions, a stock solution (= application solution of the highest test concentration) was prepared by diluting 0.557 g of the test item to 50 mL with purified water. The remaining application solutions were prepared by dilution of an aliquot of this stock solution with purified water. To achieve the desired soil concentrations, 18 mL of the respective application solution were thoroughly mixed with 220.81 g of pre-moistened artificial test soil (equivalent to 200 g dry soil), to achieve a final soil moisture content of 50% WHC. The control received purified water sufficient to make the soil up to 50% WHC. Once treated, 30 g of the soil were transferred into each replicate glass jar. An additional jar was also prepared per test item treatment and control for pH measurement at study end. Within 60 minutes of the soil being treated, moistened and placed into each test arena, 10 juvenile springtails were placed in each replicate using a low-suction aspirator (test start).

3. Sampling and measurements

In the definite test, the numbers of both surviving adults and F1 progeny in each test arena were recorded at the end of the test (day 28). The test substrate from each arena was tipped into a tray (approximately 11 cm x 17 cm in area and 6 cm in depth). Water (approximately 150-200 mL) was then added to the substrate and stirred gently, so that the soil sank and the springtails floated to the surface. Any adult springtails floating on the water were counted and removed. The water-filled arenas were left for a period of > 2 h and any further adult springtails that had surfaced were recorded. Black ink was then added to the water and the numbers of any nymphs (smaller in size to adults) left in each arena were assessed.

In a separate test, carried out by the test facility in October 2014, the efficiency of the method used to extract the springtails was determined to be 100% for adult springtails and 98.3% for juvenile springtails.

The weight of each test arena was noted at the start of the test. At day 14, the test arenas were reweighed and since the change in the mean weight was calculated to be > 2% of the soil's original water content, purified water was added to restore the arenas' original weights. The weight of each arena was also measured at the end of the test.

At the start and end of the test, the pH of the artificial soil was measured. The test temperature and humidity in the controlled-environment cabinet were recorded hourly by data logger. Light intensities were recorded at the start of the bioassays.

4. Calculation of toxicity

The percentage mortality of the springtails originally introduced was calculated for each treatment, both before and after correction for any control treatment losses using Abbott's formula (Abbott, 1925).

The mean number of offspring produced per replicate (including standard deviation) was calculated for each treatment. In addition, the percentage reduction in reproductive performance in the test item treatment groups, compared to the control group, was calculated.

5. Statistics

The 28-day mortality data for the individual test-item treatments were compared to those for the control using Fisher's Exact Test ($\alpha = 0.05$) (Sokal and Rohlf, 1981). This test was applied to identify the LOEC and NOEC values for mortality. The LC_{50} could not be calculated but was estimated by extrapolation from the data.

For reproduction at day 28, both normality of the data (Shapiro-Wilk test, $\alpha = 0.05$) and equality of variance (Levene's test, $\alpha = 0.05$) were checked, prior to comparison of the test item results to the control by one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$). The results were used to determine both

the LOEC and the NOEC with respect to effects on reproduction. The EC_{50} for reproduction could not be calculated but was estimated by extrapolation from the data.

Results and Discussion

The results of the study are presented in the following table.

Springtail mortality at the end of the test after 28 days was 19% in the control. Corrected mortality in the test item treatments was in the range of -8% and 39%. Statistically significant effects on springtail mortality were recorded at the two highest concentrations of 500 and 1000 mg product/kg dry soil (Fisher's Exact Test, $\alpha = 0.05$). Therefore, the NOEC and LOEC for mortality were determined as 250 mg product/kg dry soil (42.8 mg a.s./kg dry soil) and 500 mg product/kg dry soil (85.6 mg a.s./kg dry soil), respectively. The LC_{50} could not be calculated but was estimated to be > 1000 mg product/kg dry soil (> 171 mg a.s./kg dry soil).

The mean number of offspring per replicate determined at test termination was 217 in the control and 246, 213, 216, 140 and 131 at concentrations of 62.5, 125, 250, 500 and 1000 mg product/kg dry soil, respectively. Statistically significant effects (one-way ANOVA and Dunnett's t-test, $\alpha = 0.05$)) on offspring numbers compared to the control were recorded at 500 and 1000 mg product/kg dry soil. Therefore, the NOEC and LOEC for reproduction were determined as 250 mg product/kg dry soil (42.8 mg a.s./kg dry soil) and 500 mg product/kg dry soil (85.6 mg a.s./kg dry soil), respectively. The EC_{50} for reproduction could not be calculated but was estimated to be > 1000 mg product/kg dry soil (> 171 mg a.s./kg dry soil).

The validity of the test was fulfilled since mean mortality of adults in the control was 19% (required ≤ 20%) at the end of the test, the reproduction rate in the control was on average 217 juveniles per replicate (required ≥ 100) and the coefficient of variation of reproduction was 12.2% in the control (required ≤ 30%).

Table A 2.4.2.1.1-1: Effects of AG-T3-175 EC1 on mortality and reproduction of *Folsomia candida*

Treatment		Mortality after 28 days of exposure [%]	Corrected[a)] mortality [% ± SD]	Reproduction after 28 days	
[mg a.s./kg dry soil]	[mg product/kg dry soil]			[mean number of offspring/replicate ± SD]	Reduction compared to control [%]
0 (control)	0 (control)	19	-	217 ± 26.5[b)]	-
10.7	62.5	13	-8	246 ± 47.0	-13.4
21.4	125	20	2	213 ± 23.4	2.0
42.8	250	18	-2	216 ± 27.3	0.4
85.6	500	48*	35	140* ± 13.0	35.5
171	1000	50*	39	131* ± 32.7	39.9
Endpoints [mg a.s./kg dry soil][c)] / [mg product/kg dry soil]					
EC_{50} (reproduction)		> 171 / > 1000			
NOEC (reproduction)		42.8 / 250			
LOEC (reproduction)		85.6 / 500			
LC_{50}		> 171 / > 1000			
NOEC (mortality)		42.8 / 250			
LOEC (mortality)		85.6 / 500			

SD: standard deviation

Note: A negative value represents an increase of parameter and a positive value represents a decrease of parameter, relative to the control.

* Statistically significantly different from the control; mortality: according to Fisher's Exact Test ($\alpha = 0.05$), reproduction: according to one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$)

a) Corrected according to Abbott's formula.
b) Coefficient of variation: 12.2%
c) Calculated by the applicant

Conclusion

In this test on effects of AG-T3-175 EC1 on mortality and reproduction of *Folsomia candida*, the LC_{50} and EC_{50} (reproduction) were both determined to be > 1000 mg product/kg dry soil (> 171 mg a.s./kg dry soil). The NOEC and LOEC were found to be 250 mg product/kg dry soil (42.8 mg a.s./kg dry soil) and 500 mg product/kg dry soil (85.6 mg a.s./kg dry soil), respectively, both for mortality and reproduction. All validity criteria were fulfilled.

A 2.4.2.1.2 Study 2: Effects on *Hypoaspis aculeifer*

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.4.2.1/02
Report	AG-T3-175 EC1 (Trinexapac-ethyl 175 EC) – A laboratory test to determine the effects of fresh residues on the predatory soil mite *Hypoaspis aculeifer* (Acari, Laelapidae) in an artificial soil substrate, Geary, N., 2017, AGAN-17-39 (report number), 90020910 (sponsor report number)
Guideline(s):	Yes, OECD 226 (2016)
Deviations:	Deviations to OECD 226 (2016): The water content of the soil at the end of the test is not reported. This reporting deficiency is not considered to have affected the quality and integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and Methods

A. MATERIALS

1. Test material	AG-T3-175 EC1
Description	Light-amber fluid, EC (emulsifiable concentrate)
Lot/Batch #	8162
Purity	Trinexapac-ethyl: 175 g/L nominal; 170.5 g/L analysed Density: 0.996 g/mL

Stability of test material	Stable when stored in original packaging under normal storage conditions Expiry date: March 2019
2. Vehicle and/or positive control	Vehicle control: Purified water Reference item: The effects on reproduction of the reference item dimethoate to mites from the same culture as used in the study was evaluated in a separate bioassay run within 12 months of the study. The EC_{50} was calculated to be 5.47 mg a.s./kg dry soil (with 95% confidence limits of 4.90 and 6.07 mg a.s./kg dry soil). This is in the range of 3.0-7.0 mg a.s./kg dry soil as stated in OECD 226 (2016).
3. Test organism	
Species	Predatory mite *Hypoaspis aculeifer*
Source	In-house culture maintained at the test facility since 2015 (original source: Bias Laboratories Ldt., Kirkcaldy, Fife, UK)
Age	Adults: 33 days (range-finding test) and 30 days (definite test) after start of egg laying for synchronisation (approximately 7-14 days after becoming adult)
Acclimatisation	Prior to the range-finding and definite test, the culture was maintained at test temperature and a 16-hour light (light intensity: 450-560 lux in the range-finding test and 450-520 lux in the definite test) to 8-hour dark photoperiod.
Diet	Cheese mites (*Tyrophagus putrescentiae* (Schrank)) and juvenile springtails (*Folsomia candida* (Willem)) were added to the soil surface of each test arena, at the beginning of the test and cheese mites *ad libitum* (2-3 times per week) throughout the test.
Test units	The test arenas were 60-mL capacity glass jars (5.5 cm tall x 5.2 cm outer diameter, 4.4 cm inner diameter), with screw-top lids. An 8-mm-diameter hole was made in the lid for ventilation and this was covered with fine nylon netting (80 micron mesh).
4. Environmental conditions	
Soil	Artificial soil was prepared with the following constituents (percentage distribution on dry weight basis): Sphagnum peat 5% w/w Kaolinite clay 20% w/w Sand (> 50% of particle size 0.05-0.2 mm) 74.79% w/w Calcium carbonate ($CaCO_3$) 0.21% w/w (for adjustment to pH 6 ± 0.5) Four days (range-finding test) and six days (definitive test) prior to treatment, the dry artificial soil was partially pre-moistened by adding purified water and then during treatment, the artificial soil was made up to 50% of the maximum water holding capacity (WHC).
Temperature	Nominal: 20 ± 2°C; actual: 20.3-21.5ºC (range-finding test), 20.4-21.5ºC (definite test)

Photoperiod	16 hour light (light intensity: nominal 400-800 lux; actual 510-650 lux in the range-finding test and 490-610 lux in the definite test) to 8 hour dark photoperiod

B. STUDY DESIGN AND METHODS

1. In-life dates 26 Oct 2017 to 08 Dec 2017

2. Experimental conditions

Test design

Adult female mites were exposed to soil treated with the test item at a limit concentration for a period of 14 days. A water control (deionised water) was tested in parallel. The reference item dimethoate was tested in a separate study. At the end of the exposure period, the surviving individuals were extracted from the test units. The number of juveniles per test unit and additionally the number of surviving females were determined. The reproductive output and the mortality in the test item treatment group were compared to that of the control group.

Number of animals per treatment

For the range-finding bioassay, two replicates per test item treatment and control were used, each set up with 10 female soil mites. In the definite test, eight replicates for both the test item treatment and the control were used with 10 female soil mites per replicate.

Test conditions

By application, the soil moisture content in each test vessel was adjusted to 50% of WHC. The pH value in the test item treatment and control was 6.03 and 5.99 at the start of the test and 5.78 and 5.60 at the end of the test, respectively, for the definite test. Throughout the bioassay, the test arenas were stored in a controlled-environment room maintained at 20.3-21.5ºC in the range-finding test and 20.4-21.5ºC in the definite test and the test arenas were kept under a 16-hour light (light intensity: 510-650 lux in the range-finding test and 490-610 lux in the definite test) to 8-hour dark photoperiod.

Test concentrations

AG-T3-175 EC1 was tested at the limit concentration of 1000 mg product/kg dry soil corresponding to 171 mg a.s./kg dry soil trinexpac-ethyl (based on analysed content of active substance and product density, calculated by the applicant). The test concentrations were based on the results of a range-finding test performed at 0.1, 1, 10, 100 and 1000 mg a.s./kg dry soil. Test item solutions were prepared in purified water. A control (receiving purified water only) was tested in parallel. The reference item dimethoate was tested in a separate study.

Treatment/Application

For preparation of the application solution, 0.279 g of the test item were diluted to 25 mL with purified water. To achieve the desired soil concentration, 18 mL of the application solution were thoroughly mixed with 220.81 g of pre-moistened artificial test soil (equivalent to 200 g dry soil), to achieve a final soil moisture content of 50% WHC. The control received purified water sufficient to make the soil up to 50% WHC. Once treated, 23.88 g soil (20 g dry weight equivalent) were transferred into each replicate glass jar. An additional two abiotic replicate jars were also prepared for the test item treatment and the control for pH measurement at the start and end of the study. Within approximately 30 minutes of the soil being treated, 10 female soil mites were placed in each replicate (test start).

3. Sampling and measurements

In both the range-finding and definitive tests, the number of both surviving adults and of F1 progeny (i.e. juvenile test mites) in each test arena were assessed at day 14. For this assessment, the soil from each arena was placed into individual Tullgren funnel apparatus. This consisted of a meshed container suspended over a funnel. Above the funnel was fitted a light-bulb (25 Watts, with a 24-hour photoperiod). Over a two-day period, the heat of the bulbs slowly dried the soil from the top, forcing *H. aculeifer* to move downwards until they fell from the base of the funnels into collecting vials placed beneath. These vials contained 70% v/v methyl alcohol in which the mites drowned and were preserved.

Once the test soil had been removed from the arenas, the numbers of original adult and juvenile *H. aculeifer* that remained in the test arena were counted, with the use of a binocular microscope. In addition, the number of original adult and juvenile *H. aculeifer* in the collection vial arenas were counted, following extraction from the soil.

In a separate test, carried out by the test facility, the efficiency of the method used to extract the mites was determined to be 96.2% (98.3% for the adult female mites and 94.0% for the juvenile mites).

The weight of each test arena was noted at the start of the test. At day 7, the test arenas were reweighed and since the change in the weight was calculated to be > 2% of the soil's original water content, purified water was carefully added to restore the arenas' original weights. The weight of each arena was also measured at the end of the test.

At the start and end of the test, the pH of the artificial soil was measured. The test temperature in the controlled-environment cabinet was recorded hourly by data logger. Light intensities were recorded at the start of the bioassays.

4. Calculation of toxicity

The percentage mortality of the mites originally introduced was calculated for the test item treatment, both before and after correction for any control treatment losses using Abbott's formula (Abbott, 1925).

The mean number of offspring produced per replicate (including standard deviation) was calculated for each treatment. In addition, the percentage reduction in reproductive performance in the test item treatment, compared to the control, was calculated.

5. Statistics

The 14-day mortality data for the test-item treatment was compared to that for the control using Fisher's Exact Test ($\alpha = 0.05$) (Sokal and Rohlf, 1981). This test was applied to identify the LOEC or NOEC value for mortality. The LC_{50} could only be estimated with respect to the limit concentration tested.

For reproduction at day 14, both normality of the data (Shapiro-Wilk test, $\alpha = 0.05$) and homogeneity of variance (Levene's test, $\alpha = 0.05$) were checked, prior to comparison of the test item treatment to the control by t-test for independent samples ($\alpha = 0.05$) (Fowler et al., 1998). The results were used to determine the LOEC or NOEC value with respect to mite reproduction. The EC_{10}, EC_{20} and EC_{50} for reproduction could only be estimated with respect to the limit concentration tested.

Results and Discussion

The results of the study are presented in the following table.

Mite mortality at the end of the test after 14 days was 6% in the control and 5% at the limit concentration of 1000 mg product/kg dry soil. Therefore, no effect on mite mortality was observed at the tested concentration of 1000 mg product/kg dry soil (171 mg a.s./kg dry soil) which is the NOEC for mortality in this test. The LC_{50} was estimated to be > 1000 mg product/kg dry soil (> 171 mg a.s./kg dry soil).

The mean number of progeny per replicate determined at test termination was 271 in the control and 274 at the limit concentration of 1000 mg product/kg dry soil. Therefore, no effect on reproduction of *H. aculeifer* was observed at the tested concentration of 1000 mg product/kg dry soil (171 mg a.s./kg dry soil) which is the NOEC for reproduction in this test. The EC_{10}, EC_{20} and EC_{50} for reproduction were estimated to be > 1000 mg product/kg dry soil (> 171 mg a.s./kg dry soil).

The validity of the test was fulfilled since mortality of female adults in the control was 6% (required ≤ 20%) at the end of the test, the mean number of juveniles per replicate in the control was 271 (required ≥ 50) at the end of the test and the coefficient of variation of reproduction in the control was 9.9% (required ≤ 30%).

Table A 2.4.2.1.2-2: Effects of AG-T3-175 EC1 on mortality and reproduction of *Hypoaspis aculeifer*

Treatment		Mortality after 14 days of exposure [%]	Corrected[a] mortality [% ± SD]	Reproduction after 14 days	
[mg a.s./kg dry soil]	[mg product/kg dry soil]			[mean number of progeny/replicate]	Reduction compared to control [%]
0 (control)	0 (control)	6	-	271[b]	-
171	1000	5	-1	274	-1.1
Endpoints [mg a.s./kg dry soil][c] / [mg product/kg dry soil]					
EC_{10} (reproduction)		> 171 / > 1000			
EC_{20} (reproduction)		> 171 / > 1000			
EC_{50} (reproduction)		> 171 / > 1000			
NOEC (reproduction)		171 / 1000			
NOEC (mortality)		171 / 1000			

Note: A negative value represents an increase of parameter, relative to the control.
There were no significant differences in mortality (Fisher's Exact Test, α = 0.05) and reproduction (t-test for independent samples, α = 0.05) for the test item treatment in comparison with the control.

a) Corrected according to Abbott's formula.
b) Standard deviation: 26.8, coefficient of variation: 9.9%
c) Calculated by the applicant

Conclusion

In this test on effects of AG-T3-175 EC1 on mortality and reproduction of *Hypoaspis aculeifer*, the LC_{50}, EC_{10}, EC_{20} and EC_{50} (reproduction) were all determined to be > 1000 mg product/kg dry soil (> 171 mg a.s./kg dry soil), the limit concentration tested. The NOEC for both mortality and reproduction was 1000 mg product/kg dry soil (171 mg a.s./kg dry soil). All validity criteria were fulfilled.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.4.3 KCP 10.5 Effects on soil nitrogen transformation

A 2.4.3.1 Study 1: Effects on soil nitrogen transformation

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.5/01
Report	AG-T3-175 EC: Determinations of Effects on Soil Microflora Activity, Seyfried, B., 2009, B93227 (report number), 90018043_000081139 (sponsor report number)
Guideline(s):	Yes, OECD 216 (2000) and OECD 217 (2000)
Deviations:	Deviations to OECD 216 (2000): None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Material and Methods

A. MATERIALS

1. Test material	AG-T3-175 EC (emulsifiable concentrate formulation of the active substance trinexapac-ethyl)
Description	Yellow – red brown liquid
Lot/Batch #	D-I0703
Purity	Nominal / analysed: 175 / 180 g/L trinexapac-ethyl Density: 0.97 ± 0.02 g/cm³ (20°C)
Stability of test material	Stable under storage conditions Expiry date: January 2010
2. Vehicle and/or positive control	Vehicle/negative control: Water Positive control for carbon transformation: Dinoterb Positive control for nitrogen transformation: Nitrapyrin
Reference item 1	Dinoterb
Description	Not stated
Lot/Batch #	3209x
Purity	99.9% (GC)
Stability of reference item	Stable under storage conditions (at room temperature) Expiry date: 28 Jul 2010
Reference item 2	Nitrapyrin
Description	Not stated
Lot/Batch #	3230x
Purity	97.5% (GC)

Stability of reference item	Stable under storage conditions (at room temperature) Expiry date: 18 Aug 2010

3. Test system

Soil	Name:	Speyer 2.3
	Soil Type (USDA):	sandy loam
	Batch:	F2.3 35.08
	Organic C:	0.98%
	Nitrate content:	47 mg/kg dry soil
	Total nitrogen (N_{tot}):	0.05%
	pH ($CaCl_2$):	6.4
	pH (water):	7.82
	Cation exchange capacity:	8 mval/100 g soil
	Maximum water holding capacity (WHC):	34.4%
	Bulk density:	1291 g/1000 mL
	Particle size analyses (USDA):	
	< 0.002 mm (clay):	9.4%
	0.002-0.05 mm (silt):	29.8%
	> 0.05 (sand):	60.8%
	Microbial biomass:	211 mg microbial C/kg dry soil (2.2% of organic C)
Source	Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA, Speyer/ Germany)	
Soil history	The soil had not been subjected to any pesticide or organic fertilizer treatment in the sampling year and four former years. From 2004 to 2006, pumpkins were planted, in the year 2007 and in the sampling year, the soil was uncultivated.	
Soil sampling	End of August 2008 After sampling, the soil was 2-mm sieved.	
Soil preparation	The soil was sieved through a 5-mm sieve by the test facility. Prior to application, the soil moisture content was adjusted to just below 45% WHC and the soil was equilibrated at 20±2°C in the dark for a period of 14 days. Furthermore, all samples used in the nitrogen transformation test were amended with lucerne meal. The lucerne meal contained approximately 2.5% nitrogen.	

B. STUDY DESIGN AND METHODS

1. In-life dates 17 Sep 2008 to 05 Nov 2008

2. Experimental conditions

Test design

The influence of AG-T3-175 EC on the soil microflora was determined in a nitrogen and in a carbon transformation test. Soil samples of 150 g dry weight were set-up in 1-litre incubation flasks (stoppered with cotton wool plugs). Three replicates per treatment group and control were set up. After application, all samples were adjusted to 45% WHC, mixed thoroughly and incubated in the dark at 20±2°C for 28 days.

Test conditions

The incubation temperature was monitored continuously. The moisture content of the samples was monitored on a weekly basis and moisture loss was compensated for by the addition of purified water to maintain 45% WHC until the end of incubation.

Test concentrations

The target rates to be applied were 0.4 mg a.s./kg dry soil for the low dose and 2.0 mg a.s./kg dry soil for the high dose. This corresponds to application rates of 300 and 1500 g a.s./ha for the low and high dose, respectively, when assuming homogeneous distribution of AG-T3-175 EC in the top 5 cm soil layer and a soil bulk density of 1.5 g dry weight/cm³.

Control soil was not treated with the test substance but was incubated under identical conditions as treated soil.

Soil samples treated with the positive control for carbon transformation received 3.78 mg dinoterb/150 g dry soil, corresponding to 25 mg dinoterb/kg dry soil. Soil samples treated with the positive control for nitrogen transformation received 0.78 mg nitrapyrin/150 g dry soil, corresponding to 5 mg nitrapyrin/kg dry soil.

Treatment/Application

The application solution for the high dose was prepared by dissolving 80.58 mg AG-T3-175 EC in 50 ml double distilled water by continuously mixing in an ultrasonic bath. For the low dose application solution, 5.0 mL of the high dose application solution were diluted to 25 ml with purified water. Each soil sample (150 g dry weight) received 1000 µL of the corresponding application solution.

For the application with dinoterb, soil samples received 1.5 g quartz sand containing 3.78 mg dinoterb. For the application with nitrapyrin, soil samples received 1.5 g quartz sand containing 0.77 mg nitrapyrin. The amount of sand represented 10 g per kg soil.

All soil samples were mixed thoroughly to achieve a uniform distribution of the test substance/positive control.

3. Sampling and measurements

Carbon and nitrogen transformation were determined for all treatments at intervals of 0 (within 3 hours), 7, 14 and 28 days after treatment.

In the carbon transformation test, the microbial biomass of untreated soil was determined using soil sub-samples mixed with different concentrations of glucose and talc. The lowest amount of glucose resulting in a maximum CO_2 production, i.e. 0.58 g/kg dry soil was used for the short-term respiration experiment with the soil samples of all treatment groups. After amending the soil samples with glucose, the short-term respiration rates were measured by analysing evolved CO_2 for about 22 to 23 hours. In order to evaluate the influence of the test substance on carbon mineralisation in soil, the respiration rates of treated and control soil during the first 12 consecutive hours were compared.

In the nitrogen transformation test, the concentrations of nitrite and nitrate were determined for each sampling interval in a 2M KCl extract of the soil sample. Nitrite and nitrate (after reduction to nitrite) concentrations in the extract were quantified by triplicate measurement using a Flow Injection Analyzer (AutoAnalyzer 3, Bran+Luebbe). The initial nitrogen content (nitrate and nitrite) of the soil used in the study was measured in unamended and untreated soil samples at the start of the study.

4. Calculation of toxicity

Toxicity was evaluated based on the trigger value of 25% deviation of test parameters for treated samples when compared with the control.

5. Statistics

In the carbon and nitrogen transformation experiments, the mean of individual values at the end of their respective incubation period were statistically evaluated on a 5%-significance level ($\alpha = 0.05$, two-sided) by the Williams-test to find significant differences between control and treated samples.

Results and Discussion

A. CARBON TRANSFORMATION

The respiration rates in the control and treated samples for each assessment date are presented in Table A 2.4.3.1.1-1. The differences in respiration rate between both test substance doses and the control were found to be < 25% at any time interval. Therefore, AG-T3-175 EC tested at 0.4 mg a.s./kg dry soil (low dose) and 2.0 mg a.s./kg dry soil (high dose) had no adverse effect on carbon transformation in the tested soil.

The reference substances dinoterb had a clear inhibitory effect on the respiration rate of the soil microflora thereby showing the sensitivity of the test system and validity of the experimental design.

The variation between replicate control samples was less than 15%. Thus, the validity criterion for the test was fulfilled.

Table A 2.4.3.1.1-1: Effects of AG-T3-175 EC and dinoterb (positive control) on carbon transformation in soil (mean of 3 replicates)

Treatment	Incubation time [days]	Respiration rate [mg CO_2/h per kg dry soil]		
		mean	% SD	% deviation from control
Control	0	12.18	2.5	-
	7	12.16	1.1	-
	14	12.56	1.1	-
	28	10.99	0.1	-
Low dose (0.4 mg a.s./kg = 2.16 mg product/kg[a])	0	11.31*	1.7	-7.2
	7	11.57*	2.3	-4.8
	14	11.45*	2.5	-8.8
	28	10.53*	1.1	-4.2
High dose (2.0 mg a.s./kg = 10.8 mg product/kg[a])	0	11.55*	2.3	-5.2
	7	11.40*	1.6	-6.2
	14	11.25*	3.8	-10.4
	28	10.15*	1.9	-7.6
Dinoterb (25 mg/kg)	0	8.07*	2.1	-33.8
	7	8.05*	1.6	-33.7
	14	7.35*	2.4	-41.5
	28	5.38*	3.5	-51.0

* Value is significantly different from the control (Williams-test, two-sided, $\alpha = 0.05$)

a) Calculated by the applicant using the analysed content of 180 g/L trinexapac-ethyl and the density of 0.97 ± 0.02 g/cm³ (20°C).

B. NITROGEN TRANSFORMATION

The NO_2-N and NO_3-N contents and nitrate formation rates in the control and the test substance treated samples for each assessment date are presented in Table A 2.4.3.1.1-2. Nitrogen transformation of soil treated with the reference substance nitrapyrin was assessed in an additional test. The results are presented in Table A 2.4.3.1.1-3.

The difference in NO_2-N content between the low dose of the test substance and the control at day 0 was found to be > 25%. However at days 7, 14 and 28, the differences in NO_2-N contents between the low dose treatment and the control were < 25%. The differences in NO_2-N contents between the high dose treatment and the control were < 25% throughout the study. Therefore, the influence of the test substance on the turn-over of nitrite was negligible for both the low and the high dose.

Except for day 7 at the high dose, the differences in NO_3-N contents between the test substance treatments and the control were < 25% throughout the study. After 28 days of incubation, the differences in NO_3-N contents between both test substance doses and the control were well below the trigger value of 25%. When nitrate formation rates (mg NO_3^-/kg dry soil/day) were calculated, the differences between the test substance treatments and the control were < 25% at the end of the study, too. Thus, no influence of AG-T3-175 EC on microbial nitrogen transformation in the test soil was observed for both the low and the high dose.

The reference substances nitrapyrin had a clear inhibitory effect on nitrogen transformation of the soil microflora thereby showing the sensitivity of the test system and validity of the experimental design.

The variation between replicate control samples was less than 15%. Thus, the validity criterion for the test was fulfilled.

Table A 2.4.3.1.1-2: Effects of AG-T3-175 EC on nitrogen transformation in soil (mean of 3 replicates)

Treatment	Incubation time [days]	Nitrite [mg NO_2^-/kg dry soil]			Nitrate [mg NO_3^-/kg dry soil]			Nitrate formation rate[a)] [mg NO_3^-/kg dry soil/day]		
		mean	% SD	% dev. from control	mean	% SD	% dev. from control	mean	% SD	% dev. from control
Control	0	0.44	9.3	-	51.0	5.2	-	-	-	-
	7	0.09	9.2	-	12.7	9.5	-	-5.48	-3.1	-
	14	0.08	7.4	-	30.3	0.9	-	2.52	1.6	-
	28	0.09	n.a.	-	83.5	0.8	-	3.80	1.2	-
Low dose (0.4 mg a.s./kg = 2.16 mg product/ kg[b)])	0	0.20*	3.2	-54.5	49.9	2.8	-2.3	-	-	-
	7	0.09	9.5	-7.5	10.2*	12.4	-19.3	-5.66	-3.2	3.3
	14	0.07*	6.5	-14.1	23.0*	1.6	-24.3	1.82*	2.9	-27.9
	28	0.09	5.1	3.3	73.8*	0.6	-11.7	3.63*	0.8	-4.5
High dose (2.0 mg a.s./kg = 10.8 mg product/ kg[b)])	0	0.37*	3.4	-16.2	53.5	6.5	4.8	-	-	-
	7	0.09	6.8	-1.1	8.8*	3.1	-30.8	-6.39	-0.6	16.6
	14	0.07*	3.3	-16.5	23.1*	3.7	-23.9	2.05*	5.9	-18.8
	28	0.09	4.8	-4.4	67.3*	0.8	-19.5	3.16*	1.2	-17.0

n.a. not applicable
* Value is significantly different from the control (Williams-test, two-sided, $\alpha = 0.05$)
a) Calculated for the intervals of 0-7 days, 7-14 days and 14-28 days
b) Calculated by the applicant using the analysed content of 180 g/L trinexapac-ethyl and the density of 0.97 ± 0.02 g/cm³ (20°C).

Table A 2.4.3.1.1-3: Effects of nitrapyrin (positive control) on nitrogen transformation in soil (mean of 3 replicates)

Treatment	Incubation time [days]	Nitrite [mg NO_2^-/kg dry soil]			Nitrate [mg NO_3^-/kg dry soil]			Nitrate formation rate[a)] [mg NO_3^-/kg dry soil/day]		
		mean	%SD	% dev. from control	mean	%SD	% dev. from control	mean	% SD	% dev. from control
Control	0	0.37	2.5	-	34.8	4.7	-	-	-	-
	7	0.10	9.8	-	4.6	1.5	-	-4.32	-0.2	-
	14	n.a.	n.a.	-	14.9	0.5	-	1.47	0.7	-
	28	0.11	9.4	-	52.6	2.6	-	2.70	3.7	-
Nitrapyrin (5 mg/kg)	0	0.35*	1.3	-4.6	32.42*	6.1	-6.9	-	-	-
	7	< 0.07	n.a.	n.a.	< 0.6	n.a.	n.a.	-4.56*	-0.7	5.4
	14	< 0.05	n.a.	n.a.	4.21*	4.1	-71.6	0.53*	4.7	-64.1
	28	< 0.05	n.a.	n.a.	7.21*	1.4	-86.3	0.21*	3.4	-92.1

n.a. not applicable
* Value is significantly different from the control (Williams-test, two-sided, $\alpha = 0.05$)
a) Calculated for the intervals of 0-7 days, 7-14 days and 14-28 days

Conclusion

AG-T3-175 EC caused no adverse effects on soil nitrogen transformation (measured as NO_2-N and NO_3-N production and nitrate formation rate) and on soil carbon transformation (measured as respiration rate) after 28 days of incubation when applied to a sandy loam soil at 0.4 mg a.s./kg dry soil (low dose, corresponding to 2.16 mg product/kg) and 2.0 mg a.s./kg dry soil (high dose, corresponding to 10.8 mg product/kg). The validity criteria were fulfilled.

A 2.5 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.5.1 KCP 10.6.1 Summary of screening data

A 2.5.2 KCP 10.6.2 Testing on non-target plants

A 2.5.2.1 Study 1: Vegetative vigour

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.6.2/01
Report	Terrestrial (non-target) plant test with Trinexapac-ethyl 175 EC: Vegetative vigour test of non-target terrestrial plants, Friedrich, S., 2008a, 08 10 48 030 S (report number), 90018044_000081140 (sponsor report number)
Guideline(s):	Yes, OECD 227 (2006)
Deviations:	Deviations to OECD 227 (2006): None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Material and Methods

A. MATERIALS

1. Test material	Trinexapac-ethyl 175 EC (= AG-T3-175 EC; emulsifiable concentrate formulation of the active substance trinexapac-ethyl)
Description	Yellow – red brown liquid
Lot/Batch #	D-I0703

Purity	Nominal / analysed: 175 / 180 g/L trinexapac-ethyl Density: 0.97 ± 0.02 g/cm³ (20°C)
Stability of test material	Stable under storage conditions Expiry date: January 2010
2. Vehicle and/or positive control	Vehicle: Deionised water No positive control tested
3. Test system	Four monocotyledonous and six dicotyledonous species were selected: ▪ Corn*: Zea mays, Gramineae, Monocotyledonae* ▪ Oat: *Avena sativa, Gramineae, Monocotyledonae* ▪ Perennial ryegrass: *Lolium perenne, Gramineae, Monocotyledonae* ▪ Onion: *Allium cepa, Liliaceae, Monocotyledonae* ▪ Lettuce: *Lactuca sativa, Asteraceae, Dicotyledonae* ▪ Cabbage: *Brassica oleracea, Brassicaceae, Dicotyledonae* ▪ Carrot: *Daucus carota, Apiaceae, Dicotyledonae* ▪ Tomato: *Lycopersicon esculentum, Solanaceae, Dicotyledonae* ▪ Cucumber, *Cucumis sativa, Cucurbitaceae, Dicotyledonae* ▪ Soybean: *Glycine max, Fabaceae, Dicotyledonae*

B. STUDY DESIGN AND METHODS

1. In-life dates	21 Oct 2008 to 18 Nov 2008

2. Experimental conditions

Test design

AG-T3-175 EC was tested for its influence on plant survival and plant growth of four monocotyledonous (corn, oat, perennial reygrass and onion) and six dicotyledonous (lettuce, cabbage, carrot, tomato, cucumber and soybean) plant species.

The test plants were grown in non-porous plastic flower pots (∅ 15 cm) containing 1.4 kg of a sandy loam soil. The number of plants per pot was two for corn, cucumber, tomato and soybean, four for lettuce, cabbage and carrot and five for oat, perennial reygrass and onion. Six to 15 pots per treatment were used to result in 30-32 plants per treatment. The plants were pre-cultured for 2-3 weeks after sowing to reach a growth stage of 12 - 13 according to BBCH key (corresponding to 2 – 3 true leaves). At BBCH stage 12 - 13, the application solutions were applied once onto healthy plants. The test duration (observation period) was 21 days after application.

Test conditions

The plants were grown under greenhouse conditions. They were watered daily with tap water using a bottom watering system via pot saucers. A light-dark cycle of 16 hours light and 8 hours darkness was obtained by complementing daylight with artificial light. The air temperature and the relative humidity in the greenhouse were continuously monitored over the test period and were in the range of 13–30°C and 38-92%, respectively.

Test concentrations

Per test plant species, six application rates plus an untreated control (deionised water only) were tested: 0.024, 0.048, 0.095, 0.19, 0.38 and 0.76 kg a.s./ha. For preparation of the application solutions, the exactly weighed amount of the test substance was mixed with deionised water without addition of solubility mediators.

The analytically determined test substance concentrations in the application solutions varied from 93.4% to 107.6% based on measured trinexapac-ethyl concentrations. Therefore, the reported biological results were based on the nominal application rates of the test substance.

Treatment/Application

The application solutions were sprayed onto the leaves and the soil substrate using an automatic application cabin (Festo GmbH, nozzle: Teejet 80015 EVS, pressure: 0.45 MPa, application speed: 1.5 km/h). The spray volume was equivalent to 400 L water/ha. Prior to application, the application cabin was calibrated. The actually applied test solution per area was checked by weighing four glass plates placed at representative spots of the application cabin.

Analytics

AG-T3-175 EC concentrations in the application solutions were quantified by analysing the active substance trinexapac-ethyl using HPLC with UV-detection at 280 nm (column: Phenomenex Aqua 5µ C18 125 A; 150 x 2 mm; eluent: acetonitrile/water (50/50, v/v) + 0.1% phosphoric acid; flow rate 0.3 mL/min.; temperature: 25°C (column oven); retention time of trinexapac-ethyl: approximately 5 min.). The analytical method was validated according to SANCO/3029/99 rev.4. Details of the analytical method validation are given in dRR Part B5.

3. Sampling and measurements

Assessments of plant survival and phytotoxic symptoms were carried out on days 7, 14 and 21 of the observation period. Additionally, the shoot fresh weight was determined at study termination on day 21 of the observation period.

4. Calculation of toxicity

Mortality (in %), effects on shoot fresh weight (mean, SD, CV and % reduction) and phytotoxic effects (necrosis, chlorosis and growth inhibition in %) were calculated for each plant species and treatment group.

5. Statistics

For statistical calculation of the mortality results, the Fisher's Exact Binomial test was used. The accepted significance level was $p \leq 0.05$ (one-sided greater).

The LR_{25}/LR_{50} and ER_{25}/ER_{50} were calculated by Probit analysis according to the maximum likelihood method. The goodness-of-fit of the model was evaluated by Pearson's Chi^2 test.

The NOER values were calculated using the Dunnett`s Multiple t-test Procedure (for homogeneous variances, two-sided, alpha = 0.05).

Results and Discussion

A. PLANT SURVIVAL

Post-emergence application of AG-T3-175 EC with rates up to 0.76 kg a.s./ha did not result in a significant effect on survival of any of the tested species. On day 21 of the observation period, the LR_{25} and LR_{50} values as well as the NOER for plant survival were determined to be > 0.76 kg a.s./ha for all tested species (Table A 2.5.2.1.1-1).

B. PLANT GROWTH

On day 21 of the observation period, plant growth in terms of shoot fresh weight was not affected by AG-T3-175 EC up to the highest application rate of 0.76 kg a.s./ha in onion and carrot. Therefore for these species, the ER_{25} and ER_{50} values as well as the NOER for plant growth were > 0.76 kg a.s./ha. At study termination (day 21 of the observation period), plant growth of cucumber and soybean was affected at > 0.38 kg a.s./ha, of oat and lettuce at > 0.19 kg a.s./ha, of corn, perennial ryegrass and cabbage at > 0.095 kg a.s./ha and of tomato at > 0.048 kg a.s./ha (NOER for these species). For corn, oat, perennial ryegrass, cucumber and soybean, the fresh weight decrease was less than 25% at the highest application rate of 0.76 kg a.s./ha. Therefore, for these species the ER_{25} and ER_{50} values for plant growth were determined to be > 0.76 kg a.s./ha. For lettuce, cabbage and tomato, the calculated ER_{25} values were 0.361, 0.241 and 0.144 kg a.s./ha and the calculated ER_{50} values were 0.683, 0.627 and 0.384 kg a.s./ha, respectively (Table A 2.5.2.1.1-1).

Table A 2.5.2.1.1-1: Effect of AG-T3-175 EC on plant survival and plant growth

Plant species	AG-T3-175 EC [kg a.s./ha] / [L product/ha][a)]					
	Survival (21 days after application)			Growth (fresh weight 21 days after application)		
	NOER	LR_{25}	LR_{50}	NOER	ER_{25}	ER_{50}
Zea mays corn	> 0.76 / > 4.22	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 (n.d.) / > 4.22 (n.d.)	0.095 / 0.528	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 (n.d.) / > 4.22 (n.d.)
Avena sativa oat	> 0.76 / > 4.22	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 (n.d.) / > 4.22 (n.d.)	0.19 / 1.06	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 (n.d.) / > 4.22 (n.d.)
Lolium perenne perennial ryegrass	> 0.76 / > 4.22	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 (n.d.) / > 4.22 (n.d.)	0.095 / 0.528	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 (n.d.) / > 4.22 (n.d.)
Allium cepa onion	> 0.76 / > 4.22	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 / > 4.22	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 (n.d.) / > 4.22 (n.d.)
Lactuca sativa lettuce	> 0.76 / > 4.22	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 (n.d.) / > 4.22 (n.d.)	0.19 / 1.06	0.361 (0.324-0.395) / 2.01 (1.80-2.19)	0.683 (0.627-0.756) / 3.79 (3.48-4.20)
Brassica oleracea cabbage	> 0.76 / > 4.22	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 (n.d.) / > 4.22 (n.d.)	0.095 / 0.528	0.241 (0.200-0.279) / 1.34 (1.11-1.55)	0.627 (0.539-0.758) / 3.48 (2.99-4.21)
Daucus carota carrot	> 0.76 / > 4.22	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 / > 4.22	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 (n.d.) / > 4.22 (n.d.)
Lycopersicon esculentum tomato	> 0.76 / > 4.22	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 (n.d.) / > 4.22 (n.d.)	0.048 / 0.267	0.144 (0.124-0.163) / 0.800 (0.689-0.906)	0.384 (0.346-0.432) / 2.13 (1.92-2.40)
Cucumis sativa cucumber	> 0.76 / > 4.22	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 (n.d.) / > 4.22 (n.d.)	0.38 / 2.11	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 (n.d.) / > 4.22 (n.d.)
Glycine max soybean	> 0.76 / > 4.22	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 (n.d.) / > 4.22 (n.d.)	0.38 / 2.11	> 0.76 (n.d.) / > 4.22 (n.d.)	> 0.76 (n.d.) / > 4.22 (n.d.)

Note: 95% confidence limits in brackets
n.d. could not be determined
[a)] Calculated by the applicant using the analysed formulation content of 180 g/L trinexapac-ethyl

C. PHYTOTOXIC SYMPTOMS

For onion, no phytotoxic symptoms were observed up to the highest application rate of 0.76 kg a.s./ha. Necrotic effects occurred in tomato and cucumber at the highest application rate of 0.76 kg a.s./ha and in corn, cabbage and soybean at ≥ 0.38 kg a.s./ha. Chlorosis was observed in cabbage, tomato, cucumber and soybean at the highest application rate of 0.76 kg a.s./ha. Growth inhibitory effects occurred in carrot, cucumber and soybean at the highest application rate of 0.76 kg a.s./ha, in oat and lettuce at ≥ 0.38 kg a.s./ha, in corn, perennial ryegrass and cabbage at ≥ 0.19 kg a.s./ha and in tomato at ≥ 0.095 kg a.s./ha. These results are based on the observations made at study termination (day 21).

Treatment group Trinexapac-ethyl 175 EC (kg a.i./ha)	Phytotoxic effects, effects on growth and effects on plant development (BBCH growth stage) Plant species				
	Zea mays	*Avena sativa*	*Lolium perenne*	*Allium cepa*	*Lactuca sativa*
	Necrosis (%) after 21 days of exposure				
Control	0	0	0	0	0
0.024	0	0	0	0	0
0.048	0	0	0	0	0
0.095	0	0	0	0	0
0.19	0	0	0	0	0
0.38	2	0	0	0	0
0.76	4	0	0	0	0
	Chlorosis (%) after 21 days of exposure				
Control	0	0	0	0	0
0.024	0	0	0	0	0
0.048	0	0	0	0	0
0.095	0	0	0	0	0
0.19	0	0	0	0	0
0.38	0	0	0	0	0
0.76	0	0	0	0	0
	Growth inhibition (%) after 21 days of exposure				
Control	0	0	0	0	0
0.024	0	0	0	0	0
0.048	0	0	0	0	0
0.095	0	0	0	0	0
0.19	6	0	8	0	0
0.38	14	15	20	0	23
0.76	28	18	32	0	46
	BBCH growth stage (%) after 21 days of exposure				
Control	16	16	22	14	16
0.024	16	16	22	14	16
0.048	16	16	22	14	16
0.095	16	16	22	14	16
0.19	16	16	22	14	16
0.38	15-16	16	22	14	15-16
0.76	15	15-16	21-22	14	15

Treatment group Trinexapac-ethyl 175 EC (kg a.i./ha)	Phytotoxic effects, effects on growth and effects on plant development (BBCH growth stage) Plant species				
	Brassica oleracea	*Daucus carota*	*Lycopersicon esculentum*	*Cucumis sativa*	*Glycine max*
	Necrosis (%) after 21 days of exposure				
Control	0	0	0	0	0
0.024	0	0	0	0	0
0.048	0	0	0	0	0
0.095	0	0	0	0	0
0.19	0	0	0	0	0
0.38	2	0	0	0	1
0.76	4	0	3	4	3
	Chlorosis (%) after 21 days of exposure				
Control	0	0	0	0	0
0.024	0	0	0	0	0
0.048	0	0	0	0	0
0.095	0	0	0	0	0
0.19	0	0	0	0	0
0.38	0	0	0	0	0
0.76	2	0	4	3	2
	Growth inhibition (%) after 21 days of exposure				
Control	0	0	0	0	0
0.024	0	0	0	0	0
0.048	0	0	0	0	0
0.095	0	0	6	0	0
0.19	10	0	21	0	0
0.38	26	0	51	0	0
0.76	53	6	71	8	12
	BBCH growth stage (%) after 21 days of exposure				
Control	16	15	16	53	16
0.024	16	15	16	53	16
0.048	16	15	16	53	16
0.095	16	15	16	53	16
0.19	16	15	15-16	53	16
0.38	15	15	15	53	16
0.76	14	15	14-15	51-53	15

Zea mays

The foliar application of Trinexapac-ethyl 175 EC onto corn plants had no effects on survival up to the highest application rate of 0.76 kg a.i./ha (equivalent to 4.34 L test item/ha).

Growth in terms of fresh weight was significantly reduced at the tested application rates including and above 0.19 kg a.i./ha, a NOER of 0.095 kg a.i./ha was calculated. The 21-day ER_{25} and ER_{50} values were determined to be > 0.76 kg a.i./ha.

Necrotic effects were observed for the rates of 0.38 and 0.76 kg a.i./ha. Growth inhibitory effects were observed for the rates including and above 0.19 kg a.i./ha.

No chlorotic effects were observed on day 21 up to the highest tested application rate of 0.76 kg a.i./ha.

Avena sativa

Application of Trinexapac-ethyl 175 EC to oat plants caused no effects on survival.

Regarding fresh weight decrease, a NOER of 0.19 kg a.i./ha was calculated. The 21-day ER_{25} and ER_{50} values were determined to be > 0.76 kg a.i./ha (equivalent to 4.34 L test item/ha).

No necrotic and chlorotic effects were observed on day 21 up to the highest tested application rate of 0.76 kg a.i./ha. Growth inhibitory effects were observed for the rates of 0.38 and 0.76 kg a.i./ha.

Lolium perenne
The foliar application of Trinexapac-ethyl 175 EC onto ryegrass plants had no effects on survival up to the highest application rate of 0.76 kg a.i./ha (equivalent to 4.34 L test item/ha).
Growth in terms of fresh weight on day 21 after application was significantly reduced at the tested application rates including and above 0.19 kg a.i./ha. The NOER with respect to growth was 0.095 kg a.i./ha.
No necrotic and chlorotic effects were observed on day 21 up to the highest tested application rate of 0.76 kg a.i./ha. Growth inhibitory effects were observed on day 21 including and above 0.19 kg a.i./ha.

Allium cepa
The foliar application of Trinexapac-ethyl 175 EC onto onion plants had no effects on survival and fresh weight up to the highest application rate of 0.76 kg a.i./ha (equivalent to 4.34 L test item/ha).
The 21-day NOER, ER_{25} and ER_{50} values were determined to be > 0.76 kg a.i./ha.
No necrotic, chlorotic and growth inhibitory effects were observed on day 21 up to the highest tested application rate of 0.76 kg a.i./ha.

Lactuca sativa
Application of Trinexapac-ethyl 175 EC to lettuce plants caused no effects on survival.
Growth in terms of fresh weight on day 21 after application was significantly reduced at the tested application rates of 0.38 and 0.76 kg a.i./ha (equivalent to 2.17 and 4.34 L test item/ha). The NOER with respect to growth was0.19 kg a.i./ha. The 21-day ER_{25} and ER_{50} values for fresh weight decrease were 0.361 kg a.i./ha and0.683 kg a.i./ha, respectively.
No necrotic and chlorotic effects were observed on day 21 up to the highest tested application rate of 0.76 kg a.i./ha. Growth inhibitory effects were observed for the rates of 0.38 and 0.76 kg a.i./ha.

Brassica oleracea
Application of Trinexapac-ethyl 175 EC to cabbage plants caused no effects on survival at any test rate.
Regarding growth in terms of fresh weight decrease, a NOER of 0.095 kg a.i./ha was calculated. The 21-day ER_{25} and ER_{50} values were determined to be 0.241 and 0.627 kg a.i./ha, respectively.
Necrotic effects were observed for the rates of 0.38 and 0.76 kg a.i./ha (equivalent to 2.17 and 4.34 L test item/ha). Slight chlorotic effects were observed for the rate of 0.76 kg a.i./ha. Growth inhibitory effects were observed on day 21 including and above 0.19 kg a.i./ha.

Daucus carota
The foliar application of Trinexapac-ethyl 175 EC onto carrot plants had no adverse effects on survival and fresh weight up to the highest application rate of 0.76 kg a.i./ha (equivalent to 4.34 L test item/ha).
The 21-day NOER, ER_{25} and ER_{50} values were determined to be > 0.76 kg a.i./ha.
No necrotic and chlorotic effects were observed on day 21 up to the highest tested application rate of 0.76 kg a.i./ha. Slight growth inhibitory effects were observed for the rate of 0.76 kg a.i./ha.

Lycopersicon esculentum
Application of Trinexapac-ethyl 175 EC to tomato plants caused no effects on survival.
Growth in terms of fresh weight on day 21 after application was significantly reduced at the tested application rates including and above 0.095 kg a.i./ha (equivalent to 0.54 L test item/ha). The NOER with respect to growth was 0.048 kg a.i./ha. The 21-day ER_{25} and ER_{50} values for fresh weight decrease were 0.144 kg a.i./ha and 0.384 kg a.i./ha, respectively.
Slight necrotic and chlorotic effects were observed for the rate of 0.76 kg a.i./ha. Growth inhibitory effects were observed on day 21 including and above 0.095 kg a.i./ha.

Cucumis sativa
Application of Trinexapac-ethyl 175 EC to cucumber plants caused no effects on survival at any test rate.
Regarding fresh weight decrease, a NOER of 0.38 kg a.i./ha was calculated. The 21-day ER_{25} and ER_{50} values were determined to be > 0.76 kg a.i./ha, respectively (equivalent to 4.34 L test item/ha).
Slight necrotic, chlorotic and growth inhibitory effects were observed on day 21 up to the highest tested application rate of 0.76 kg a.i./ha.

Glycine max
The foliar application of Trinexapac-ethyl 175 EC onto soybean plants had no adverse effects on survival and fresh weight up to the highest application rate of 0.76 kg a.i./ha (equivalent to 4.34 L test item/ha).

Growth in terms of fresh weight on day 21 after application was significantly reduced at the tested application rates including and above 0.76 kg a.i./ha (equivalent to 4.34 L test item/ha). The NOER with respect to growth was0.38 kg a.i./ha.
Necrotic effects were observed for the rates of 0.38 and 0.76 kg a.i./ha. Chlorotic effects and growth inhibitory effects were observed on day 21 up to the highest tested application rate of 0.76 kg a.i./ha.

D. VALIDITY CRITERIA

The seedling emergence in the control was in the range of 90-98% (required ≥ 70%) and the mean survival of emerged control seedlings was 100% (required ≥ 90%). Furthermore, control plants did not exhibit visible phytotoxic effects. Environmental conditions for a particular species were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source. Therefore, the validity criteria of the guideline were met.

Conclusion

Post-emergence application of AG-T3-175 EC resulted in no effects on plant survival of all test plants with LR_{25}, LR_{50} and NOER values being > 0.76 kg a.s./ha (> 4.22 L product/ha). No effects on plant growth based on shoot fresh weight were observed for onion and carrot (ER_{25}, ER_{50} and NOER > 0.76 kg a.s./ha, equivalent to > 4.22 L product/ha) while cucumber and soybean were affected at > 0.38 kg a.s./ha (> 2.11 L product/ha), oat and lettuce were affected at > 0.19 kg a.s./ha (> 1.06 L product/ha), corn, perennial ryegrass and cabbage were affected at > 0.095 kg a.s./ha (> 0.528 L product/ha) and tomato was affected at > 0.048 kg a.s./ha (> 0.267 L product/ha) (NOER for these species). For corn, oat, perennial ryegrass, lettuce, cabbage, tomato, cucumber and soybean, the ER_{25} values were > 0.76, > 0.76, > 0.76, 0.361, 0.241, 0.144, > 0.76 and > 0.76 kg a.s./ha (> 4.22, > 4.22, > 4.22, 2.01, 1.34, 0.800, > 4.22 and > 4.22 L product/ha) and the ER_{50} values were > 0.76, > 0.76, > 0.76, 0.683, 0.627, 0.384, > 0.76 and > 0.76 kg a.s./ha (> 4.22, > 4.22, > 4.22, 3.79, 3.48, 2.13, > 4.22 and > 4.22 L product/ha), respectively. All validity criteria were fulfilled.

A 2.5.2.2 Study 2: Seedling emergence

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.6.2/02
Report	Terrestrial (non-target) plant test with Trinexapac-ethyl 175 EC: Seedling emergence and seedling growth test of non-target terrestrial plants, Friedrich, S., 2008b, 08 10 48 029 S (report number), 90018045_000081141 (sponsor report number)
Guideline(s):	Yes, OECD 208 (2006)
Deviations:	Deviations to OECD 208 (2006): None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Material and Methods

A. MATERIALS

1. Test material	Trinexapac-ethyl 175 EC (= AG-T3-175 EC; emulsifiable concentrate formulation of the active substance trinexapac-ethyl)
Description	Yellow – red brown liquid
Lot/Batch #	D-I0703
Purity	Nominal / analysed: 175 / 180 g/L trinexapac-ethyl Density: 0.97 ± 0.02 g/cm³ (20°C)
Stability of test material	Stable under storage conditions Expiry date: January 2010
2. Vehicle and/or positive control	Vehicle: Deionised water No positive control tested
3. Test system	Four monocotyledonous and six dicotyledonous species were selected: ▪ Corn*: Zea mays, Gramineae, Monocotyledonae* ▪ Oat: *Avena sativa, Gramineae, Monocotyledonae* ▪ Perennial ryegrass: *Lolium perenne, Gramineae, Monocotyledonae* ▪ Onion: *Allium cepa, Liliaceae, Monocotyledonae* ▪ Lettuce: *Lactuca sativa, Asteraceae, Dicotyledonae* ▪ Oilseed rape: *Brassica napus, Brassicaceae, Dicotyledonae* ▪ Carrot: *Daucus carota, Apiaceae, Dicotyledonae* ▪ Tomato: *Lycopersicon esculentum, Solanaceae, Dicotyledonae* ▪ Cucumber, *Cucumis sativa, Cucurbitaceae, Dicotyledonae* ▪ Pea: *Pisum sativum, Fabaceae, Dicotyledonae*

B. STUDY DESIGN AND METHODS

1. In-life dates	28 Oct 2008 to 26 Nov 2008

2. Experimental conditions

Test design

AG-T3-175 EC was tested for its influence on seedling emergence and seedling growth of four monocotyledonous (corn, oat, perennial ryegrass and onion) and six dicotyledonous (lettuce, oilseed rape, carrot, tomato, cucumber and pea) plant species.

The test plants were sown into non-porous plastic flower pots (∅ 15 cm) containing 1.4 kg of a sandy loam soil. Between two and five plants per pot were sown depending on the plant species. Six to 15 pots per treatment were used to result in 30-32 plants per treatment. After sowing, the surface applications were made by spraying onto the soil.

The test duration (observation period) was 21 days after emergence of 50% of the control plants. The control plants of all the species reached an emergence rate of at least 50% between 3 and 8 days after sowing.

Test conditions

The plants were grown under greenhouse conditions. They were watered daily with tap water using a bottom watering system via pot saucers. A light-dark cycle of 16 hours light and 8 hours darkness was obtained by complementing daylight with artificial light. The air temperature and the relative humidity in the greenhouse were continuously monitored over the test period and were in the range of 13–30°C and 38–90%, respectively.

Test concentrations

Per test plant species, six application rates plus an untreated control (deionised water only) were tested: 0.025, 0.05, 0.10, 0.20, 0.40 and 0.80 kg a.s./ha. For preparation of the application solutions, the exactly weighed amount of the test substance was mixed with deionised water without addition of solubility mediators.

The analytically determined test substance concentrations in the application solutions varied from 97.2% to 109.5% based on measured trinexapac-ethyl concentrations. Therefore, the reported biological results were based on the nominal application rates of the test substance.

Treatment/Application

The application solutions were sprayed onto the soil substrate using an automatic application cabin (Festo GmbH, nozzle: Teejet 80015 EVS, pressure: 0.45 MPa, application speed: 1.5 km/h). The spray volume was equivalent to 400 L water/ha. Prior to application, the application cabin was calibrated. The actually applied test solution per area was checked by weighing four glass plates placed at representative spots of the application cabin.

Analytics

AG-T3-175 EC concentrations in the application solutions were quantified by analysing the active substance trinexapac-ethyl using HPLC with UV-detection at 280 nm (column: Phenomenex Aqua 5µ C18 125 A; 150 x 2 mm; eluent: acetonitrile/water (50/50, v/v) + 0.1% phosphoric acid; flow rate 0.3 mL/min.; temperature: 25°C (column oven); retention time of trinexapac-ethyl: approximately 5 min.). The analytical method was validated according to SANCO/3029/99 rev.4. Details of the analytical method validation are given in dRR Part B5.

3. Sampling and measurements

Assessments of seedling emergence, plant survival and phytotoxic symptoms were carried out on days 7, 14 and 21 of the observation period. Additionally, the shoot fresh weight was determined at study termination on day 21 of the observation period.

4. Calculation of toxicity

The seedling emergence (in %), effects on shoot fresh weight (mean, SD, CV and % reduction) and phytotoxic effects (necrosis, chlorosis and growth inhibition in %) were calculated for each plant species and treatment group.

5. Statistics

For statistical calculation of the emergence results, the Fisher's Exact Binomial test was used. The accepted significance level was $p \leq 0.05$ (one-sided greater).

The effect rates (LR_{25}/ER_{25} and LR_{50}/ER_{50}) could not be calculated due to the absence of an inhibitory effect of the test substance on seedling emergence and seedling growth of all test species up to the highest test concentration.

The NOER values were determined using the Dunnett`s Multiple t-test Procedure (for homogeneous variances, two-sided, alpha = 0.05).

Results and Discussion

A. SEEDLING EMERGENCE

Pre-emergence application of AG-T3-175 EC with rates up to 0.80 kg a.s./ha did not result in a significant decrease in seedling emergence in any of the tested species. On day 21 of the observation period, the LR_{25} and LR_{50} values as well as the NOER for seedling emergence were determined to be > 0.80 kg a.s./ha for all tested species (Table A 2.5.2.1.2-1).

B. SEEDLING GROWTH

Pre-emergence application of AG-T3-175 EC with rates up to 0.80 kg a.s./ha did not result in a significant decrease in seedling growth (measured as shoot fresh weight) in any of the tested species. On day 21 of the observation period, the ER_{25} and ER_{50} values as well as the NOER for seedling growth were determined to be > 0.80 kg a.s./ha for all tested species (Table A 2.5.2.1.2-1).

Table A 2.5.2.1.2-1: Effect of AG-T3-175 EC on seedling emergence and growth

Plant species	AG-T3-175 EC [kg a.s./ha] / [L product/ha][a)]					
	Seedling emergence (21 days after 50% seedling emergence)			Growth (21 days after 50% seedling emergence)		
	NOER	LR_{25}	LR_{50}	NOER	ER_{25}	ER_{50}
Zea mays corn	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)
Avena sativa oat	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)
Lolium perenne perennial reygrass	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)
Allium cepa onion	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)
Lactuca sativa lettuce	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)
Brassica napus oilseed rape	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)
Daucus carota **carrot**	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)
Lycopersicon esculentum tomato	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)
Cucumis sativa cucumber	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)
Pisum sativum pea	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 / > 4.44	> 0.80 (n.d.) / > 4.44 (n.d.)	> 0.80 (n.d.) / > 4.44 (n.d.)

Note: 95% confidence limits in brackets
n.d. could not be determined
a) Calculated by the applicant using the analysed formulation content of 180 g/L trinexapac-ethyl

C. PHYTOTOXIC SYMPTOMS

Pre-emergence application of AG-T3-175 EC with rates up to 0.80 kg a.s./ha had no phytotoxic effects on any tested species when observed on day 21 after 50 % emergence of the seedlings.

D. VALIDITY CRITERIA

The seedling emergence in the control was in the range of 90-98% (required ≥ 70%) and the mean survival of emerged control seedlings varied of 93-100% (required ≥ 90%). Furthermore, control seedling did not exhibit visible phytotoxic effects and the plants exhibited normal variation in growth and morphology. Environmental conditions for a particular species were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source. Therefore, the validity criteria of the guideline were met.

Conclusion

Pre-emergence application of AG-T3-175 EC with rates up to 0.80 kg a.s./ha did not result in a significant decrease in seedling emergence and seedling growth (measured as shoot fresh weight) in any of the tested species. On day 21 of the observation period, the LR_{25}/ER_{50} and LR_{50}/ER_{50} values as well as the NOER for seedling emergence and seedling growth were determined to be > 0.80 kg a.s./ha (> 4.44 L product/ha) for all tested species. All validity criteria were fulfilled.

A 2.5.3 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.6 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.7 KCP 10.8 Monitoring data