European Commission

Combined Draft (Renewal) Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

GIBBERELLINS (GA4, GA7)

Volume 3 – B.9 (PPP) – Novagib

Rapporteur Member State: Slovenia
Co-Rapporteur Member State: Slovakia

Version History

When	What
2019/April	Initial DRAR

Table of contents

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

Introduction

This document has been prepared to evaluate the European Gibberellin Task Force (Valent Biosciences Corporation (Sumitomo Chemical Agro Europe), Fine Agrochemicals Ltd, Globachem NV) application for EU renewal of the Annex I inclusion of active substance gibberellins (GA4, GA7). The document supplements and updates the corresponding Annex B section of the Draft Assessment Report produced during the first review of gibberellins (2005 - 2011).

In this report studies submitted for the first inclusion of gibberellin in Annex I to Directive 91/414/EEC and for the renewal of the approval of gibberellin have been evaluated.

The representative formulation "Novagib" contains 10 g/L pure gibberellin and is formulated as soluble concentrate (SC). The formulation is plant growth regulator used on apples and pears.

Previous EU assessment

The dossier to support the first inclusion of gibberellin in Annex I to Directive 91/414/EEC was submitted to Hungary as the Rapporteur Member State in June 2005. The Draft Assessment Report is dated August 2006. Final Addendum to Draft Assessment Report, containing all individually submitted addenda on gibberellins, was compiled by EFSA in October 2011.

Structure of this document

In each section of this document, the following headings (a)-b)) occur:

a) Previous evaluation (2005-2011)

Under this heading study reports submitted for the first inclusion of gibberellin in Annex I to Directive 91/414/EEC are summarised. These studies have been re-evaluated for the purpose of the renewal in the light of current scientific and technical knowledge. The endpoints from the studies were also re-assessed and if considered relevant, re-calculated. However, full details from each study have not been repeated in this DRAR - therefore this DRAR is not a "stand-alone document" and for full reference sometimes the reader needs to consult the DAR (2005-2011).

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

Under this heading studies submitted prior to Annex I inclusion, but no evaluation of such material was presented in the form of Addenda to the DAR and studies that were submitted to support the application for renewal of Annex I inclusion are evaluated, i.e. new studies.

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

Effects on birds

Avian toxicity

a) Previous evaluation (2005-2011)

Novagib (GA4/GA7 10g/L SL) was one of three representative formulations for the first inclusion of gibberellins GA4/GA7. Formulation toxicity data for effects of GA4/GA7 10g/L SL on birds was not submitted for the first inclusion of gibberellins GA4/GA7.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No new studies are available assessing the effects of the representative formulation, Novagib, on birds. All other components of the formulation Novagib (please see the corresponding DRAR Vol.4) are inert and/or of no ecotoxicological relevance, and hence are not expected to present any hazards to the environment. Therefore, data on the technical grade active substance, GA4/GA7, are considered applicable and relevant with regard to the evaluation of effects on birds of the formulated product. However, no data on acute toxicity of gibberellins GA4/GA7 on birds are available. Acute toxicity data for gibberellic acid GA3 are considered acceptable for risk assessment of GA4/GA7, based on similarities between GA3 and GA4/GA7. Additionally, there are available rat acute toxicity studies with gibberellic acid GA3 and gibberellins GA4/GA7 which show comparable toxicity. The acute toxicity endpoint LD_{50} is > 5000 mg/kg bw for both substances, GA3 and GA4/GA7. This further supports the read across approach.

No metabolites are considered relevant and hence no further evaluation of metabolite toxicity is considered necessary. GA4/GA7 is naturally occurring and any metabolites formed will also be naturally occurring and of low risk to terrestrial organisms.

Higher tier data on birds

An acceptable risk to birds was concluded at the first-tier risk assessment and a refined risk assessment is therefore not considered necessary.

Effects on terrestrial vertebrates other than birds

Acute oral toxicity to mammals

a) Previous evaluation (2005-2011)

Toxicity data for the representative formulation, Novagib, were previously submitted for the Annex I evaluation of GA4/GA7. This study is considered appropriate for the current assessment to support renewal of GA4/GA7 and no new studies are submitted assessing the acute oral toxicity to mammals. Details of this study can be found in DRAR Vol.3 B.6 (CP 7.1.1/01), demonstrating the low acute oral toxicity of Novagib to mammals (LD_{50} > 5000 mg/kg bw).

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No new studies have been submitted for evaluation of effects of the representative formulation, Novagib, on mammals.

Higher tier data on mammals

An acceptable risk to mammals was concluded at the first-tier risk assessment and a refined risk assessment is therefore not considered necessary.

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

Risk assessment for birds

A summary of the toxicity data for birds is provided below in Table 9.2.1- 1. No data are available assessing the toxicity of GA4/GA7 to birds. However, on the basis of the similarities between gibberellic acid (GA3) and GA4/GA7, and the high margin of safety obtained with the risk assessment for GA3 (see DRAR Vol.3 ProGibb 40SG B.9.2.1) the toxicity data for GA3 is considered acceptable to address the risk to GA4/GA7. This approach was considered acceptable during the Annex I approval of GA4/GA7 (EFSA Journal 2012;10(1):2502).

Table 9.2.1- 1 : Endpoints and effect values relevant for the risk assessment for birds

Species	Substance	Exposure system	Results	Reference
Colinus virginianus Bobwhite quail	GA3	Oral Acute	**LD_{50} > 2000 mg/kg bw**	CA 8.1.1.1/03
Colinus virginianus Bobwhite quail	GA3	Dietary Short-term	LDD_{50} > 1376 mg/kg bw/d **NOEL = 1376 mg/kg bw/d**	CA 8.1.1.2/01

Endpoints in bold are used in the risk assessment

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 risk assessment is based on the critical GAP of 1 x 12 g a.s./ha in apples and pears (BBCH 62 – 74).

The results of the acute and reproductive first-tier risk assessments are summarised in Table 9.2.1- 2.

Table 9.2.1- 2 : Screening assessment of the acute and long-term/reproductive risk for birds due to the use of Novagib in apples and pears

Intended use	**Apples and pears**				
Active substance/product	GA4/GA7/ Novagib				
Application rate (kg/ha)	1 x 0.012 BBCH 62-74				
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**
Orchards (all growth stages- screening step)	Small insectivorous bird	46.8	1	0.562	> 3651
Reprod. toxicity (mg/kg bw/d)	13.76*				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Orchards (all growth stages- screening step)	Small insectivorous bird	18.2	1 x 0.53	0.116	119

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.

* As a protective worst-case, the long-term NOEL for birds has been assumed to be 100-fold lower than the NOEC for short-term toxicity. Given that the NOEL for short-term toxicity was found to equate to the highest dose rate tested in the study, this is considered to be an acceptable approach.

The TERa and TERlt values are well above the relevant triggers (10 and 5, respectively) for all relevant growth stages in apples and pears. In the absence of long-term toxicity data for birds the long-term risk assessment was based on a worst-case toxicity value assuming the long-term NOEL to be 100-fold lower than the NOEL for short-term toxicity. Given that the NOEL for short-term toxicity was found to equate to the highest dose rate tested in the study, this is considered to be an acceptable approach. An acceptable long-term risk is concluded even under this worst-case assumption and further studies on sub-chronic and reproductive toxicity to birds and the associated expenditure of vertebrate test animals are therefore not justified. At the given application rate of 0.012 kg a.s./ha, the NOEL would have to be below 0.58 mg/kg bw, for TER_{lt} to be below 5 indicating high risk. The RMS considers that it is unlikely that gibberellins GA4/GA7 would have such high toxicity. Especially considering that GA4/GA7 is ubiquitous in the tissues of plants (CA 8.9/01) and therefore represents a habitual component of the diet in herbivorous birds and insectivorous birds that feed upon herbivorous arthropod prey. As GA4/GA7 is a natural dietary component of birds it is expected that the long-term toxicity value is indeed higher than 13.76 mg a.s./kg bw/d.

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). Since Novagib 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. Due to the characteristics of the puddle 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 0.5747 L/kg, GA4/GA7 belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	12		
Acute toxicity (mg/kg bw)	=	>2000	quotient =	<0.006
Reprod. toxicity (mg/kg bw/d)	=	13.76*	quotient =	0.872

* As a protective worst-case, the long-term NOEL for birds has been assumed to be 100-fold lower than the NOEC for short-term toxicity. Given that the NOEL for short-term toxicity was found to equate to the highest dose rate tested in the study, this is considered to be an acceptable approach.

The quotients are well below the trigger of 50 and therefore no further assessment is considered necessary to address the risk to birds due to exposure to GA4/GA7 via contaminated drinking water in puddles.

The log P_{ow} values of GA4 and GA7 are 2.34 and 2.25, respectively (EFSA Journal 2012;10(1):2502) and thus do not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

An acceptable risk to birds is concluded at the first tier following the proposed use of Novagib in apples and pears, without the need for specific risk mitigation measures. No further data are considered necessary.

Risk assessment for other terrestrial vertebrates

A summary of the toxicity data for mammals is provided below in Table 9.2.2- 1.

Table 9.2.2- 1 : Endpoints and effect values relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference
Rat	GA4/GA7	Oral Acute	**LD_{50} >5000 mg/kg bw**	CA 5.2.1/02
Rat	GA4/GA7	Dietary Two-generation reproductive	**NOAEL = 300 mg/kg bw/d**	CA 5.6.1/01
Rat	GA4/GA7 10 g/L formulation (identical to Novagib)	Oral Acute	LD_{50} >5000 mg/kg bw	CP 7.1.1/01

Endpoints in bold are used in the risk assessment

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 risk assessment is based on the critical GAP of 1 x 12 g a.s./ha in apples and pears (BBCH 62 – 74).

The results of the acute and reproductive first-tier risk assessments are summarised in Table 9.2.2- 2.

Table 9.2.2- 2 : Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of Novagib in apples and pears

Intended use	**Apples and pears**				
Active substance/product	GA4/GA7/ Novagib				
Application rate (kg/ha)	1 x 0.012 BBCH 62-74				
Acute toxicity (mg/kg bw)	> 5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Orchards (all growth stages- screening step)	Small herbivorous mammal	136.4	1	1.64	> 3049
Reprod. toxicity (mg/kg bw/d)	300				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Orchards (all growth stages- screening step)	Small herbivorous mammal	72.3	1 x 0.53	0.460	652

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.

The TERa and TERlt values are well above the relevant triggers (10 and 5, respectively) for all relevant growth stages in apples and pears, concluding an acceptable acute and long-term risk to mammals following the proposed use of Novagib in apples and pears. Furthermore, GA4/GA7 is ubiquitous in the tissues of plants (CA 8.9/01) and therefore represents a habitual component of the diet in herbivorous mammals.

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). 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 0.5747 L/kg, GA4/GA7 belongs to the group of less sorptive substances.

Effective application rate (g/ha)	=	12		
Acute toxicity (mg/kg bw)	=	>5000	quotient =	<0.0024
Reprod. toxicity (mg/kg bw/d)	=	300	quotient =	0.04

The quotients are below the trigger of 50 and therefore no further assessment is considered necessary to address the risk to mammals due to exposure to GA4/GA7 via contaminated drinking water.

The log P_{ow} values of GA4 and GA7 are 2.34 and 2.25, respectively (EFSA Journal 2012;10(1):2502) and thus do not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

An acceptable risk to mammals is concluded at the first tier following the proposed use of Novagib in apples and pears, without the need for specific risk mitigation measures. No further data are considered necessary.

B.9.3. EFFECTS ON AQUATIC ORGANISMS

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

a) Previous evaluation (2005-2011)

Novagib (GA4/GA7 10g/L SL) was one of three representative formulations at first inclusion of gibberellins GA4/GA7. Formulation toxicity data for effects of GA4/GA7 10g/L SL on fish, aquatic invertebrates and algae were submitted for the first inclusion of gibberellins GA4/GA7. For re-registration the applicant has submitted five new formulation toxicity studies with fish, aquatic invertebrates, algae and aquatic macrophytes. For the sake of completeness the original summaries of the studies submitted for the first inclusion of gibberellins GA4/GA7 on Annex I are provided below. All studies have been evaluated in the original DAR and have been considered in the first EU peer review. They were found to be valid. All studies give endpoints that are higher than the endpoints from the studies submitted for re-registration, therefore their results will not be used for risk assessment.

Summaries of the studies with GA4/GA7 10g/L SL submitted for first inclusion of gibberellins GA4/GA7:

Study 1 (III A 10.2.1/01) Rainbow trout

Reference:	[redacted] **(2004)**. The acute toxicity of GA_4/GA_7 10g/l SL to Rainbow Trout (*Oncorhynchus mykiss*) over a 96-hour exposure period, Report No. [redacted]
Testing laboratory:	[redacted]
Guideline(s):	OECD Guideline No. 203 (1993).
GLP:	Yes.
Deviations:	According to the OECD guideline the total hardness of the test water is recommended to be 10 and 250 mg $CaCO_3$ per litre. In this study it was 288 mg $CaCO_3$ per litre.
Validity:	Study is considered to be valid and is accepted.
Material and	**methods:**
Test substance:	Formulation GA_4/GA_7) 10g/l SL. CAS no.: 468-44-0 (GA_4), 510-75-8 (GA_7). Purity: 10 g/L, 0.97% w/w Lot/Batch no.: 1140.27.
Test organism:	Rainbow trout (*Oncorhynchus mykiss).* Juvenile, mean total length 51.3mm, mean wet weight 1.20 g.
Treatments:	Nominal concentrations were 0 (control) and 200 mg test item/L. Mean measured concentration were 134 mg test item/l.
Number of animals:	7 fish per treatment.
Duration:	Fish were exposed over a period of 96 hours to formulation GA_4/GA_7) 10g/l SL.
Test conditions:	A limit test was performed under semi-static conditions with daily replacement of the exposure media. Glass aquarium contained 12 L medium. Measurements of temperature, pH and dissolved oxygen concentrations were made at initiation and at daily intervals in all vessels, with measurements made in both the expired and fresh media. Environmental parameters (temperature: 14.5-15.5 ºC, pH: 8.2-8.5, total hardness: 288 mg $CaCO_3$/l, dissolved oxygen: 96-100 % ASV, photoperiod: 16

	hours light/8 hours dark) remained within acceptable limits throughout the test with the exception of total hardness. The fish were not fed throughout the exposure period or during the 48 hours that immediately preceded it.
Analytical methods:	A HPLC method of analysis was used to measure GA_4/GA_7) 10g/L SL in the exposure test solutions. Analyses were conducted for GA_4 only as no commercial standard was available for GA_7. The method was verified under the test conditions before exposure of the organisms to the test substance. Good recoveries were obtained for the 0.5 mg/L standard (101 to 107%) and consistent recoveries (71 to 75%) from the test solutions.
Biological observations:	Daily check for mortality, occurrence of sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in behaviour).
Statistical evaluation:	Statistical analysis was not needed.

Results:

Table B.9.2.1.3-3: Effects of formulation GA_4/GA_7) 10g/L SL on mortality of O. mykiss following 96-hour exposure in an acute toxicity test under semi-static conditions

Formulation GA_4/GA_7) 10g/L SL Mean measured concentration (mg/L)	**Observation period**			
	24 hours[a] Cumulative mortality[b] [%]	**48 hours Cumulative mortality [%]**	**72 hours Cumulative mortality [%]**	**96 hours Cumulative mortality [%]**
Control	0	0	0	0
134	0	0	0	0

[a] Including observations made at 0, 3 and 6 hours.
[b] Out of 7 fish per treatment.

Findings:

Non-lethal toxicity endpoints:	No sign of abnormalities occurred in the control and treated groups.
Analytical results:	The percentage recovery of GA_4 was consistent over the full test period with no significant reduction in recovery from aged solutions. However the percentage recovery calculated from the mean measured exposure concentrations was not within 20% of the nominal concentrations and therefore, the results are expressed in mean measured concentrations.
LC_{50} of positive control :	Potassium dichromate 96 hour LC_{50} 140 mg/L [127-157] Historical mean: 104.3 mg/L [SD: 29.23, %RSD 96 hour LC_{50} 28.02 %]

Conclusions

LC_{50}:	Based on mean measured exposure concentrations, the 96-hour LC_{50} of formulation GA_4/GA_7) 10g/l SL to *Oncorhynchus mykiss* was greater than 134 mg/L, the highest concentration tested.
NOEC:	The no observed effect concentration (NOEC) of formulation GA_4/GA_7) 10g/l SL after 96 h was 134 mg /L, the highest concentration tested at and below which there was no toxicant related mortality, or behavioural abnormalities.

Study 1 (III A 10.2.1/02) Daphnia magna

Reference:	**Craig, W. J. (2004a)**. The toxicity to *Daphnia magna* of GA_4/GA_7 10 g/l SL., Report No. ENV6921/050407. Globachem N.V. report.
Testing laboratory:	Chemex Environmental International Ltd, Cambridge, UK.
Guideline(s):	OECD Guideline No. 202 (1984). Method C.2 of Commission Directive 92/69EEC.
GLP:	Yes.
Deviations:	None.
Validity:	Study is considered to be valid and is accepted.
Material and	**methods:**
Test substance:	Formulation GA_4/GA_7) 10 g/l SL. CAS no.: 468-44-0 (GA_4), 510-75-8 (GA_7). Purity: 10 g/L, 0.97% w/w Lot/Batch no.: 1140.27.
Test organism:	Daphnia magna. Young Daphnia - less than 24 hours old.
Treatments:	Dissolution of the test substance without the need for co-solvents was achieved by mixing. Nominal concentrations were 0 (control) and 200 mg/L. Mean measured concentration was 146 mg/L.
Number of animals:	Twenty daphnids (4 replicates of 5) per treatment.
Duration:	48 h.
Test conditions:	For the test limit concentration a glass tube was used to transfer five Daphnia into four replicate 50 ml crystallising dishes containing 25 ml of the test sample concentration under semi-static condition. Four control vessels were prepared as 25 ml of dilution water, each containing five Daphnia. The test vessels were then placed into an incubator at a temperature of 20±1 °C with a light cycle of sixteen hours light and eight hours dark. The pH value, dissolved oxygen and temperature were measured on all solutions at the start of the study and at the end of the 48 hour exposure period. Environmental parameters (temperature: 20.0 °C, pH: 7.4-7.8, total hardness: 231 mg $CaCO_3$/l, dissolved oxygen: 99-100 % ASV) remained within acceptable limits throughout the test. The daphnids received no food during exposure and the test vessels were not aerated.
Analytical methods:	Samples of the test solutions in which the organisms were exposed were taken for analytical verification at 0 hours, before and after solution replacement at 24 hours and at the end of the 48 hour exposure period. An HPLC method of analysis was used to measure GA4/GA7 10 g/L SL in the exposure test solutions. Analyses were conducted for GA_4 only as no commercial standard was available for GA_7. The limit of detection was not determined, however the instrument detection limit was considered to be approximately 1/5 of the bottom calibration standard, i.e. 1 ug/ml (1/5 the limit of quantitation).
Biological observations:	The number of immobilized daphnids in each replicate test vessel was recorded at 24 and 48 hours of exposure. Immobilization was defined as those animals not able to swim within 15 seconds after gentle agitation of the test vessel. Biological observations and observations of the physical characteristics of each replicate test solution were also made and recorded at 0, 24 and 48 hours. The mean measured concentrations tested and the corresponding immobilization data derived from the definitive toxicity test were used to estimate the 24- and 48-hour median effective concentrations (EC50).

Statistical evaluation:	During this study, no concentration tested resulted in 50% immobilization, therefore the EC50 value was empirically estimated to be greater than the highest mean measured concentration tested.
Results:	Following 48 hours of exposure, no immobilization or sublethal effects were observed among daphnids exposed to the treatment level tested.

Table B.9.2.4.6-3: Effect of Formulation GA_4/GA_7) 10 g/l SL on immobilisation of Daphnia magna

Formulation GA_4/GA_7) 10 g/l SL Mean measured concentration (mg/L)	**Observation period (Initial population: 20 per treatment)**					
	3 Hours		**24 Hours**		**48 Hours**	
	No. immobile	**Cumulative immobilisation [%]**	**No. immobile**	**Cumulative immobilisation [%]**	**No. immobile**	**Cumulative immobilisation [%]**
Control	0	0	0	0	0	0
146	0	0	0	0	0	0

Findings:

Non-lethal toxicity endpoints:	Following 48 hours of exposure, no immobilization or sublethal effects were observed among daphnids exposed to the treatment level tested.
EC_{50} of positive control :	A positive control study using potassium dichromate as the reference material was conducted. Exposure conditions were the same as those in the definitive test. The 48 hour EC_{50} for potassium dichromate to D. magna was 0.72 mg/L. The 48 hour NOEC was 0.56 mg/L. These results are considered to be in the normal range for this material.
Analytical results:	The percentage recovery of GA_4 was consistent over the full test period with no significant reduction in recovery from aged solutions. However the percentage recovery (73.2 %) calculated from the mean measured exposure concentrations was not within 20% of the nominal concentrations and therefore, the results are expressed in mean measured concentrations.
Conclusions	
EC_{50}:	Based on mean measured exposure concentrations the acute 48-hour EC_{50} for Formulation GA_4/GA_7) 10 g/l SL to Daphnia magna was greater than 146 mg/L, the highest concentration tested.
NOEC:	The no observed effect concentration (NOEC) after 48 hours was 146 mg/L. The NOEC is defined as the highest concentration tested at and below which there were no toxicant-related immobilization or physical and behavioural abnormalities (e.g., lethargy), with respect to the control organisms.

Study 1 (IIIA 10.2.1/03) Pseudokirchneriella subcapitata

Reference:	**Craig, W. J. (2004b)**. The Growth Inhibition of the alga *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*) by GA4/GA7 10g/l SL., Report No. ENV6920/050407. Globachem N. V. Report.
Testing laboratory:	Chemex Environmental International Ltd, Cambridge, England.
Guideline(s):	OECD guideline No. 201 (1984). Method C3 of Commission Directive 92/69/EEC.
GLP:	Yes.

Deviations:	None.
Validity:	Study is considered to be valid and is accepted.
Material and	**methods:**
Test substance:	Formulation GA_4/GA_7) 10 g/l SL. CAS no.: 468-44-0 (GA_4), 510-75-8 (GA_7). Purity: 10 g/L, 0.97% w/w. Lot/Batch no.: 1140.27.
Test organism:	*Pseudokirchneriella subcapitata*.
Treatments:	Algae were exposed over a period of 72 hours to Gibberellins (GA_4/GA_7) at a nominal limit concentration of 200 mg/L. There was also a control treatment that comprised nutrient medium only.
Number of cells (initial) / replicates:	At test initiation the culture contained a nominal cell density of 10^4 cells per ml.
Duration:	72 hours.
Test conditions:	The test material was dissolved directly in culture medium. 6 test vessels for the control and 3 test vessels for each of the Gibberellins (GA_4/GA_7) exposure concentrations were prepared containing 100 mL of the test media. Measurements of pH were made at initiation and at termination. The temperature level and illumination was recorded every 24 hours. The temperature was 21 to 25 ^oC, with continuous illumination (6000 to 10000 Lux) and a shaking rate of 200 rpm. The pH was 7.8 -7.9.
Measurements:	Cell densities were counted directly, with a haemocytometer and microscope, in samples taken from each vessel at test initiation and after 24, 48, and 72 exposure.
Analytical method:	Where present in samples, algal cells were removed from the samples by centrifugation. Extraction of the sample was carried out immediately. An HPLC method of analysis was used to measure GA4/GA7 10 g/L SL in the exposure test solutions. Analyses were conducted for GA_4 only as no commercial standard was available for GA_7. The limit of detection was not determined, however the instrument detection limit was considered to be approximately 1/5 of the bottom calibration standard, i.e. 1 ug/ml (1/5 the limit of quantitation).
Statistical evaluation:	The NOEC, the concentration that caused no significant adverse effect, was determined by Bonferroni T test.
Results:	The control cultures increased by a factor of 145 during the test in line with the guidelines which state at least a factor of 16 should be attained after 72 hours.

Table B.9.2.6.1-6: Effects of Formulation GA_4/GA_7) 10 g/l SL on growth of Pseudokirchneriella subcapitata

Formulation GA_4/GA_7) 10 g/l SL mean measured concentration (mg/l)		**Cell densities ($\times 10^4$ cells/ml)[a]**	**Mean area under growth curves (% of control)[b]**	**Mean growth rate (% of control)[b]**
Control	24 h	5.83	-	-
	48 h	28.39	-	-
	72 h	145.28	-	-
151	24 h	4.0	-	-
	48 h	28.78	-1 [a]	9
	72 h	133.33	1	7

[a]Negative values indicate growth stimulation relative to the solvent control.

Findings:	
Non-lethal toxicity endpoints:	There were no abnormalities detected in the control or test cultures.
Analytical results:	The percentage recovery of GA_4 was consistent over the full test period with no significant reduction in recovery from aged solutions. However the percentage recovery calculated from the mean measured exposure concentrations was not within 20% of the nominal concentrations and therefore, the results are expressed in mean measured concentrations.
EC50 of positive control:	Potassium dichromate 72 hour E_bC_{50} = 0.47 mg/, E_rC_{50} = 1.13 mg/L. Historical mean: E_bC_{50} = 0.53 mg/, E_rC_{50} = 0.88 mg/L [RSD: 17.11 % for biomass integral, RSD: 16.22 % for growth rate].
Conclusions	
E_bC_{50}:	Based on mean measured exposure concentrations, the 72-hour E_bC_{50} of formulation GA_4/GA_7) 10g/l SL to *Pseudokirchneriella subcapitata* was greater than 151 mg/l, the highest concentration tested.
E_rC_{50}:	Based on mean measured exposure concentrations, the 72-hour E_rC_{50} of formulation GA_4/GA_7) 10g/l SL to *Pseudokirchneriella subcapitata* was greater than 151 mg/l, the highest concentration tested.
NOEC:	The NOEC based on mean measured test concentrations was 151 mg/l.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

New studies are submitted here to support the representative formulation Novagib in the renewal process.

A new study testing the acute toxicity of Novagib to the Rainbow trout, *Oncorhynchus mykiss*, is summarised below (CP 10.2.1/01) and referenced in DRAR Vol.2.

Data point addressed:	CP 10.2.1/01
Author(s) (year):	[redacted] (2010a)
Title:	Novagib: Acute Toxicity To Rainbow Trout (*Oncorhynchus mykiss*)
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	OECD 203 (1992)
Deviations:	None
GLP:	Yes
Endpoint:	96 h LC_{50} > 9700 mg product/L, equivalent to > 100 mg a.s./L

Executive Summary

In a 96-h acute toxicity study, rainbow trout (*Oncorhynchus mykiss*) were exposed to Novagib formulation at the limit test concentration of 100 mg a.s./L and a control under static conditions. One aquarium was maintained in each treatment and control group, with seven fish per test chamber. Observations for mortality were performed 3, 6, 24, 48, 72 and 96 hours after the start of the test. The numbers of individuals exhibiting signs of toxicity or abnormal behaviour also were evaluated. The 96-hour LC_{50} value was estimated to be > 100 mg a.s./L. The no mortality concentration and the NOEC were 100 mg a.s./L. Endpoints were based on the nominal concentration.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material: Novagib
Description: Clear colourless liquid
Lot/Batch #: 1401437008
Active substance: gibberellins GA4/GA7 at nominal 10 g/L
Content: 10.3 g a.s./L (measured)

Test animals
Species: Rainbow trout (*Oncorhynchus mykiss*)
Source: [redacted]
Number: 7 fish in the test item and control group
Age: Juvenile
Length: 4.6 – 5.4 cm
Weight: 1.17 – 1.99 g
Acclimation period: From 14 July to 26 July 2010 (start of the study)
Feeding: The fish were not fed during the test.

B. STUDY DESIGN AND METHODS

Test design
System: Static system
Duration: 96 hours
Test vessel: 20 litre glass exposure vessels
Dilution water: Dechlorinated laboratory tap water (circulated, filter and cooled)
Concentrations: 0 (control) and 100 mg/L (nominal concentration)

Environmental conditions
Oxygen content: ≥ 8.7 mg O_2/L
Temperature: 14-15 °C
pH: 7.4-8.6
Hardness: 140 mg/L as CaCO3
Photoperiod: 16 hours of light / 8 hours of darkness daily

Animal assignment and treatment
Two aquaria, each with seven fish were prepared for the treatment and control group. The nominal concentration of Novagib was (on the basis of the preliminary study) 9700 mg Novagib/L (equivalent to 100.0 mg a.s./L).

Dose preparations
The test medium was prepared just before introduction of the fish. The test item was dissolved into the test water as homogeneously as possible by intense stirring. No solvent substance was used. The control consisted of dilution water only.

Measurements/observations
Observations were performed at 3, 6, 24, 48, 72 and 96 hours after the start of the test. Mortality was determined at each observation period. The water temperature, pH values and dissolved oxygen concentrations were determined daily in the test medium of the treatment and control group. Water samples were collected from the treatment group and the control group at the start and at the end of the test to determine concentrations of the test substance. The samples were diluted and analysed by high performance liquid chromatography mass spectrometry using an external standard.

Statistics

Probit analysis as well as statistical comparisons was not deemed appropriate as no mortality occurred at the test concentration of 100 mg/L.

II. RESULTS AND DISCUSSION

The results of the study were based on the nominal concentration of the test item. Daily observations of mortality during the test are presented in Table 9.3.1- 1 below. No mortalities or symptoms of intoxication were observed in the control group and in the 100 mg a.s./L treatment throughout the test. Therefore, the 96h-LC_{50} was estimated to be > 100 mg a.s./L. The NOEC was 100 mg a.s./L (based on the nominal concentrations).

Table 9.3.1- 1 : Summary of results from acute fish toxicity study with Novagib

Nominal concentration (mg a.s./L)	Mean measured conc. (% of nominal)	Mortality rate of treated fish (dead fish/treated fish)					
		3 h	6 h	24 h	48 h	72 h	96 h
Control	-	0/7	0/7	0/7	0/7	0/7	0/7
100	95	0/7	0/7	0/7	0/7	0/7	0/7

III. CONCLUSION

The 96-hour LC_{50} value of Rainbow trout (*Oncorhynchus mykiss*) was estimated to be > 9700 mg Novagib/L (equivalent to > 100 mg a.s./L) and the corresponding NOEC was 9700 mg Novagib/L (equivalent to 100 mg a.s./L). Endpoints were based on the nominal concentration.

RMS comments and conclusions :

The OECD 203 Fish acute toxicity test aims to determine toxicity of test substance to fish by determining LC_{50} at 24h, 48h, 72h and 96h.

Validity: For the test to be valid, the following performance criteria should be met:

- The mortality of the control should not exceed 10% (or one fish if less than 10 are used) at the end of test **(in test: control mortality = 0%, condition fulfilled)**
- Constant conditions should be maintained as far as possible throughout the test and, if necessary, semi-static or flow-through procedures should be used **(in test: temperature from 14 to 15°C, dissolved oxygen from 8.7 to 9.7 mg/L, pH from 7.4 to 8.6, condition fulfilled)**
- The dissolved oxygen must have been at least 60% of the air saturation value throughout the test **(dissolved oxygen concentration of 6.2 mg/l represents 60% saturation at 14ºC in freshwater, in test: 8.7 to 9.7 mg/L, condition fulfilled)**
- There must be evidence that the concentration of the substance being tested has been satisfactory maintained and preferably it should be at least 80% of the nominal concentration throughout the test **(in test: from 94% to 98% of nominal value, condition fulfilled)**

The guideline requires that fish should be in good health and free from any apparent malformations. This was not reported in the study report.

According to the Commission regulation (EU) No 284/2013 Section 10 Point 9 of Introduction if the study can

be designed to determine an effective concentration (EC_x), the study should be conducted to determine an EC_{10} and EC_{20} along with corresponding 95 % confidence intervals. If EC_{10} and EC_{20} cannot be estimated a justification should be provided. This was not done.

Acceptability of the analytical methods used in the test:

Endpoints: The 96-hour LC_{50} is > 100 mg a.s./L (nominal), the limit concentration tested. In the absence of treatment-related mortality or behavioural abnormalities, the NOEC is considered to be 100 mg a.s./L.

Conclusion of the RMS: The fish acute toxicity study is valid.

A new study testing the acute toxicity of Novagib to *Daphnia magna* is summarised below (CP 10.2.1/02) and referenced in DRAR Vol.2.

Data point addressed:	CP 10.2.1/02
Author(s) (year):	Goodband, T. J. and Mullee, D. M. (2010b)
Title:	Novagib: Acute Toxicity To *Daphnia magna*
Laboratory report / project number:	0673/0014
Testing facility:	Harlan Laboratories Ltd., Shardlow, UK
Published:	No
Test guideline used:	OECD 202 (2004)
Deviations:	None
GLP:	Yes
Endpoint:	48 h EC_{50} > 9700 mg product/L, equivalent to > 100 mg a.s./L

Executive Summary

In a 48-h acute toxicity study, *Daphnia magna* were exposed to Novagib at the limit test concentration of 100 mg a.s./L and a control under static conditions. There were 20 daphnids, divided into 5 replicates, in the concentration and control group. Immobilisation was observed 24 and 48 hours after the start of the test. The 48-hour EC_{50} value was estimated to be > 100 mg a.s./L. The no immobility concentration and the NOEC were 100 mg a.s./L. Endpoints were based on the nominal concentration.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material: Novagib
Description: Clear colourless liquid
Lot/Batch #: 1401437008
Active substance: gibberellins GA4/GA7 at nominal 10 g/L
Content: 10.3 g a.s./L (measured)

Test animals

Species: *Daphnia magna*
Source: In-house laboratory cultures
Number: 20 daphnids (4 replicates of 5 animals) in the test item and control group
Age: < 24 h old at the beginning of the test
Acclimation period: No acclimatisation as the water used was similar to the culture water
Feeding: The test animals were not fed during the test

B. STUDY DESIGN AND METHODS

Test design

System:	Static system
Duration:	48 hours
Test vessel:	Glass jars (250 mL), containing 200 mL test media
Dilution water:	Reconstituted water
Concentrations:	0 (control) and 100 mg/L (nominal concentration)

Environmental conditions

Oxygen content:	8.6 – 8.9 mg/L
Temperature:	21 – 22 °C
pH:	7.1 – 8.1
Photoperiod:	16 hours of light / 8 hours of darkness daily

Animal assignment and treatment

Four vessels were used in the test item and control group. Each group comprised of twenty *Daphnia*, five in each of the four replicate vessels, each containing 200 mL test dilution. The nominal concentration of Novagib was 9700 mg Novagib/L (equivalent to 100.0 mg a.s./L).

Dose preparations

The test medium was prepared just before introduction of the daphnids. The test item was dissolved into the test water as homogeneously as possible inverting several times. No solvent substance was used. The control consisted of dilution water only.

Measurements/observations

The number of mobile and immobilised test animals was observed and recorded 24 and 48 hours after the start of the test. The oxygen concentrations and pH values of the control and the test solution were measured at the beginning and at the end of the test. The water temperature was measured daily. Water samples were taken from each test group at the start and from each replicates of the test and control group at the end of the test, to determine concentrations of the test substance. The samples were diluted and analysed by high performance liquid chromatography mass spectrometry using an external standard.

Statistics

Statistical analysis of the results as well as calculations of the EC50 was not deemed appropriate as no immobilisation occurred at the test concentration of 100 mg/L.

II. RESULTS AND DISCUSSION

The results of the study were based on the nominal concentration of the test item. Daily observations of immobility during the test are presented in Table 9.3.1- 2 below. No immobility was observed in the control group and in the 100 mg a.s./L treatment throughout the test. Therefore, the 48-hour-EC_{50} was estimated to be > 100 mg a.s./L. The NOEC was 100 mg a.s./L (based on the nominal concentration).

Table 9.3.1- 2 : Summary of results from acute *Daphnia* toxicity study with Novagib

Nominal concentration (mg a.s./L)	Mean measured conc. (% of nominal)	No. of treated animals	No. of immobilised animals	
			24 h	48 h
Control	-	20	0	0
100	95	20	0	0

III. CONCLUSION

The 48-hour EC_{50} value of *Daphnia magna* was estimated to be > 9700 mg Novagib/L (equivalent to > 100 mg a.s./L) and the corresponding NOEC was 9700 mg Novagib/L (equivalent to 100 mg a.s./L). Endpoints were based on the nominal concentration.

RMS comments and conslusions:

The OECD TG 202 *Daphnia* sp. acute toxicity test is designed to assess toxicity of chemicals towards daphnids. Immobilisation is recorded at 24 hours and 48 hours in order to calculate the EC_{50} at 48h.

Validity: According to OECD TG 202 for the test to be valid the following conditions should be fulfilled:

- In the control, including the control containing the solubilising agent, not more than 10 percent of the daphnids should have been immobilised **(in study: control daphnids immobilised: 0%, condition fulfilled)**

- The dissolved oxygen concentration at the end of the test should be ≥3 mg/l in control and test vessels **(in test: 8.6 – 8.9 mg/L, condition fulfilled)**

The RMS found no deviations from the test guideline.

According to the Commission regulation (EU) No 284/2013 Section 10 Point 9 of Introduction if the study can be designed to determine an effective concentration (EC_x), the study should be conducted to determine an EC_{10} and EC_{20} along with corresponding 95 % confidence intervals. If EC_{10} and EC_{20} cannot be estimated a justification should be provided. This was not done.

Acceptability of the analytical methods used in the test:

Endpoints: The 48h EC_{50}< 9700 mg/L (nominal), corresponding to 100 mg a.s./L.

Conclusion of the RMS: The *Daphnia* sp. acute toxicity test is considered valid.

A new study testing the effects of Novagib on algal growth is summarised below (CP 10.2.1/03) and referenced in DRAR Vol.2.

Data point addressed:	CP 10.2.1/03
Author(s) (year):	Vryenhoef, H. and Mullee, D. M. (2010)
Title:	Novagib: Algal Growth Inhibition Test
Laboratory report / project number:	0673/0015
Testing facility:	Harlan Laboratories Ltd., Shardlow, UK
Published:	No
Test guideline used:	OECD 201 (2006)
Deviations:	None
GLP:	Yes
Endpoint:	72 h E_rC_{50} = 6080 mg product/L, equivalent to 60 mg a.s./L 72 h E_yC_{50} = 6384 mg product/L, equivalent to 63 mg a.s./L

Executive Summary

Exponentially growing cultures of the unicellular green alga *Desmodesmus subspicatus* were exposed to Novagib at nominal concentrations of 0 (control), 1.0, 3.2, 10, 32 and 100 mg a.s./L under constant illumination and shaking at 24 ± 1 °C. The inhibition of growth compared to the control was determined over a period of 72 hours. The results of the test are based on the nominal concentrations. The 72 h E_rC_{50}, was found to be 60 mg a.s./L and the 72 h E_yC_{50} found to be 63 mg a.s./L.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material: Novagib
Description: Clear colourless liquid
Lot/Batch #: 1401437008
Active substance: gibberellins GA4/GA7 at nominal 10 g/L
Content: 10.3 g a.s./L (measured)

Test organisms
Species: *Desmodesmus subspicatus*, strain CCAP 276/20
Source: Culture collection of Algae and Protozoa (CCAP), Scotland
Cell concentration: Initial cell concentration = 4 x 10^3 cells/mL

B. STUDY DESIGN AND METHODS

Test design
System: Constant shaking at 100 – 150 rpm
Duration: 72 hours
Test vessel: 250 mL glass flask each containing 100 mL of medium
Dilution water: Culture medium
Concentrations: 0 (control), 1.0, 3.2, 10, 32 and 100 mg a.s./L (nominal concentration)

Environmental conditions
Temperature: 23 – 25 °C
pH: 4.1 – 7.6
Photoperiod: Continuous illumination (7000 lux)
Agitation: Shaking at approximately 150 rpm

Assignment and treatment

On the basis of a preliminary range finding study, *Desmodesmus subspicatus* were exposed to Novagib at nominal concentrations of 1.0, 3.2, 10, 32 and 100 mg a.s./L. An untreated control group was tested in parallel. The test design included three replicates at each test concentration and six replicates for the untreated control. At the start of the study the alga cell concentration was 4 x 10^3 cells/mL in each of the test cultures. To keep the algae in suspension, test flasks were placed on an orbital shaker table at approximately 150 rpm into the climate chamber.

Dose preparations

Nominal concentrations of Novagib were 101, 324, 1013, 3243 and 10134 mg Novagib/L (equivalent to 1, 3.2, 10, 32 and 100 mg a.s./L, respectively). Stock solutions at 100 and 32 mg a.s./L were prepared by dispersing 9708 and 3107 mg Novagib in 1000 mL of algal medium. The remaining test solutions were prepared from these stock solutions by serial dilution and distributed into the appropriate test flasks.

Measurements/observations

Samples were taken and the cell numbers counted, using a Coulter Multisizer Particle Counter at 0, 23, 48 and 72 in each testing flask during the 72-hour test. The pH was recorded at the beginning and at the end of the test in the controls and at each test concentration. The temperature was checked within the incubators daily. For analytical determination of the test concentrations samples were taken from each concentration level at the start of the test and from each testing flask at the end of the test. The samples were analysed by high performance liquid chromatography Mass Spectrometry using an external standard.

Statistics

One way analysis of variance incorporating Bartlett's test for homogeneity of variance (Sokal and Rohlf 1981) and Dunnett's multiple comparison procedure for comparing several treatments with a control (Dunnett 1955) was carried out on the growth rate and yield data after 72 hours for the control and all test concentrations to determine and statistically significant differences between the test and control groups. All statistical analyses were performed using SAS computer software package (SAS 1999 – 2001).

II. RESULTS AND DISCUSSION

The analysed test item concentrations varied between 85 and 100 percent of the nominal concentrations at the test start and end of the test so the results are based on nominal concentrations only. A summary of the endpoints from this study are presented in the table below.

Table 9.3.1- 3 : Toxicity of Novagib on the growth of *Desmodesmus subspicatus* (based on nominal concentrations)

Response Variable (0-72 h)	**Endpoints (mg a.s./L)**		
	EC_{50}	**NOEC**	**LOEC**
Growth rate (r)	60	32	100.0
Yield (y)	63	32	100.0

Validity criteria

All validity criteria were met as follows:

- The biomass in the control cultures increased exponentially by a factor of at least 16 within the 72-hour test period (increased by a factor of 63).
- The mean coefficient of variation for section-by-section specific growth rates in the control cultures (23%) did not exceed 35%.
- The coefficient of variation of average specific growth rates during the whole test period in the control cultures (2%) did not exceed 7%.

III. CONCLUSION

The 72 h E_rC_{50} and the 72 h E_yC_{50} values of *Desmodesmus subspicatus* were determined to be 6080 and 6384 mg product/L, respectively, which is equivalent to 60 and 63 mg a.s./L, respectively. The corresponding NOEC values were determined to be 3243 mg product/L, which is equivalent to 32 mg a.s./L. The results of the test are based on the nominal concentrations.

RMS comments and conclusion (for CA 8.2.6.1/01a and CA 8.2.6.1/01b):

The purpose of OECD 201 Freshwater alga and cyanobacteria growth inhibition test is to determine the effects of a substance on the growth of freshwater microalgae and/or cyanobacteria.

Validity: According to the OECD 201 test guideline the alga growth inhibition test is considered acceptable if the following validity criteria are met:

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 day-1. For the most frequently used species the growth rate is usually substantially higher **(in test: exponential growth factor 63, condition fulfilled)**

- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%. This criterion applies to the mean value of coefficients of variation calculated for replicate control cultures (**in test: 23%, condition fulfilled)**

- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. For other less frequently tested species, the value should not exceed 10% **(in test: 2%, condition fulfilled)**

Potassium dichromate was used as reference item. E_rC_{50} was 0.74 mg/L. E_yC_{50} was 0.37 mg/L. Both values are in the normal range for the substance.

According to the Commission regulation (EU) No 284/2013 Section 10 Point 9 of Introduction if the study can be designed to determine an effective concentration (EC_x), the study should be conducted to determine an EC_{10} and EC_{20} along with corresponding 95 % confidence intervals. If EC_{10} and EC_{20} cannot be estimated a justification should be provided. This was not done.

Acceptability of the analytical methods used in the test:

Endpoints: The 72h E_rC_{50} = 6080 mg product/L, corresponding to 60 mg a.s./L. The 72h E_yC_{50} = 6384 mg product/L, corresponding to 63 mg a.s./L.

Conclusion of the RMS: The freshwater alga and cyanobacteria growth inhibition test is considered valid.

A new study testing the toxicity of Novagib on the aquatic macrophyte, *Lemna minor*, is summarised below (CP 10.2.1/04) and referenced in DRAR Vol.2.

Data point addressed:	CP 10.2.1/04
Author(s) (year):	Scheerbaum, D. (2012)
Title:	Novagib: Aquatic plant toxicity test, *Lemna minor*, limit-test, semi-static, 7 days
Laboratory report / project number:	120508FM/ SLM15085
Testing facility:	Dr. U. Noack-Laboratorien, Sarstedt, Germany
Published:	No
Test guideline used:	OECD 221 (2006)
Deviations:	None
GLP:	Yes
Endpoint:	EC_{50} for all parameters > 100 mg product/L, equivalent to > 0.96 mg a.s./L

Executive summary

In a 7-day toxicity study, *Lemna minor* plants were exposed to Novagib formulation at the limit test concentration of 100 mg product/L and a control under semi-static conditions. Six replicates, each containing 3 plants with 4 fronds, were prepared for the treatment and control group. Frond numbers were determined at the start and end of the test and every 2-3 days (days 2 and 4), along with qualitative observations. At the end of the test dry weight was determined. The 7-day EC_{50} values for yield and growth rate for both frond numbers and biomass were > 100 mg product/L. Endpoints were based on analytically confirmed nominal concentrations.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material: Novagib
Description: Clear colourless liquid
Lot/Batch #: 1021437001
Active substance: Gibberellins A4 & A7
Content: 9.98 g a.s./L (measured)
Relative density: 1.04

Test organisms

Species: *Lemna minor*
Source: Federal Environment Agency (UBA), Berlin, Germany
Number: 3 plants per replicate
Stage: 4 fronds per plant
Weight: 0.9 mg per replicate at the start of the test
Acclimation period: 10 days under test conditions

B. STUDY DESIGN AND METHODS

Test design

System:	Semi-static system
Duration:	7 days
Test vessel:	500 mL crystallisation dishes
Dilution water:	Swedish Standard (SIS) Medium
Concentrations:	0 (control) and 100 mg/L (nominal concentration)

Environmental conditions

Temperature:	22-23°C
pH:	6.31-6.84
Light intensity:	114 μE.m-2.s-1

Organism assignment and treatment

Six replicates, each containing 3 plants with 4 fronds, were prepared for the treatment and control group. The nominal concentration of Novagib was (on the basis of the preliminary study) 100.0 mg product/L.

Dose preparations

The stock solution (100 mg product/L in test medium) was prepared on test start and every water renewal day. The control consisted of dilution water only.

Measurements/observations

Fronds were determined at the start and end of the test and every 2-3 days (days 2 and 4), along with qualitative observations. Fronds that visibly projected beyond the edge of a parent frond were counted as separate fronds. Fronds without pigmentation were not counted. At the end of the test dry weight was determined by drying the colonies from each test vessel (including root fragments) at 60°C. The starting biomass was determined based on a sample of colonies (same number of fronds as at the start of the test) taken from the same batch used to inoculate the test vessels. Concentrations of the active ingredient Gibberellin A4 were analysed via LC-MS/MS at the beginning and end of exposure and on every renewal day. The analytical method was sufficiently validated by procedural recoveries (97-99% at 50 and 100 mg product/L). pH was measured on start and end of the test and every renewal day. Room temperature was measured continuously and light intensity was measured at test start.

Statistics

NOEC values were determined using one way ANOVA following a normality test and an equal variance test.

II. RESULTS AND DISCUSSION

Validity Criteria: Environmental conditions were acceptable. Doubling time of frond number in the control is less than 60 hours with an average specific growth rate of 0.299 (mean of 6 replicates), which meets the validity criteria.

Reference Test: 2011-10-24 to 2011-10-31 with 3,5-Dichlorophenol achieved EC_{50} values of 2.49 and 2.24 mg/L for frond number (growth rate and yield inhibition respectively) and 2.82 and 1.66 mg/L for dry weight (growth rate and yield inhibition respectively).

Test results: Measured concentrations in the fresh and old media were 106-109% and 105-113% nominal, respectively. The effect values are therefore based on nominal. No effects were observed on growth. After 7 days, slight phytotoxic effects were seen in the treated groups (fronds vaulted and break up of plants). Test endpoints are summarised in Table 9.3.1- 4 below.

Table 9.3.1- 4 : Endpoints from a *Lemna* toxicity study with Novagib

	Frond number	**Dry weight**
Growth-rate inhibition (mg product/L)		
NOEC	100	100
LOEC	> 100	> 100
E_rC_{10}	> 100	> 100
E_rC_{20}	> 100	> 100
E_rC_{50}	> 100	> 100
	Frond number	**Dry weight**
Yield inhibition (mg product/L)		
NOEC	100	100
LOEC	> 100	> 100
E_yC_{10}	> 100	> 100
E_yC_{20}	> 100	> 100
E_yC_{50}	> 100	> 100

III. CONCLUSION

The NOEC was 100 mg product/L for all parameters. The EC_{50} was >100 mg product/L for all parameters. These endpoints are equivalent to 0.96 mg total a.s./L (calculated using the reported active substance content and density of the product).

RMS comments and conclusion:

The OECD TG 221 *Lemna* sp. growth inhibition test is designed to assess the toxicity of substances to freshwater aquatic plants of the genus *Lemna* (duckweed). The test endpoint is inhibition of growth expressed as EC_{50}.

Validity: According to OECD TG 221 for the test to be valid, the doubling time in control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an specific growth rate of 0.275 d^{-1}. In test: control average specific growth rate = 0.299 d^{-1}, corresponding to doubling time = 2.32 days, **condition fulfilled**.

No information is given regarding the contamination of monocultures (free from algae and protozoa), health or age of plants (young rapidly growing without lesions or discoloration). However, the study report states that the plants were healthy. The RMS considers this to be acceptable.

Acceptability of the analytical methods used in the test:

Endpoints: The 7-day EC_{50}, EC_{10}, EC_{20} values for frond number, biomass and growth rates based on frond number and biomass of *Lemna gibba* Novagib are > 100 mg product/L, corresponding to 0.96 mg a.s./L.

Conclusion of the RMS: The *Lemna* sp. growth inhibition test is considered valid.

A new study testing the toxicity of Novagib on the aquatic macrophyte, *Myriophyllum spicatum*, is summarised below (CP 10.2.1/05) and referenced in DRAR Vol.2.

Data point addressed:	CP 10.2.1/05
Author(s) (year):	Hermes, H. & Wydra, V. (2014)
Title:	Toxicity of Novagib to the aquatic plant *Myriophyllum spicatum* in a static growth inhibition limit test with a prior rooting phase
Laboratory report / project number:	90251215
Testing facility:	IBACON GmbH, Rossdorf, Germany
Published:	No
Test guideline used:	OECD Draft Guideline for a Proposed Test Method for the Rooted Aquatic Macrophyte, *Myriophyllum* sp., in a water-sediment System, July 22, 2013 and Revision of 22nd July 2013 Draft, December 02, 2013; OECD 219 (2013)- Sediment-Water Chironomid toxicity Using Spiked Water Also compliant with OECD 239: Water-Sediment *Myriophyllum spicatum* Toxicity Test, September 26, 2014
Deviations:	None
GLP:	Yes
Endpoint:	14 d EC_{50} for all parameters > 100 mg product/L, equivalent to > 0.95 mg a.s./L

Executive summary

The influence of Novagib on the growth of the dicotyl freshwater plant *Myriophyllum spicatum* was assessed in a static limit test. The 14-day E_yC_{50} and the 14-day E_rC_{50} were calculated to be > 100 mg Novagib/L for shoot length, wet weight and dry weight. The 14-day NOE_yC and the NOE_rC were both determined to be 100 mg Novagib/L.

I MATERIALS AND METHODS

A MATERIALS

Test item:	Novagib
Lot No.:	1021437010
CAS No. of ingredients:	GA4: 468-44-0 GA7: 510-75-8 GA4/GA7: 8030-53-5
Active ingredient content:	10 g/L (nominal); 9.92 g/L (analytical)
Relative density:	1.04
Expiry date:	December 2015
Description:	Colourless liquid

Test organisms

Species:	*Myriophyllum spicatum*
Source:	The sterile plants were obtained from IBACON's in-house culture. Originally the cultures were obtained from the Bundesanstalt für Gewässerkunde, Referat Biochemie, Ökotoxikologie [G3], Am Mainzer Tor 1, 56068 Koblenz.

Test water

Medium:	Smart and Barko Medium ($NaHCO_3$: 58.4 mg/L; $MgSO_4$ x 7 H_2O: 69.0 mg/L; $KHCO_3$: 15.4 mg/L; $CaCl_2$ x 2 H_2O: 91.7 mg/L)

Sediment

Preparation:	According to OECD 219, based on dry weight: 5% sphagnum moss, 75% quartz sand, 20% kaolin clay, $CaCO_3$ to adjust pH of final mixture to 6.9
Moisture content:	37.67% (measured)
Organic carbon content:	2.185%

Environmental conditions

Temperature:	19 -21 °C
Light intensity:	8930 - 9850 lux at water surface
Photoperiod:	16 h light; 8 h darkness
Dissolved oxygen:	8.7 – 9.0 mg/L at test start; 7.7 – 13.2 mg/L on day 7; 7.7 – 13.9 mg/L at test end
pH:	7.9 at test start; 8.8 – 9.6 on day 7; 9.4 – 9.8 at test end

B STUDY DESIGN

Introduction of Sediment: 350 g (± 10 %) sediment was filled into standard planting pots of a diameter which just fit into the glass vessels (the sediment covered a minimum of 70 % of the vessel bottom surface). Prior to that a filter paper was put on the bottom of the planting pot to avoid loss of sediment. A very thin layer of coarse quartz sand was added on the sediment surface in order to reduce suspension of sediment into the water.

Introduction of Plants: Healthy shoot apices from healthy culture plants (without any flowers) were clipped off at a length of 6 cm (± 0.5 cm). Five shoot tips were planted into each pot containing the sediment with two nodes covered into the sediment. In order to induce root development they were kept prior to the test start for 7 days in test water.

Introduction of Test Water: After potting the plants into the sediment the pots were set into the test beakers and the test water without any test item concentration was added very carefully in order to avoid any disturbance of the sediment. The level of test water was marked at the outside of the test beaker. If necessary, the water levels were filled up with deionised water during the study to the original starting volume to prevent concentration of the test item.
Exposure: After the pre-culture period on Day 0, two of the five plants in each test beaker were removed to leave with three largely homogeneously (size and appearance) performing individuals. The day of application of the test item was designated as Day 0 (= start of the test). The only concentration tested was nominal 100 mg test item/L. Additionally, a control was tested in parallel (test water without addition of the test item).

Replicates: 3 replicates per treatment and 6 control replicates, each replicate with 3 plants.

Duration of Prior Rooting Phase: 7 days

Exposure Time: 14 days

Observations and measurements

Total Shoot Length: The shoot length above the sediment and the length of any side shoot were measured from all plants used in the exposure phase at day 0 and at test end.

Wet and Dry Weight: For the determination of the wet and dry weight five additional pots each with five plants were prepared and kept simultaneously with the test beakers in the pre-culture period. After the pre-culture period these five pots were harvested and the plant biomass was determined from the three most homogeneous individuals of every pot to obtain the wet weight (from the whole plant) of the test start. For the weighing the plants were carefully blotted to remove the remaining test medium. Subsequently the dry weight of these plants was determined by drying them at 60 °C for two hours. At test end the fresh and dry weight of every test plant was measured in the same manner as at the test start.

Observations: Any sublethal symptoms e.g. chlorosis or necrosis were recorded once during the test (Day 7) and at the end of the test. At test end the existence of roots and their appearance were also recorded.

Analysis of Test Item Concentrations

One sample from the freshly prepared stock solution and duplicate samples from the freshly prepared test media of the only test concentration and the control were taken at the start of the test. For the determination of the stability of the test item under the test conditions and of the maintenance of the test item concentration during the test period, duplicate samples were taken at the end of the test from the test medium and the control by pouring together the contents of each treatment. The concentrations of the test item Novagib were analysed by HPLC-UV in the duplicate test media samples from the only test concentration of nominal 100 mg/L from both sampling times. From the control samples duplicate samples were analysed from both sampling times.

II RESULTS AND DISCUSSION

A FINDINGS

Mean recovery of test item in the test samples: Freshly prepared: 104%; Aged test media: 94%

There was no inhibiting but a promoting effect on shoot lengths (Table 9.3.1- 5). Therefore, the 14-day EC_{50}, EC_{20} and EC_{10} for yield and growth rate based on shoot length were determined to be > 100 mg Novagib/L.

There was no statistically significant reduction in dry weight at 100 mg Novagib/L relative to the control (Table 9.3.1- 6). Therefore, the 14-day EC_{50}, EC_{20} and EC_{10} for yield and growth rate based on dry weight were determined to be > 100 mg Novagib/L.

A significant reduction was observed in wet weight in plants exposed to 100 mg Novagib/L relative to the control, for both yield and growth rate (Table 9.3.1- 7). However, as there is a very clear promoting effect on shoot length and no inhibitory effect on dry weights this slight reduction is not considered to be substance related. The most probable explanation for this discrepancy may be handling uncertainties during weighing. As the plants were first measured and then weighed the significantly longer plants at the test item concentration of 100 mg Novagib/L were longer air-dried than control plants (as the measurements took more time) which might have influenced the wet weights and caused the slight effects. Therefore, the 14-day EC_{50}, EC_{20} and EC_{10} for yield and growth rate based on wet weight were determined to be > 100 mg Novagib/L.

In the treatment group of 100 mg Novagib/L pink shoot tips were observed after 7 days of exposure and at test end. Furthermore, internodes were longer and side shoots were thinner compared to the control plants.

Table 9.3.1- 5 : Summary of effects on shoot length of *Myriophyllum spicatum* exposed to Novagib

Treatment rate (mg Novagib/L)	Mean shoot length (cm)		Yield, y, based on shoot length (cm) [a]	Growth rate, μ, based on shoot length (1/day) [a]
	0 days	14 days	14 days	14 days
Control	11.6	56.7	45.2	0.114
100	11.0	211.1	200.1 (-343.2%)	0.212 (-85.5%)

[a] Value in parentheses is the % inhibition relative to the control (negative values show an increase in yield/growth relative to the control)

* Mean value significantly different from the control (tested with Welch t-test (yield) and Student t-test, α = 0.05, one-sided). No notation depicts no significant effects.

Table 9.3.1- 6 : Summary of effects on dry weight of *Myriophyllum spicatum* exposed to Novagib

Treatment rate (mg Novagib/L)	Mean dry weight (mg)		Yield, y, based on dry weight (mg) [a]	Growth rate, μ, based on dry weight (1/day) [a]
	0 days	14 days	14 days	14 days
Control	68.4	173.4	105.0	0.066
100	68.4	191.0	122.5 (-16.7%)	0.073 (-10.0)

[a] Value in parentheses is the % inhibition relative to the control (negative values show an increase in yield/growth relative to the control)

* Values in bold are significantly different from the control (tested with Welch t-test (yield) and Student t-test, α = 0.05, one-sided). No notation depicts no significant effects.

Table 9.3.1- 7 : Summary of effects on wet weight of *Myriophyllum spicatum* exposed to Novagib

Treatment rate (mg Novagib/L)	Mean wet weight (mg)		Yield, y, based on wet weight (mg) [a]	Growth rate, μ, based on wet weight (1/day) [a]
	0 days	14 days	14 days	14 days
Control	592.4	2004.6	1412.2	0.087
100	592.4	1747.5	**1155.0 (18.2%)***	**0.077 (11.3%)***

[a] Value in parentheses is the % inhibition relative to the control (negative values show an increase in yield/growth relative to the control)

* Values in bold are significantly different from the control (tested with Welch t-test (yield) and Student t-test, α = 0.05, one-sided). No notation depicts no significant effects.

B VALIDITY

The test was considered valid as the following validity criteria were met:

- The mean total shoot length and mean total shoot fresh weight in control plants at least doubled during the exposure phase. (Compared to initial values (100%) control values after 14 days were 489% and 338% for shoot length and wet weight, respectively).
- The mean coefficient of variation in yield of fresh weight in the controls did not exceed 35% between replicates (15.7%).

III CONCLUSIONS

The influence of Novagib on the growth of the dicotyl freshwater plant *Myriophyllum spicatum* was assessed in a static limit test. The 14-day E_yC_{50} and the 14-day E_rC_{50} were calculated to be > 100 mg Novagib/L for shoot length, wet weight and dry weight. The 14-day NOE_yC and the NOE_rC were both determined to be 100 mg Novagib/L. These endpoints are equivalent to 0.95 mg a.s./L (calculated using the reported active substance content and density of the product).

RMS comments and conclusion:

The study was performed according to the

- OECD Draft Guideline for a Proposed Test Method for the Rooted Aquatic Macrophyte, *Myriophyllum* sp., in a water-sediment System, July 22, 2013 and Revision of 22nd July 2013 Draft, December 02, 2013;

- OECD 219 (2013)- Sediment-Water Chironomid toxicity Using Spiked Water

- OECD 239: Water-Sediment *Myriophyllum spicatum* Toxicity Test, September 26, 2014.

The study was evaluated according to the OECD test guideline 239 "Water sediment *Myriophyllum spicatum* toxicity test" (OECD, 2014). The OECD TG 239 Water-sediment *Myriophyllum spicatum* toxicity test is designed to assess chemical-related effects on the vegetative growth of the rooted *Myriophyllum* plants growing in standardised media in a water-sediment system. The test allows the distribution of the test chemical between water and sediment and enables an estimation of toxicity resulting from exposure via root uptake. Both average specific growth and yield of untreated and treated plants should be determined.

Validity: According to OECD test guideline 239 for the test to be valid, the following conditions must be fulfilled:

- the mean total shoot length and mean total shoot fresh weight in control plants at least double during the exposure phase of the test **(in test: control shoot length after 14 days was 489% of initial value, control wet weight after 14 days was 338% of initial value, condition fulfilled)**

- control plants must not show any visual symptoms of chlorosis and should be visually free from contamination by other organisms such as algae and/or bacterial films on the plants, at the surface of the sediment and in the test medium **(in test: not reported, condition NOT fulfilled)**

- The mean coefficient of variation for yield based on measurements of shoot fresh weight (i.e. from test initiation to test termination) and the additional measurement variables in the control cultures do not exceed 35% between replicates **(in test: coefficient of variation for yield in control based on measurements of shoot fresh weight = 15.7%, condition fulfilled)**

The pH value of control media increased for 1.7 units form 7.6 at start of the test to 9.6 at end of the test. This is more than 1.5 as recommended by the test guideline. However, the test guideline states that deviation of more than 1.5 does not invalidate the test if the validity criteria were met.

The appearance of the control plants is not reported in the study report, only appearance of treated plants, however it is stated in the study report that any sub-lethal symptoms e.g. chlorosis or necrosis were recorded once during the test (Day 7) and at the end of the test. The RMS assumes that control plants were also examined and that if any symptoms had occurred they would have been reported.

The results of this study show that gibberellins GA4/GA7 causes an increase in yield and growth rate based on shoot length. No concentration dependency was observed as only one concentration was tested. The study report provided by the applicant does not specify if these effects were statistically significant. Treatment groups that were significantly different from the control are marked with asterisk (*) in the results tables of the study report. It is stated in the study report that lack of notation indicates a lack of significance. Results of the treatment group were not noted with *, therefore the RMS presumes that the effects on yield and growth rate

were not statistically significant. However the observed effects were very high, e.g. a 343.2% increase in yield based on shoot length, shedding doubt on the presumed lack of significance. If the stimulative effects on growth were in fact statistically significant, the RMS is not comfortable stating that Novagib (gibberellins GA4/GA7) has no adverse effect on non-target aquatic plants. Considering that gibberellins GA4/GA7 is a plant growth regulator the observed effects could be a result of GA4/GA7 action. Gibberellins GA4/GA7 has the same mode of action as gibberellic acid GA3. Gibberellic acid was first identified as a metabolic by-product of the plant pathogen *Gibberella fujikuroi*, which afflicts rice plants. Infected plants grow so much taller than normal that they eventually die from no longer being able to support their own weight. The observed effect in rice plants shows that even an increase in plant growth can be an adverse effect. The RMS would like to ask other MS and EFSA for comments regarding this issue.

The study report states that a preliminary range finding test has been performed, but the test was not done in compliance with the GLP-regulations and is excluded from the Statement of Compliance in the final report. If the preliminary range finding test indicated adverse effects (also increase in yield and growth rate) at the highest tested concentration a dose-response design should have been used for the definitive test.

Acceptability of the analytical methods used in the test:

Endpoints: The EC_{50}, EC_{10}, EC_{20} values for all endpoints are estimated to be < 100 mg product/L, corresponding to < 0.95 mg a.s./L.

Conclusion of the RMS: The water sediment *Myriophyllum spicatum* test fulfils majority of validity criteria defined in OECD test guideline 239 and is thus considered to be valid. However, the study is not sufficient to address the risk of gibberellins GA4/GA7 to aquatic plants. A study with appropriate dose-response design is needed to determine toxicity of GA4/GA7 to aquatic plants. The RMS therefore concludes a data gap for adequate data to address effects of GA4/GA7 on aquatic plants.

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

a) Previous evaluation (2005-2011)

The studies were not submitted nor required for the first inclusion of gibberellins GA4/GA7.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No chronic toxicity studies are available assessing the effects of the representative formulation, Novagib, on aquatic organisms. All other components of the formulation Novagib are inert and of no ecotoxicological relevance, and hence are not expected to present any hazards to the environment. This is supported by the available acute data for the representative formulation, which show no evidence that Novagib is of greater acute toxicity than the active substance. Therefore, data on the technical grade active substance, GA4/GA7, are considered applicable and relevant with regard to the evaluation of long-term effects on aquatic organisms of the formulated product.

Further testing on aquatic organisms

a) Previous evaluation (2005-2011)

Further testing on aquatic organisms was not submitted nor required for the first inclusion of gibberellins GA4/GA7.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

An acceptable risk to aquatic organisms was concluded at the first-tier risk assessment and a refined risk assessment is therefore not considered necessary.

B.9.4. RISK ASSESSMENT FOR AQUATIC ORGANISMS

A summary of the toxicity data for aquatic organisms is provided below in Table 9.4- 1. As stated in the residue definition in CA 7.4.1, there are no surface water metabolites of environmental concern and the data provided are therefore limited to the active substance.

Table 9.4- 1 : Endpoints and effect values relevant for the acute risk assessment for aquatic organisms

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	GA4/GA7	96 h, ss	**LC_{50} >100 mg a.s./L $_{nom}$**	CA 8.2.1/01
Oncorhynchus mykiss	Novagib	96 h, s	**96 h LC_{50} >9700 mg product/L, equivalent to > 100 mg a.s./L $_{nom}$**	CP 10.2.1/01
Pimephales promelas	GA3	33 d, f, (ELS)	**NOEC = 11 mg a.s./L $_{mm}$**	CA 8.2.2.1/01
Daphnia magna	GA4/GA7	48 h, s	**EC_{50} >100 mg a.s./L $_{nom}$**	CA 8.2.4.1/01
Daphnia magna	Novagib	48 h, s	**48 h EC_{50} >9700 mg product/L,** equivalent to > 100 mg a.s./L $_{nom}$	CP 10.2.1/02
Daphnia magna	GA4/GA7	21 d, ss	**NOEC = 3.00 mg a.s./L $_{nom}$**	CA 8.2.5.1/01
Pseudokirchneriella subcapitata	GA4/GA7	72 h, s	E_rC_{50} >100 mg a.s./L $_{nom}$ E_bC_{50} >100 mg a.s./L $_{nom}$	CA 8.2.6.1/01
Navicula pelliculosa	GA4/GA7	72 h, s	E_rC_{50} >91.35 mg a.s./L $_{nom}$	CA 8.2.6.2/01
Desmodesmus subspicatus	Novagib	72 h, s	**E_rC_{50} = 6080 mg product/L, equivalent to 60 mg a.s./L $_{nom}$** E_yC_{50} = 6384 mg product/L, equivalent to 63 mg a.s./L $_{nom}$	CP 10.2.1/03
Lemna minor	Novagib	7 d, ss	**E_rC_{50} > 100 mg product/L, equivalent to > 0.96 mg a.s./L $_{nom}$** E_yC_{50} > 100 mg product/L, equivalent to > 0.96 mg a.s./L $_{nom}$	CP 10.2.1/04
Myriophyllum spicatum	Novagib	14 d, ss	**E_rC_{50} < 100 mg product/L, equivalent to < 0.95 mg a.s./L $_{nom}$** E_yC_{50} < 100 mg product/L, equivalent to < 0.95 mg a.s./L $_{nom}$	CP 10.2.1/05
Higher-tier studies (micro- or mesocosm studies)				
Not required				

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

Endpoints in bold are used in the risk assessment

The endpoints listed above indicate low toxicity of gibberellins GA4/GA7 and Novagib to aquatic organisms, except for aquatic plants. No studies with technical active substance were performed on aquatic plants. To address the risk to aquatic plants two studies with formulated product Novagib were performed, one with *Lemna minor* and the second with *Myriophyllum spicatum*. The first study with *Lemna minor* indicates low toxicity of gibberellins GA4/GA7 to aquatic plants (E_rC_{50} > 100 mg product/L, equivalent to > 0.96 mg a.s./L $_{nom}$). The secons study with *Myriophyllum spicatum* was designed as a limit-test and was not sufficient to determine toxicity of gibberellins GA4/GA7 to aquatic plant *Myriophyllum spicatum*. Based on study results (E_rC_{50} < 100 mg product/L) it is possible that effects occur at or below 1 mg/L. If effects had occurred at concentrations ≤ 1 mg/L, the product would be classified as category acute 1 and would not meet criteria for classfification as low risk substance.

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 chronic toxicity endpoint for fish used in the risk assessment is based on read-across from a study testing gibberellic acid GA3. This approach was used also for the avian risk assessment, as previously accepted by EFSA during Annex I (EFSA Journal 2012;10(1):2502), to avoid further unnecessary vertebrate testing. Read across between GA3 and GA4/GA7 is considered acceptable based on the similarities between GA3 and GA4/GA7, comparable low acute toxicity endpoints for fish (>100 mg a.s./L for both active substances), as well as the high margin of safety obtained in the risk assessment.

The relevant global maximum FOCUS Step 1 and 2 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the tables below. As discussed in CP 9.2.5, as the ratio of gibberellins GA4 and gibberellins GA7 in the active substance can vary between sources, for the determination of PECs of the active substance the approach adopted as a precautionary worst-case has been to consider alternate situations where the active substance is 100% gibberellins GA4 and separately 100% gibberellins GA7.

Table 9.4- 2 : Aquatic organisms: acceptability of risk (PEC/RAC < 1) for GA4/GA7 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of Novagib in apples and pears

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Macrophytes**	**Macrophytes**
Test species		***Oncorhynchus mykiss***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Desmodesmus subspicatus***	***Lemna minor***	***Myriophyllum spicatum***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}/E_yC_{50}	E_rC_{50}/E_yC_{50}
(µg/L)		>100000	11000	>100000	3000	60000	>960	<950
AF		100	10	100	10	10	10	10
RAC (µg/L)		>1000	1100	>1000	300	6000	>96	<95
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	8.58	<0.009	0.008	<0.009	0.029	0.001	<0.089	**>0.090**
Step 2								
S-Europe	1.66	<0.002	0.002	<0.002	0.006	<0.001	<0.017	**>0.018**

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

For the intended uses of Novagib, calculated PEC/RAC ratios indicate an acceptable risk for the most sensitive group of aquatic organisms, that is aquatic plants, based on *Lemna minor* endpoint E_rC_{50} of >960 µg/L in connection with an assessment factor of 10 in FOCUS Steps 1-2 scenarios. Risk assessment based on toxicity data for *Myriophyllum spicatum* (E_rC_{50} <950 µg/L) shows potentially unacceptable risk. The ratio PEC/RAC is > 0.018 in FOCUS Step 2 and could therefore be above 1. The risk to rooted aquatic macrophytes cannot be excluded. A further dose-response study needs to be performed to determine toxicity endpoint for *Myriophyllum spicatum* and to finalize the risk assessment.

Table 9.4- 3 : Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Novagib for each organism group based on FOCUS Step 3 calculations for its use in apples and pears

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Macrophytes**	**Macrophytes**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Desmodesmus subspicatus***	***Lemna minor***	***Myriophyllum spicatum***
Endpoint **(µg product/L)**		LC_{50} > 9700000	EC_{50} > 9700000	E_rC_{50} 6080000	E_rC_{50}/E_yC_{50} > 100000	E_rC_{50}/E_yC_{50} < 100000
AF		100	100	10	10	10
RAC (µg/L)		> 97000	> 97000	608000	> 10000	< 10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**					
Step 3 at default distance						
	163.6	<0.002	<0.002	<0.001	<0.016	**>0.016**

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

For the intended uses of Novagib, calculated PEC/RAC ratios indicate an acceptable risk for the most sensitive group of aquatic organisms (aquatic plants) based on *Lemna minor* endpoint E_rC_{50} of >100000 µg/L in connection with an assessment factor of 10 in FOCUS Step 3 scenarios. Risk assessment based on toxicity data for *Myriophyllum spicatum* (E_rC_{50} <10000 µg/L) shows potentially unacceptable risk. The ratio PEC/RAC is > 0.016 in FOCUS Step 3 and could therefore be above 1. The risk to rooted aquatic macrophytes cannot be excluded. A further dose-response study needs to be performed to determine toxicity endpoint for *Myriophyllum spicatum* and to finalize the risk assessment.

No acceptable risk to aquatic organisms can be concluded following the proposed use of Novagib in apples and pears. Further data are considered necessary to show acceptable risk.

B.9.5. EFFECTS ON ARTHROPODS

Effects on bees

Acute toxicity to bees

a) Previous evaluation (2005-2011)

A study with the current representative formulation Novagib was not submitted for the first inclusion of gibberellins GA4/GA7.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No acute toxicity studies are available assessing the effects of the representative formulation, Novagib, on bees. All other components of the formulation Novagib are inert and of no ecotoxicological relevance, and hence are not expected to present any hazards to the environment. Therefore, data on the technical grade active substance, GA4/GA7, are considered applicable and relevant with regard to the evaluation of acute effects on bees of the formulated product.

Chronic toxicity to bees

a) Previous evaluation (2005-2011)

Formulation toxicity data for chronic effects of Novagib on bees was not submitted nor required for the first inclusion of gibberellins GA4/GA7.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No chronic toxicity studies are available assessing the effects of the representative formulation, Novagib, on bees. All other components of the formulation Novagib are inert and of no ecotoxicological relevance, and hence are not expected to present any hazards to the environment. Therefore, data on the technical grade active substance, GA4/GA7, are considered applicable and relevant with regard to the evaluation of chronic effects on bees of the formulated product.

Effects on honey bee development and other honey bee life stages

a) Previous evaluation (2005-2011)

Formulation toxicity data for effects of Novagib on honey bee development was not submitted nor required for the first inclusion of gibberellins GA4/GA7.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No larval toxicity studies are available assessing the effects of the representative formulation, Novagib, on bees. All other components of the formulation Novagib are inert and of no ecotoxicological relevance, and hence are not expected to present any hazards to the environment. Therefore, data on the technical grade active substance, GA4/GA7, are considered applicable and relevant with regard to the evaluation of developmental effects on bees of the formulated product.

Sub-lethal effects

No further data available.

Cage and tunnel tests

An acceptable risk to bees was concluded at the first-tier risk assessment and further data is therefore not considered necessary.

Field tests with honey bees

An acceptable risk to bees was concluded at the first-tier risk assessment and further data is therefore not considered necessary.

Effects on non-target arthropods other than bees

Standard laboratory testing for non-target arthropods

a) Previous evaluation (2005-2011)

No study with the current representative formulation Novagib was submitted for the first inclusion of gibberellins GA4/GA7.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

Toxicity data for the representative formulation, Novagib, were not previously submitted for the Annex I evaluation of GA4/GA7. Therefore new studies are submitted here to support the representative formulation in the renewal process. A new study testing the toxicity of Novagib to the predatory mite, *Typhlodromus pyri*, is summarised below (CP 10.3.2.1/01) and referenced in DRAR Vol.2.

Data point addressed:	CP 10.3.2.1/01
Author(s) (year):	Jeker, L. (2010)
Title:	Novagib: Toxicity to the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae) under Worst-Case Laboratory Conditions
Laboratory report / project number:	C94908
Testing facility:	Harlan Laboratories Ltd., Itingen, Switzerland
Published:	No
Test guideline used:	Blumel *et al.* (2000)
Deviations:	None
GLP:	Yes
Endpoint:	LR_{50} > 80 L product/ha, equivalent to > 800 g a.s./ha

Executive Summary

In a worst-case laboratory study, *Typhlodromus pyri* (Acarina: Phytoseiidae) were exposed to dried residues of Novagib on glass plates at rates of 50, 100, 200, 400 and 800 g a.s./ha corresponding to 5, 10, 20, 40 and 80 L product/ha. The endpoints of the study were mortality after 7 days of exposure, including determination of the LR_{50}. Assessment of mortality was carried out 3 and 7 days after application. No reproduction phase was performed. After 7 days, mortality in the highest test item treatment was 2.99% (corrected mortality to the control). The LR_{50} was determined to be > 800 g a.s./ha or > 80 L product/ha.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:	Novagib
Description:	Clear colourless liquid
Lot/Batch #:	1401437008
Active substance:	gibberellins GA4/GA7 at nominal 10 g/L
Content:	10.3 g a.s./L (measured)
Test animals	
Species:	Predatory mites *Typhlodromus pyri* SCHEUTEN
Source:	Originally supplied by Syngenta Crop Protection, Switzerland and maintained in house as breeding stock since 2003.
Age:	Protonymphs, < 24 hours old after the moulting of the larvae
Number:	80 mites per treatment, reference substance and control (4 replicates of 20 protonymphs)

B. STUDY DESIGN AND METHODS

Test design	
Duration:	7 days (mortality)
Test units:	Two glass cover slides (approximately 24 x 50 mm) fixed together in longitude by a small strip of adhesive tape. The gap between the two sides was close enough to prevent escaping but sufficient to ensure availability of drinking water. The test arena was approx 10-13 cm^2 made of pure tangle-foot-glue in which the mites were confined. The test units were placed individually on a layer of wet filter paper covering a glass plate on a water soaked foam sponge.
Concentrations:	Control: deionised water applied at 200 L/ha Test substance: 50, 100, 200, 400 and 800 g a.s./ha, corresponding to 5, 10, 20, 40 and 80 L product/ha. applied at 200 L/ha Toxic standard: 16 mL product/ha applied at 200 L/ha
Application:	With a calibrated sprayer (Schachtner Spray-Lab) at 2 ± 0.2 mg/cm^2
Food:	Apple pollen from *Malus vulgaris* was supplied at day 0 and day 3, tap water was provided *ad libitum*

Environmental conditions

Temperature:	24.0 – 25.9 °C
Relative humidity:	79 – 88%
Photoperiod:	16 hours light : 8 hours dark (1192 - 1485 lux)

Animal assignment and treatment

The test units were treated and placed separately in a tray with tap water. After the spray residue had dried the protonymphs were transferred to test arenas and left to develop under continuous exposure to the residue.

Dose preparations

The test substance was prepared by mixing 100 mL of test item in 250 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 treatments were applied with calibrated spraying equipment.

Observations

The number of living, dead and escaped mites was counted on days 3 and 7 after application. A reproduction phase was not performed.

Statistics

The percentage mortality in the test substance and the reference treatment groups was corrected for mortality in the control group using Abbott's formula with improvements by Schneider-Orelli. The mean survival was compared between the test item treatment and the control. Due to low mortality (<5% in all the test item rates), the LR_{50} and its 95% confidence interval could not be calculated and were determined directly from the raw data.

II. RESULTS AND DISCUSSION

The cumulative mean mortality after 7 days was 16.3% in the water control group and 87.5% in the toxic reference group. The corrected mortality in the test substance treatments was -1.5-3% after 7 days. A summary of the results obtained for mortality is given in the following table.

Table 9.5.2.1- 1 : Mortality of *Typhlodromus pyri* exposed to fresh residues of Novagib in the laboratory

Treatment rate (g a.s./ha)	Mean mortality (%) Day 7	Corrected mortality (%)[1]
Control	16.3	-
50	17.5	1.49
100	17.5	1.49
200	13.8	-2.99 (A)
400	15.0	-1.49 (A)
800	18.8	2.99
Toxic reference	87.5	85.07

[1] Calculated using Abbott's/ Schneider-Orelli formula

(A) Note: negative effect % hence no adverse effect

The 7-day LR_{50} value was estimated to be > 800 g a.s./ha (> 80 L Novagib/ha) applied at 200 L/ha.

III. CONCLUSIONS

In a worst-case laboratory study with *Typhlodromus pyri* the 7-day LR_{50} value was estimated to be > 80 L Novagib/ha (800 g a.s./ha) applied at 200 L water/ha.

RMS comments and conclusion:

Test guideline 'The Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (*Acari: Phytoseiidae*) for regulatory testing of plant protection products' by Blümel et al (2000) aims to evaluate lethal and sub-lethal effects of a test item (spray formulation) on the predatory mite *Typhlodromus pyri*.

Validity: According to the test guideline the test is considered acceptable if the following validity criteria are met:

- The arithmetic mean mortality (dead and escaped individuals) in the control should not exceed 20% on day 7 after treatment application **(in test: control mortality = 16.3%, condition fulfilled)**
- The cumulative mean number of eggs per female in the control (from day 7 to day 14) should be ≥ 4 eggs/female **(in test: reproduction phase was not performed)**
- The cumulative mean mortality (control corrected) of protonymphs on day 7 exposed to toxic reference item should range between 50% and 100% **(in test: dimethoate mortality = 85.07%, condition fulfilled)**

Stability of the test compound was not tested in this study.

Acceptability of the analytical methods used in the test: Not applicable.

Endpoints: The 7 d LR_{50} is > 800 g a.s./ha, equivalent to > 80L product/ha.

Conclusion of the RMS: The test is considered valid.

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

a) Previous evaluation (2005-2011)

No study with the current representative formulation Novagib was submitted for the first inclusion of gibberellins GA4/GA7.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

An acceptable risk to non-target arthropods was concluded at the first-tier risk assessment and a refined risk assessment is therefore not considered necessary. However, a further extended laboratory study testing the effects of Novagib on the predatory bug, *Orius laevigatus*, is available. This study was submitted to support the assessment. This new study is summarised below (CP 10.3.2.2/01) and referenced in DRAR Vol.2.

Data point addressed:	CP 10.3.2.2/01
Author(s) (year):	Schmidt, T. (2011)
Title:	Novagib: Toxicity to the Predatory bug *Orius laevigatus* (Heteroptera: Anthocoridae) under Extended Laboratory Conditions
Laboratory report / project number:	C94910
Testing facility:	Harlan Laboratories Ltd., Itingen, Switzerland
Published:	No
Test guideline used:	Hassan, S.A (1992), Barrett, K.L. et al (1994), Bakker, F.M et al (2000)
Deviations:	None
GLP:	Yes
Endpoint:	LR_{50} = 43.1 L product/ha, equivalent to 431 g a.s./ha ER_{50} > 40 L product/ha, equivalent to > 400 g a.s./ha

Executive Summary:

The toxicity of Novagib to the predatory bug *Orius laevigatus* was determined in 10 days of exposure to the test item under extended laboratory conditions i.e. bean leaf discs as substrate, according to the IOBC/WPRS Guideline. The test item rates were 50, 100, 200, 400 and 800 g a.s/ha, corresponding to 5, 10, 20, 40 and 80 L Novagib/ha. A control, deionised water, and a reference item treatment, Roxion, containing 400 g Dimethoate/L, were tested in parallel. Eight replicates per treatment were set up. The mortality and reproduction values of the control and reference item were within the study validity criteria. The 10-day LR_{50} and ER_{50} were estimated to be 43.1 L Novagib/ha (431 g a.s./ha) and > 40 L Novagib/ha (> 400 g a.s./ha), respectively.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material: Novagib
Description: Clear colourless liquid
Lot/Batch #: 1401437008
Active substance: gibberellins GA4/GA7 at nominal 10 g/L
Content: 10.3 g a.s./L (measured)

Test animals
Species: *Orius laevigatus* (Fieber) (Heteroptera: Anthocoridae)
Source: Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth/Germany
Age: Nymphs in the second nymphal stage
Number: 5 nymphs/replicate, 8 replicates for each treatment group in the exposure phase; one mated *O. laevigatus* female/replicate, 15 replicates per treatment for the reproduction phase.

B. STUDY DESIGN AND METHODS

Test design
Duration: 10 days
Test units: The test units used to assess effects of the test item consisted of small plastic petri dishes covered with a lid. The inner part of the lid was cut out and covered with a fine mesh netting to allow air exchange in the test unit. The test unit was filled up with 20 mL of agar. Afterwards a bean leaf disc (French bean, *Phaseolus vulgaris* L., Fabaceae) was fixed on a solidified agar with a thin layer of fresh agar. The leaf was positioned with the lower leaf side facing upwards.
Concentrations: Control: deionised water applied at 200 L/ha
Test substance: 50, 100, 200, 400 and 800 g a.s./ha, corresponding to
5, 10, 20, 40 and 80 L Novagib/ha applied at 200 L/ha
Toxic standard: 25 mL product/ha
Application: With a calibrated sprayer (Schachtner Spray-Lab) at 2 ± 0.2 mg/cm^2
Food: Fed *ad libitum* with a supply of *Ephestia sp*. eggs

Environmental conditions
Temperature: 24 -25 °C
Relative humidity: 60-85%
Photoperiod: 2510 - 2900 lux, 16:8 day/dark rhythm

Endpoints
Test organisms were counted as dead when they were motionless even after touching them with a fine hairbrush. Mortality was calculated by adding the number of bugs which had escaped to the number of those that had died.

Statistical analysis
The LR_{50} and ER_{50} were determined directly from the raw data.

II. RESULTS AND DISCUSSION

After 10 days exposure (endpoint of the mortality assessment), the mean mortality values in the control and in the reference item treatment were 15 and 100%, respectively, and were therefore within the set validity criteria (i.e. mean mortality in the control: up to and including 20%; mean mortality in the reference item treatment: between 50 and 100%).

The mean corrected mortality in the test item treatments was 5.9% in the lowest test item treatment, varied between 23.5 and 29.4% in the treatments from 100 to 400 g a.s./ha and was 82.4% in the highest test item rate with 800 g a.s./ha.
The LR_{50} was calculated to be 43.1 L product/ha, which is equivalent to 431 g a.s./ha (95% confidence interval: 194-960 g a.s./ha).
Mean egg production in the control was 15.8 eggs per female and per two days and was therefore within the validity criterion for fecundity (i.e. at least 2 eggs per female per day). The hatch of the larvae from eggs in the control was 93.0% equal to 14.6 successfully hatched larvae per female and was therefore within the validity criterion for fertility (i.e. at least 70% hatching success in the control).
The overall reproduction values in the test item treatments up to and including 200 g a.s./ha ranged between 14.5 and 19.6 fertile eggs per female, which is 99-134% of the control values. In the treatment with 400 g a.s./ha, reproduction was reduced by 38% (i.e. 9.0 fertile eggs per female). A reproduction assessment was not performed at the highest test item rate since mortality was >50% in this assessment. The ER_{50} for reproduction was therefore set at >40 L product/ha, which is equivalent to >400 g a.s./ha.

III. CONCLUSIONS

In an extended laboratory study with *Orius laevigatus* the 10-day LR_{50} and ER_{50} were estimated to be 43.1 L Novagib/ha (431 g a.s./ha) and >40 L Novagib/ha (>400 g a.s./ha), respectively.

RMS comments and conclusions:

The study was conducted according to the test guideline 'A laboratory test for evaluating the effects of plant protection products on the predatory bug, *Orius laevigatus*', by Bakker et al (2000). The objective of the test is to identify potential adverse effects of a plant protection product on non-target leaf-dwelling insects. The test measures the mortality of *Orius laevigatus* nymphs and fertility of adults.

Validity: According to the currently valid test guideline by Bakker et al. (2000) the study is considered valid if the following criteria are met:

- the mortality in the control treatment does not exceed 25% and the mortality in the toxic reference is greater than 40% **(in test: mean control mortality = 15%, mortality of the toxic reference dimethoate = 100%, condition fulfilled)**
- the bugs in the control treatment produce a minimum of 2.0 eggs per female per day **(in test: 7.9 eggs per female per day, condition fulfilled)**
- there are less than five bugs in the control producing zero values **(in test: not reported)**
- the hatching rate in the control is greater than 70% **(in test: control hatching rate = 93%, condition fulfilled)**

In the reproduction phase 1 to 4 replicates per treatment were used, not 15 as stated in the summary provided by the applicant. This is less than 15 as required by the test guideline. The RMS considers this to be a major deviation. The results of reproduction phase are considered not reliable. This is also reflected in standard deviations for the reproduction part of the study, e.g. mean fecundity for treatment with 50 g a.s./ha is 14.5 with standard deviation 20.5 (14.5±20.5).

Temperature during the study was 24.4 – 25.4°C, not 24 – 25°C as stated in the summary provided by applicant.

There were 5 nymphs per replicate and 8 replicates per treatment used in this study; this is less than 10 nymphs

per replicate and 8 replicates per treatment as required by the test guideline. The RMS considers this a major deviation. The results are considered not reliable. This is also reflected in standard deviation for mortality, e.g. mean control mortality is 15% with standard deviation 14.1 (15±14.1 %).

The test lasted for 14 days, which is less than 21 days as defined in the test guideline.

Stability of the test compound was not tested in this study.

Due to several deviations from the test guideline the results of the study are considered not reliable.

Acceptability of the analytical methods used in the test: Not applicable.

Endpoints: The 10-day LR_{50} is 43.1 L product/ha, equivalent to 431 g a.s./ha.

Conclusion of the RMS: The test meets majority of the validity criteria and is thus considered to be valid. However, due to several deviations from the test guideline the results of the study are considered not reliable and will not be used in risk assessment.

Semi-field studies with non-target arthropods

An acceptable risk to non-target arthropods was concluded at the first-tier risk assessment and further data is therefore not considered necessary.

Field studies with non-target arthropods

An acceptable risk to non-target arthropods was concluded at the first-tier risk assessment and further data is therefore not considered necessary.

B.9.6. RISK ASSESSMENT FOR ARTHROPODS

Risk assessment for bees

A summary of the toxicity data for bees is provided below in Table 9.6.1- 1.

Table 9.6.1- 1 : Endpoints and effect values relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
Apis mellifera	GA4/GA7	Acute oral, 48 h	**LD_{50} >87 µg a.s./bee**	CA 8.3.1.1/01
Apis mellifera	GA4/GA7	Acute contact, 48 h	**LD_{50} >100 µg a.s./bee**	CA 8.3.1.1/02
Apis mellifera	GA4/GA7	Chronic oral, 10 days	LC_{50} >150 mg a.s./kg diet LDD_{50} >5.644 µg a.s./bee/day	CA 8.3.1.2/01
Apis mellifera	GA4/GA7	Larval, single exposure, 72 h	LD_{50} >100 µg a.s./larva	CA 8.3.1.3/01
Higher-tier studies (tunnel test, field studies)				
Not required				

Endpoints in bold are used in the risk assessment

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 draft EFSA bee guidance document (EFSA Journal 2013;11(7):3295) has not yet been formally agreed and noted.

The risk assessment is based on the critical GAP of 1 x 12 g a.s./ha in apples and pears (BBCH 62 – 74).

The results of the risk assessment are summarised in Table 9.6.1- 2.

Table 9.6.1- 2 : First-tier assessment of the risk for bees due to the use of Novagib in apples and pears

Intended use	Apples and pears		
Active substance	GA4/GA7		
Application rate (g/ha)	1 x12		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>87	12	<0.14
Contact toxicity	>100		<0.12

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

The acute oral and contact hazard quotients are below the trigger of 50, indicating an acceptable risk to bees following the proposed use of Novagib in apples and pears and a large margin of safety. Furthermore, GA4/GA7 is ubiquitous in the tissues of plants (CA 8.9/01) and therefore bees are likely naturally exposed to gibberellins when foraging for nectar, pollen, propolis and other botanical sources.

A formal risk assessment is not conducted for the chronic risk to honey bee adults and larvae as no agreed risk assessment scheme is available at the time of submission. However, the available data indicate low chronic toxicity of gibberellins GA4/GA7 to honey bee adults. No effects on mortality were observed at the highest concentration tested in the chronic adult toxicity study (LC_{50} >150 µg a.s./kg, corresponding to LDD_{50} >5.644 µg a.s./bee/day). A low larval toxicity was also observed for GA4/GA7, with no effects on mortality at the highest concentration tested (LD_{50} >100 µg a.s./larva). Furthermore, GA4/GA7 is ubiquitous in the tissues of plants (CA 8.9/01) and therefore adults and larvae are likely naturally exposed to gibberellins in nectar, pollen, propolis and other botanical sources.

An acceptable risk to bees is concluded at the first tier following the proposed use of Novagib in apples and pears, without the need for specific risk mitigation measures. No further data are considered necessary.

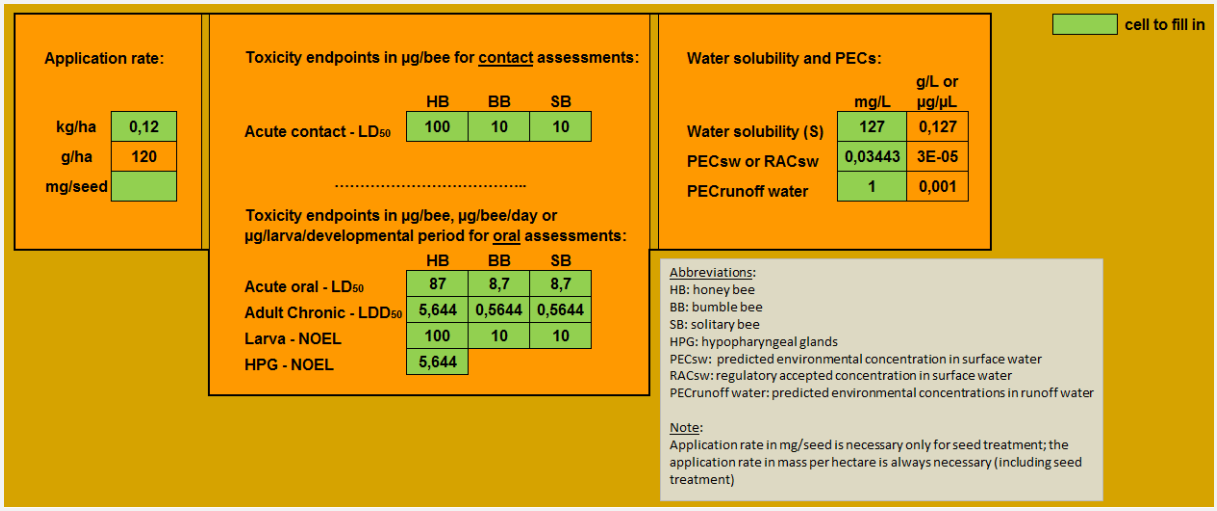
Additional RMS comments:

The RMS performed the risk assessment for bees according to "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002). The risk to bees was assessed as low with a large margin of safety. The HQ values were calculated to be <0.14, that is 300 times lower than the trigger value 50. No further studies with bees are required. No risk mitigation measures are required.

For illustrative purposes only, the RMS also performed screening and First tier risk assessment according to the

not yet noted draft EFSA bee guidance document (EFSA Journal 2013;11(7):3295). For risk assessment active substance toxicity data were used, as no formulation toxicity data were available. The results are presented below.

The input parameters are the following:



No data are available regarding HPG toxicity, therefore the lowest value form other toxicity studies with bees was used (5.644 µg a.s./bee/day).

Contact risk is acceptable for all species at First Tier risk assessment, as shown in the teble below.

scenario	BBCH	Honeybee		Bumble bee		Solitary bee	
		HQ	trigger	HQ	trigger	HQ	trigger
treated crop	≥ 40	0,0	85	0,0	14	0,0	16
weeds	≥ 40	0,4	42	3,6	7	3,6	8
field margin	≥ 40	0,2	42	1,9	7	1,9	8

Oral risk assessment is presented in the table below. Bold values represent unacceptable risk.

category	scenario	BBCH	Honeybee		Bumble bee		Solitary bee	
			ETR	trigger	ETR	trigger	ETR	trigger
acute	treated crop	40 - 69	0,01	0,2	**0,18**	0,036	**0,10**	0,04
acute	treated crop	≥ 70	0,00	0,2	0,00	0,036	0,00	0,04
acute	weeds	40 - 69	0,00	0,2	0,03	0,036	0,01	0,04
acute	weeds	≥ 70	0,00	0,2	0,03	0,036	0,01	0,04
acute	field margin	40 - 69	0,00	0,2	0,00	0,036	0,00	0,04
acute	field margin	≥ 70	0,00	0,2	0,00	0,036	0,00	0,04
acute	adjacent crop	40 - 69	0,00	0,2	0,00	0,036	0,00	0,04
acute	adjacent crop	≥ 70	0,00	0,2	0,00	0,036	0,00	0,04
acute	next crop	40 - 69	0,00	0,2	0,01	0,036	0,01	0,04
acute	next crop	≥ 70	0,00	0,2	0,01	0,036	0,01	0,04
chronic	treated crop	40 - 69	**0,13**	0,03	**1,75**	0,0048	**1,12**	0,0054
chronic	treated crop	≥ 70	0,00	0,03	0,00	0,0048	0,00	0,0054
chronic	weeds	40 - 69	0,01	0,03	**0,27**	0,0048	**0,11**	0,0054
chronic	weeds	≥ 70	0,01	0,03	**0,27**	0,0048	**0,11**	0,0054
chronic	field margin	40 - 69	0,00	0,03	**0,05**	0,0048	**0,02**	0,0054

chronic	field margin	≥ 70	0,00	0,03	**0,05**	0,0048	**0,02**	0,0054
chronic	adjacent crop	40 - 69	0,00	0,03	**0,05**	0,0048	**0,03**	0,0054
chronic	adjacent crop	≥ 70	0,00	0,03	**0,05**	0,0048	**0,03**	0,0054
chronic	next crop	40 - 69	0,01	0,03	**0,12**	0,0048	**0,08**	0,0054
chronic	next crop	≥ 70	0,01	0,03	**0,12**	0,0048	**0,08**	0,0054
larva	treated crop	40 - 69	0,01	0,2	**0,30**	0,2	0,12	0,2
larva	treated crop	≥ 70	0,00	0,2	0,00	0,2	0,00	0,2
larva	weeds	40 - 69	0,00	0,2	0,09	0,2	0,11	0,2
larva	weeds	≥ 70	0,00	0,2	0,09	0,2	0,11	0,2
larva	field margin	40 - 69	0,00	0,2	0,02	0,2	0,02	0,2
larva	field margin	≥ 70	0,00	0,2	0,02	0,2	0,02	0,2
larva	adjacent crop	40 - 69	0,00	0,2	0,02	0,2	0,01	0,2
larva	adjacent crop	≥ 70	0,00	0,2	0,02	0,2	0,01	0,2
larva	next crop	40 - 69	0,00	0,2	0,02	0,2	0,01	0,2
larva	next crop	≥ 70	0,00	0,2	0,02	0,2	0,01	0,2
HPG	treated crop	40 - 69	0,07	1	N/R	N/R	N/R	N/R
HPG	treated crop	≥ 70	0,00	1	N/R	N/R	N/R	N/R
HPG	weeds	40 - 69	0,01	1	N/R	N/R	N/R	N/R
HPG	weeds	≥ 70	0,01	1	N/R	N/R	N/R	N/R
HPG	field margin	40 - 69	0,00	1	N/R	N/R	N/R	N/R
HPG	field margin	≥ 70	0,00	1	N/R	N/R	N/R	N/R
HPG	adjacent crop	40 - 69	0,00	1	N/R	N/R	N/R	N/R
HPG	adjacent crop	≥ 70	0,00	1	N/R	N/R	N/R	N/R
HPG	next crop	40 - 69	0,00	1	N/R	N/R	N/R	N/R
HPG	next crop	≥ 70	0,00	1	N/R	N/R	N/R	N/R

Oral acute, larval and HPG risk to honeybees is acceptable. Chronic oral risk in the treated crop is unacceptable for honey bees.

Chronic oral risk is unacceptable for bumblebees in all scenarios (treated crop, weeds, field margin, adjacent crop, next crop). Larval risk is unacceptable for bumblebees in treated crop scenario.

Chronic oral risk is unacceptable for solitary bees in all scenarios (treated crop, weeds, field margin, adjacent crop, next crop).

In risk assessment for guttation the RMS assumed that the formulated product will be applied after the guttation period. It is our understanding that guttation rarely occurs in trees. Trees are considered to be too large to create the force needed to push xylem upward hard enough to cause guttation. Plants that most commonly experience guttation are non-woody and smaller than 3 feet tall, however, some shrubs and vines can show guttation as well. The exposute of bees to guttation water in apple and pear trees is therefore considered negligible and the risk is low.

Risk from drinking water is low for all species. There are no accumulatory effects.

Accumulative effects

	Choose answer	
Has the substance a potential for accumlative toxicity (see section 8.1.1.3 and pertinent part of Appendix O in the GD)?	No	assessment finished - low risk

Exposure to contaminated water

Guttation

	Choose answer	
Is the substance applied after the guttation period?	Yes	assessment finished - low risk (exposure considered negligible)
Does guttation water occurs for less than 10% of the location/calendar year combinations?	Unclear	Go to 1st tier ! ► ► ► ► ►

Surface water

	water consumption (µL)	ETR	Trigger	Risk indicator
acute	11,4	0,00	0,2	OK
chronic	11,4	0,000	0,03	OK
larvae	111	0,00	0,2	OK
HPG	11,4	0,0	1	OK

Puddle water

	water consumption (µL)	ETR	Trigger	Risk indicator
acute	11,4	0,00	0,2	OK
chronic	11,4	0,002	0,03	OK
larvae	111	0,00	0,2	OK
HPG	11,4	0,0	1	OK

The risk assessment according to the not yet noted draft EFSA bee guidance document (EFSA Journal 2013;11(7):3295) is considered by the RMS to be unrealistically conservative. Gibberellins GA4/GA7 is a naturally occurring substance, secreted by many plants and other organisms. Is is reasonable to assume that bees are exposed to gibberellins GA4/GA7 in their natural environment and are adjusted to their effects. Additionally, gibberellins GA4/GA7 functions as a plant growth regulator. It is not expected that a plant growth regulator will have strong toxic effects on insects. The RMS considers it is unlikely gibberellins GA4/GA7 will have any undesirable effects on non-target arthropods, bees or other.

Risk assessment for non-target arthropods other than bees

A summary of the toxicity data for non-target arthropods other than bees is provided below in Table 9.6.2- 1.

Table 9.6.2- 1 : Endpoints and effect values relevant for the risk assessment for non-target arthropods other than bees

Species	**Substance**	**Exposure System**	**Results**	**Reference**
Aphidius rhopalosiphi (adults)	GA4/GA7	Laboratory test glass plates (2D)	**LR_{50} > 40 g a.s./ha**	CA 8.3.2/01
Typhlodromus pyri (protonymphs)	GA4/GA7	Laboratory test glass plates (2D)	**LR_{50} > 40 g a.s/ha**	CA 8.3.2/02
Typhlodromus pyri (protonymphs)	Novagib	Laboratory test glass plates (2D)	LR_{50} > 80 L product/ha, equivalent to > 800 g a.s./ha	CP 10.3.2.1/01
Field or semi-field tests				
Not required				

Endpoints in bold are used in the 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 in-field risk assessment is based on the worst-case PER value based on the critical GAP of 4 x 5 g a.s./ha (see Table 9.6.2- 2).

Table 9.6.2- 2 : $PER_{in\text{-}field}$ values for all supported uses of Novagib

Use No.	**Maximum number of applications**	**Maximum rate per application (g a.s./ha)**	**MAF[a]**	**$PER_{in\text{-}field}$ (g/ha)***
1	4	5	2.7	13.5
2	1	12	1.0	12.0
3	2	6	1.7	10.2

[a] MAF based on Appendix V of ESCORT II, with a default dissipation DT_{50}: Spray interval of 2.3:1

* $PER_{in\text{-}field}$ = Application rate × MAF

The results of the first-tier assessment of the in-field risk are summarised in Table 9.6.2- 3.

Table 9.6.2- 3 : First-tier assessment of the in-field risk for non-target arthropods due to the use of Novagib in apples and pears

Intended use	**Apples and pears**		
Active substance/product	GA4/GA7/ Novagib		
Application rate (g/ha)	4 x 5 (minimum interval 7 days) BBCH 62-74		
MAF	2.7 (based on ESCORT II guidance for 4 applications, and a default dissipation DT_{50}: Spray interval of 2.3:1)		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)***	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 40	13.5	< 0.338
Aphidius rhopalosiphi	> 40		<0.338

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

* $PER_{in\text{-}field}$ = Application rate × MAF

The hazard quotients ($HQ_{in\text{-}field}$) are well below the trigger of 2 for both standard indicator species, concluding an acceptable in-field risk to non-target arthropods following the proposed use of Novagib in apples and pears.

The off-field risk assessment is based on the worst-case PER value based on the critical GAP of 1 x 12 g a.s./ha (see Table 9.6.2- 4).

Table 9.6.2- 4 : $PER_{off\text{-}field}$ values for all supported uses of Novagib

Use No.	Maximum number of applications	Maximum rate per application (g a.s./ha)	MAF[a]	Drift values for x no. of applications[b]	$PER_{off\text{-}field}$ (g/ha)*
1	4	5	2.7	10.12	1.37
2	1	12	1.0	15.73	1.89
3	2	6	1.7	12.13	1.24

[a] MAF based on Appendix V of ESCORT II, with a default dissipation DT_{50}: Spray interval of 2.3:1

[b] Drift values for x no. of applications for late fruit crops at 3 m (Appendix VI of ESCORT II)

* $PER_{off\text{-}field}$ = [Application rate x MAF x (drift factor/VDF)]/CF

The results of the first-tier assessment of the off-field risk are summarised in Table 9.6.2- 5.

Table 9.6.2- 5 : First-tier assessment of the off-field risk for non-target arthropods due to the use of Novagib in apples and pears

Intended use	**Apples and pears**					
Active substance/product	GA4/GA7/ Novagib					
Application rate (g/ha)	1 x 12 BBCH 62-74					
MAF	1.0					
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift factor**	**VDF**	**CF**	**$PER_{off\text{-}field}$ (g/ha)***	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 40	15.73 (based on late fruit crops, ESCORT II)	10	10	1.89	< 0.047
Aphidius rhopalosiphi	> 40					< 0.047

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; VDF: Vegetation Distribution Factor; CF: Correction Factor; Criteria values shown in bold breach the relevant trigger.
* $PER_{off\text{-}field}$ = [Application rate x MAF x (drift factor/VDF)]/CF

The hazard quotients ($HQ_{off\text{-}field}$) are well below the trigger of 2 for both standard indicator species, concluding an acceptable off-field risk to non-target arthropods following the proposed use of Novagib in apples and pears.

A higher tier assessment is not required for in-field or for off-field. None the less an extended laboratory study with *Orius laevigatus* was submitted for re-registration. The results of the study confirm an acceptable risk, demonstrating an LR_{50} of 431 g a.s./ha, which is well above the $PER_{off\text{-}field}$ of 1.89 g a.s./ha. However, the study was assessed as unreliable by the RMS and the results are not used in the risk assessment.

An acceptable risk to non-target arthropods is concluded at the first tier following the proposed use of Novagib in apples and pears, without the need for specific risk mitigation measures. No further data are considered necessary.

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

Earthworms

Earthworms – sub-lethal effects

a) Previous evaluation (2005-2011)

Formulation toxicity data for effects of Novagib on earthworms was not submitted for the first inclusion of gibberellins GA4/GA7. Low risk was concluded based on active substance data.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No data on the chronic toxicity of the representative formulation, Novagib, to earthworms is available. However, an acute earthworm toxicity test with Novagib is available and submitted here in support of the low toxicity of the representative formulation. This new study is summarised below (CP 10.4.1.1/01) and referenced in DRAR Vol.2. The available data show no evidence that Novagib is of greater acute toxicity than the active substance. Therefore, data on the technical grade active substance, GA4/GA7, are considered applicable and relevant with regard to the evaluation of long-term effects on earthworms of the formulated product. As stated in the residue definition in CA 7.4.1, there are no surface water metabolites of environmental concern and the data provided are therefore limited to the active substance.

Data point addressed:	CP 10.4.1.1/01
Author(s) (year):	Goodband, T.J. (2011)
Title:	Novagib: Acute toxicity to earthworms (*Eisenia foetida)*
Laboratory report / project number:	41004741
Testing facility:	Harlan Laboratories Ltd., Shardlow, UK
Published:	No
Test guideline used:	OECD 207 (1984)
Deviations:	None Note, the claimed test concentration (1000 mg a.s./kg soil dw) could not be recalculated from the reported preparation method. The nominal concentration of a.s. obtained by dissolving 48.54 g product in 500 mL was stated to be equal to 10 g a.s./L. However, the product contains 10.3 g a.s./L, and the product density is 1.0438 kg/L. This means that 48.54 g product contains 48.54 g product * 10.3 g a.s./1043.8 g product = 0.48 g a.s. The stock solution thus contained a concentration of 0.48 g a.s./500 mL = 0.96 g a.s./L. The test soil was prepared by adding 350 mL of this stock to 3.5 kg soil dw: 1000 * (0.35 L * 0.96 g a.s./L) / 3.5 kg soil dw = 96 mg a.s./kg soil dw. The tested concentration was 96 mg a.s./kg soil dw, and not 1000 mg a.s./kg soil dw as stated in the study report.
GLP:	Yes
Endpoint:	14 d LC_{50} > 96 mg a.s./kg dw soil LC_{50CORR} > 48 mg a.s./kg dw soil (in accordance with SANCO/10329/2002 rev 2 final, as the log P_{ow} > 2, the toxicity endpoint was divided by 2 to take account of the different amount of organic carbon between laboratory and natural soils)

Executive Summary

The toxicity of Novagib to earthworms *Eisenia foetida* was determined in 14 days of exposure under worst case laboratory conditions. The test item rate was 96 mg a.s./kg of soil and was tested for a period of 14 days at a temperature of 20 to 24°C. The number of mortalities was determined after 7 and 14 days. The 14-day LC_{50} based on nominal test concentration was > 96 mg a.s./kg. The no observed effect concentration was 96 mg a.s./kg.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:	Novagib
Description:	Clear colourless liquid
Lot/Batch #:	1401437008
Active substance:	gibberellins GA4/GA7 at nominal 10 g/L
Content:	10.3 g a.s./L (measured)

Test organism

Species:	*Eisenia fetida*
Age/growth stage:	Adult, with clitellum
Source:	Original Organics Limited, Devon, UK
Weight:	0.46 to 0.50 g
Acclimation:	24 hours in artificial soil, under laboratory conditions.

B. STUDY DESIGN AND METHODS

Test design

System:	Static system
Duration:	14 days
Test vessel:	1 L glass beakers
Artificial Soil:	Defined artificial soil; 70% w/w Industrial quartz sand, 20% Kaolinite clay, 10% Sphagnum moss peat
Concentrations:	0 (control) and 96 mg a.s./kg (nominal concentration)
Number of animals:	60 earthworms, six replicates of 10 worms

Environmental conditions

Soil Temperature:	≥ 9.6 mg O_2/L
Soil Moisture:	30% of dry weight (26 – 29%)
Soil pH:	6.0 ± 0.5
Photoperiod:	Continuous illumination at 657 to 742 lux

Animal assignment and treatment

A weighed group of ten randomly allocated worms was placed on the soil surface in each container. The test vessels were covered with perforated plastic film and incubated. The earthworms were not individually identified and received no food during exposure.

Dose preparations

The report stated that an amount of Novagib (48.54 g) was dissolved in deionised reverse osmosis water and the volume adjusted to 500 mL to give a 10 g a.s./L stock solution which was inverted several times to ensure adequate mixing and homogeneity. An aliquot (350 mL) of this stock solution was added to 3.5 kg (dry weight) of artificial soil with 540 mL of water and mixed using a Hobart A200N mixer to give the 1000 mg a.s./kg test concentration with a nominal moisture content of 30% of dry weight.

The claimed test concentration (1000 mg a.s./kg soil dw) could not be recalculated from the reported preparation method. The nominal concentration of a.s. in soil obtained by dissolving 48.54 g product in 500 mL was stated to be equal to 10 g a.s./L. However, the product contains 10.3 g a.s./L, and the product density is 1.0438 kg/L. This means that 48.54 g product contains 48.54 g product * 10.3 g a.s./1043.8 g product = 0.48 g a.s. The stock solution thus contained a concentration of 0.48 g a.s./500 mL = 0.96 g a.s./L. The test soil was prepared by adding 350 mL of this stock to 3.5 kg soil dw: 1000 * (0.35 L * 0.96 g a.s./L) / 3.5 kg soil dw = 96 mg a.s./kg soil dw. The tested concentration was 96 mg a.s./kg soil dw, and not 1000 mg a.s./kg soil dw as stated in the study report.

The control was prepared in an identical manner using 2.5 kg (dry weight) of artificial soil and 640 mL of deionised reverse osmosis water.

Measurements/observations

Worms were exposed over a period of 14 days to soil-incorporated at a single concentration of 96 mg a.s./kg. The weight of each worm was recorded on Day 0 before addition to the test or control vessels. Each surviving earthworm was weighed on Day 14. The control group was maintained under identical conditions but not exposed to the test item. Any mortalities were recorded 7 and 14 days after the start of treatment and any sub-lethal effects recorded daily. The criterion of death was taken to be the absence of any reaction to a physical stimulus at either end of the body.

Statistics

Statistical analysis of the earthworm weight data was performed using Bartlett's test for homogeneity of variance (Sokal and Rohlf 1981) and a Students t-test. All statistical analyses were performed using the SAS computer software package (SAS 1999 – 2001).

II. RESULTS AND DISCUSSION

Cumulative mortality data from the exposure of *Eisenia foetida* to Novagib based on nominal concentrations are given in the table below.

Table 9.7.1.1- 1 : Summary of results from acute toxicity to earthworms with Novagib

Nominal conc. (mg a.s./kg)	Cumulative Mortality (initial population: 10)		% Mortality	
	Day 7	Day 14	Day 7	Day 14
Control	0	0	0	0
96	0	0	0	0

III. CONCLUSIONS

The 14-day LC_{50} of Novagib to *Eisenia foetida* is > 96 mg a.s./kg dry soil. This value was divided by 2 to give a corrected LC_{50} of > 48 mg a.s./kg dry soil for risk assessment purposes since the log P_{ow} values for gibberellins GA4 and GA7 are > 2.

RMS comments and conclusions:

The study was performed according to the OECD 207 Earthworm acute toxicity test.

Validity: According to the OECD 207 the study is considered valid if the following criteria are met:

- the mortality in the controls does not exceed 10 per cent **(in test: 0%, condition fulfilled)**

The moisture content in soil during the test was 26%-29%. The moisture content was set at 30% at the beginning of the test, as it was discovered that moisture content of 35% (as required by the test guideline) causes formation of sludge in artificial soil. The RMS considers this to be a minor deviation that does not affect the validity of the results.

The temperature during the test was 20°C-24°C, which is outside the required range of 18°C-22°C. The RMS considers this to be a minor deviation that does not affect the validity of the results.

In the test 6 replicates were used for gibberellins GA4/GA7 treatment and 4 replicates for the control. This is in line with the test guideline.

The reference substance chloroacetamide was used as positive control. The LC_{50} was determined to be 32 mg/kg, which is in the normal range for this substance (10-50 mg/kg).

In the test 3500 g of artificial soil was divided into 6 replicates, which gives 580 g of soil per glass beaker. This is 170g less than 750g as required by the test guideline. Despite this deviation the RMS considers the results to be reliable.

The stability of the test substance was not tested.

Acceptability of the analytical methods used in the test: Not applicable.

Endpoints: The 14-day LC_{50}>96 mg a.s./kg dry soil.

Conclusion of the RMS: The Earthworm acute toxicity test is considered valid.

Earthworms – field studies

An acceptable risk to earthworms was concluded at the first-tier risk assessment and further data is therefore not considered necessary.

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

Species level testing

a) Previous evaluation (2005-2011)

Formulation toxicity data for effects of Novagib on non-target meso- and macrofauna was not submitted for the first inclusion of gibberellins.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

Data are available on both *Aphidius rhopalosiphi* and *Typhlodromus pyri* and no concerns are raised with either of these species (DRAR Vol.3 CP Novagib B.9.6.2). In accordance with the data requirements set out by EU Reg. 283/2013 and EU Reg. 284/2013, no further data on effects on non-target soil meso- and macrofauna (other than earthworms) are considered necessary.

Furthermore, the springtail *Folsomia candida* is an omnivorous, free-living soil organism. Springtails do not directly engage in the decomposition of organic matter, but contribute to it indirectly through the fragmentation of organic matter. They commonly consume fungal hyphae and spores, but also have been found to consume plant material and pollen, animal remains, colloidal materials, minerals and bacteria. Through their feeding on organic matter, *Folsomia* are naturally exposed to gibberellins GA4/GA7, especially through their feeding on fungal hyphae that actively produce gibberellins. *Hypoaspis aculeifer* is a soil dwelling mite that feeds on small arthropods and nematodes, including bulb mites, springtails, thrips pupae and fungus gnats. Thus *Hypoaspis* feed

on arthropods which feed on plants. In the soil, mites will come into direct contact with natural sources of GA4/GA7 and other gibberellins through plant roots, falling leaves, etc.

Higher tier testing

No further data available.

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

A summary of the toxicity data for earthworms is provided below in Table 9.8- 1.

Table 9.8- 1 : Endpoints and effect values relevant for the risk assessment of non-target soil meso- and macrofauna

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	GA4/GA7	Acute 14 d	14 d LC_{50} > 1250 mg a.s./kg dw soil LC_{50corr} > 625 mg a.s./kg dw soil	CA 8.4.1/01
Eisenia fetida	Novagib	Acute 14 d	14 d LC_{50} > 96 mg a.s./kg dw soil **LC_{50corr} > 48 mg a.s./kg dw soil**	CP 10.4.1.1/01
Eisenia fetida	GA3	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 250 mg GA3/kg dw **$NOEC_{corr}$* = 125 mg GA3/kg dw**	CA 8.4.1/02

Endpoints in bold are used in the risk assessment

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002 as GA4 and GA7 have log Pow values >2

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).

The chronic toxicity endpoint used in the risk assessment is based on read-across from a study testing gibberellic acid GA3. This approach was used also for the avian risk assessment, as previously accepted by EFSA during Annex I (EFSA Journal 2012;10(1):2502). Read across between GA3 and GA4/GA7 is considered acceptable based on the similarities between GA3 and GA4/GA7 and the high margin of safety obtained in the risk assessment below.

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from section B.8 (DRAR Vol.3 CP Novagib B.8.2) (Environmental Fate). According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for GA4/GA7. As discussed in B.8.2, as the ratio of gibberellins GA4 and gibberellins GA7 in the active substance can vary between sources, for the determination of PECs of the active substance the approach adopted as a precautionary worst-case has been to consider alternate situations where the active substance is 100% gibberellins GA4 and separately 100% gibberellins GA7.

The results of the acute and reproductive first-tier risk assessments are summarised in Table 9.8- 2.

Table 9.8- 2 : First-tier assessment of the acute and chronic risk for earthworms due to the use of Novagib in apples and pears

Intended use	Apples and pears		
Acute effects on earthworms			
Product/active substance	**LC_{50} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_a (criterion TER ≥ 10)**
Novagib	>48	0.0133[a]	>3609
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
GA4/GA7	125	0.0133[a]	9398

[a] Worst case PEC based on critical use pattern of 4 x 5 g a.s./ha (7 d minimum interval).
TER values shown in bold fall below the relevant trigger.

The acute and long-term TER values are well above the relevant triggers of 10 and 5 respectively. An acceptable risk to earthworms is concluded at the first tier following the proposed use of Novagib in apples and pears, without the need for specific risk mitigation measures. No further data are considered necessary.

B.9.9. EFFECTS ON SOIL NITROGEN TRANSFORMATION

a) Previous evaluation (2005-2011)

Formulation toxicity data for effects of Novagib on nitrogen mineralisation was not submitted for the first inclusion of gibberellins GA4/GA7. Low risk was concluded based on active substance data.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

Toxicity data for the representative formulation, Novagib, were not previously submitted for the Annex I evaluation of GA4/GA7. A new study assessing the effects of Novagib on soil carbon and nitrogen transformation is available and submitted here to support the representative formulation in the renewal process. The study is summarised below (CP 10.5/01) and referenced in DRAR Vol.2.

Data point addressed:	CP 10.5/01
Author(s) (year):	Clarke, N. (2011)
Title:	Novagib: Effect on soil microorganisms: Nitrogen transformation test and carbon transformation test
Laboratory report / project number:	41004742
Testing facility:	Harlan Laboratories Ltd., Shardlow, UK
Published:	No
Test guideline used:	OECD 216 (2000) and OECD 217 (2000)
Deviations:	None Note, the claimed exposure concentrations of 0.040 and 0.21 mg a.s./kg soil dw could not be recalculated from the reported preparation method. At the low rate, the nominal concentration of a.s. in the stock solution obtained by dissolving 388.3 mg product in 1000 mL was stated to be equal to 40 mg a.s./L. However, the product contains 10.3 g a.s./L, and the product density is 1.0438 kg/L. This means that 388.3 mg product/L contains 388.3 mg product/L * 10.3 g a.s./1043.8 g product = 3.83 mg a.s./L. This stock was reported to be diluted 10 times before preparation of the test soil (i.e. 0.383 mg a.s./L). The test soil was then prepared by adding 15 mL of the 10-fold diluted stock to 1.5 kg soil dw: (0.015 L * 0.383 mg a.s./L) / 1.5 kg soil dw = 0.0038 mg a.s./kg soil dw. For the high rate, 407.8 mg product was dissolved in 1000 mL water and then diluted 2 times before addition to soil (15 mL to 1.5 kg soil). The concentration at the high rate thus was 0.5 * 407.8 mg product/L * 10.3 g a.s./1043.8 g product * 0.015 L / 1.5 kg soil dw = 0.020 mg a.s./kg soil.
GLP:	Yes
Endpoint:	No effect on nitrogen and carbon transformation at 28 d at 0.0038 and 0.020 mg a.s./kg dw soil

Executive Summary

This study was performed to determine the effects of Novagib on nitrogen transformation and carbon transformation by soil micro-organisms. Novagib was tested at two treatment concentrations; equivalent to 0.0038 and 0.020 mg a.s./kg soil dw. The inhibitory effect of the test item on nitrogen transformation was assessed by the determination of nitrate concentration in the soil samples on Days 0, 7, 14 and 28 and compared to data obtained from control soil samples. The inhibitory effect of the test item on carbon transformation was assessed by the determination of the glucose-induced respiration rate in the soil samples on Days 0, 7, 14 and 28 and compared to data obtained from control soil samples. The results showed no significant effect on carbon

transformation activity of soil microorganisms at the test concentrations of 0.0038 and 0.020 mg a.s./kg over a 28-day period. An effect >25% on nitrogen transformation rate (27%) was observed during the last sampling interval at 0.0038 mg a.s./kg dw but not at 0.020 mg a.s./kg dw. This effect was therefore considered to be not biologically significant. The test item is considered to have no long term effect on nitrogen and carbon transformation in soil.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material: Novagib
Description: Clear colourless liquid
Lot/Batch #: 1401437008
Active substance: gibberellins GA4/GA7 at nominal 10 g/L
Content: 10.3 g a.s./L (measured)

Test Soil
Type: Sandy loam soil
Source: LUFA Speyer, Germany
Site history at time of soil collection: The sampling site had not been treated with crop protection products or organic fertiliser for at least 3 years prior to sampling.
pH: 6.7
Organic carbon: 0.99 ± 0.08%
Sand content: 60.0%
% sand/silt/clay (>0.063 / 0.002-0.063 / <0.002 mm): 60.1 / 31.2 / 8.7
Maximum water holding capacity: 35.6 g/100 g

B. STUDY DESIGN AND METHODS

Test design

Concentrations: The claimed exposure concentrations were 0.040 and 0.21 mg a.s./kg soil dw. The claimed exposure concentrations of 0.040 and 0.21 mg a.s./kg soil dw could not be recalculated from the reported preparation method. At the low rate, the nominal concentration of a.s. in soil obtained by dissolving 388.3 mg product in 1000 mL was stated to be equal to 40 mg a.s./L. However, the product contains 10.3 g a.s./L, and the product density is 1.0438 kg/L. This means that 388.3 mg product/L contains 388.3 mg product/L * 10.3 g a.s./1043.8 g product = 3.83 mg a.s./L. This stock was reported to be diluted 10 times before preparation of the test soil (i.e. 0.383 mg a.s./L). The test soil was then prepared by adding 15 mL of the 10-fold diluted stock to 1.5 kg soil dw: (0.015 L * 0.383 mg a.s./L) / 1.5 kg soil dw = 0.0038 mg a.s./kg soil dw. For the high rate, 407.8 mg product was dissolved in 1000 mL water and then diluted 2 times before addition to soil (15 mL to 1.5 kg soil). The concentration at the high rate thus was 0.5 * 407.8 mg product/L * 10.3 g a.s./1043.8 g product * 0.015 L / 1.5 kg soil dw = 0.020 mg a.s./kg soil.

Soil microorganisms were exposed to the test item for 28 days at a temperature of 21 ± 2°C, in the dark. For the nitrogen transformation part of the test, powdered Lucerne-green-grass meal was added to the soil to act as a respiratory substrate (0.5% w/w).

Preparation of soil: The moisture content of the soil was determined prior to the start of the test by drying an amount of soil (100.00 g) at 105°C (± 10%) until a constant weight was obtained. The moisture content of the soil, expressed as a percentage of the dry weight, was determined to be 9%. The Water Holding Capacity (WHC) of the soil supplied was 35.6 g/100g and hence 50.2 ml of deionised reverse osmosis water per 1.0 kg of soil was added. This gave a final water content of 14.0 g/100g wet weight i.e. 39% of the WHC as recommended by the Test Guidelines.

Exposure: The soil samples were incubated in glass jars, each containing 50 g dry weight of soil. The test vessels were covered with loosely fitted lids in order to minimise moisture loss by evaporation and maintained in a temperature controlled room at 21 ± 2°C, in darkness.

Sampling and analysis: On days 0, 7, 14 and 28 three control and test item vessels were sacrificed for nitrate analysis by spectrophotometry after extraction with KCl (0.1 M). A further three control and test item vessels were sacrificed for measurement of the glucose-induced respiration rate on days 0, 7, 14 and 28, after addition of 4000 mg/kg glucose.

Validation Criteria: The variation between replicate control sample nitrification rates and glucose-induced respiration rates should be less than ± 15% for the study to be accepted as valid.

II. RESULTS AND DISCUSSION

The microbial biomass of the soil was shown to be 108 µg C/g. This was equivalent to 1.1% of the total soil organic carbon content. The variation between replicate control nitrate levels and glucose-induced respiration rates was less than 15% and therefore satisfied the validation criterion given in the Test Guidelines.

The difference in nitrogen transformation activity of soil microorganisms exposed to the test item at concentrations of 0.0038 and 0.020 mg a.s./kg soil was less than 25% after 28 days when compared to controls (Table 9.9- 1).

The study report presented nitrogen transformation rates, which were calculated for days 0-7, days 0-14 and days 0-28. According to the guideline, transformation rates should be calculated for consecutive intervals, i.e. days 0-7, days 7-14 and days 14-28. These rates have been calculated from the reported nitrate levels and are shown in Table 10.5/01-1. After 28 days, the difference in nitrate formation rate was slightly higher than 25% (i.e. 27%) at 0.0038 mg a.s./kg soil. However, as the difference at the higher rate of 0.020 mg a.s./kg soil was <25% (i.e. 18%), and the differences in absolute nitrate levels were <25% at both test concentrations, the effect at 0.0038 mg a.s./kg soil is not considered to be biologically significant.

Table 9.9- 1 : Effect of Novagib on nitrification in a sandy loam soil

Treatment rate (mg a.s./kg)	**Nitrate levels (mg/kg) on day:**			
	0	**7**	**14**	**28**
Control	114.93	138.80	176.30	252.23
0.0038	145.27	170.53	206.40	302.87
0.020	142.87	174.40	210.00	299.50
	% deviation (nitrate levels) from control on day:			
	0	**7**	**14**	**28**
0.0038	+26	+23	+17	+20
0.020	+24	+26	+19	+19
	% deviation (nitrate formation rates) from control at days:			
		0-7	**7-14**	**14-28**
0.0038		+5.8	-5.1	+27
0.020		+32	-5.7	+18

III. CONCLUSIONS

Novagib showed no significant effect on the nitrogen and carbon transformation activity of soil microorganisms at the test concentrations of 0.0038 and 0.020 mg a.s./kg after 28 days. Novagib can therefore be considered to have no long-term effect on nitrogen and carbon transformation in soil.

RMS comments and conclusions:

The study was performed according to the OECD 216 Soil microorganisms: Nitrogen transformation test and OECD 217 test guidelines. The OECD 216 test guideline describes a laboratory test method designed to investigate the long-term effects of chemicals on nitrogen transformation activity of soil microorganisms after a single exposure.

Validity: According to the OECD 216 the study is considered valid if the following criteria are met:

- the variation between replicate control samples should be less than ±15% **(in test: 4%, condition fulfilled)**

The test guideline requires the lower test concentration to reflect at least the maximum amount expected to reach the soil (PEC) and the higher test concentration should be 5 times the lower concentration. In the submitted study the lower test concentration (0.0038 mg a.s./kg) was below PEC (0.0133 mg/kg dw) and the higher test concentration (0.02 mg a.s./kg) was just above PEC.

The test guideline states: 'Substances that are expected to be applied to soils several times in one season should be tested at concentrations derived from multiplying the PEC by the maximum anticipated number of applications. The upper concentration tested, however, should not exceed ten times the maximum single application rate.' As Novagib is intended to be applied more than once in apple and pear, higher test concentrations should have been used for nitrogen transformation test.

The experiment lasted 28 days. The difference between nitrogen formation rates of gibberellins GA4/GA7 concentration 0.0038 mg a.s./kg and of the control on day 28 was 27%, therefore, according to the test guideline, the experiment should continue up to 100 days.

The test guideline instructs on how to interpret the results of the test: 'When results from tests with agrochemicals are evaluated, and the difference in the rates of nitrate formation between the lower treatment (i.e. the maximum predicted concentration) and control is equal to or less than 25 % at any sampling time after day 28, the product can be evaluated as having no long-term influence on nitrogen transformation in soils.' This condition is not fulfilled in the submitted study. Additionally, the above interpretation refers to lower treatment concentration being equal to PEC, which is not the case in this study. It can't be said with certainty that Novagib (gibberellins GA4/GA7) has no long-term influence on nitrogen transformation in soils.

The stability of the test substance was not tested.

Acceptability of the analytical methods used in the test: Not applicable.

Endpoints: No endpoint can be determined.

Conclusion of the RMS: The nitrogen transformation test is considered valid according to the OECD 216 test guideline validity criteria. However, the test concentrations were poorly chosen. It can't be said with certainty that Novagib (gibberellins GA4/GA7) has no long-term influence on nitrogen transformation in soils. The results of this study will not be used in risk assessment.

B.9.10. RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION

A summary of the effects data for soil nitrogen transformation is provided below in Table 9.10- 1.

Table 9.10- 1 : Endpoints and effect values relevant for the risk assessment for soil microorganisms

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	GA4/GA7	28 d, aerobic soil type	**< 15 % at 28 d at 0.013 and 0.13 mg/kg dw soil**	CA 8.5/01

Endpoints in bold are used in the 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 B.8 (DRAR Vol.3 CP Novagib B.8.2) (Environmental Fate) and were already used in the risk assessment for earthworms (see B.9.8).

Table 9.10- 2 : Assessment of the risk for effects on soil micro-organisms due to the use of Novagib in apples and pears

Intended use	Apples and pears		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg a.s./kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
GA4/GA7	0.13 (at 28 d)	0.0133	yes

The results of the active substance study showed no effects of >25% compared to the control (trigger value according to SANCO/10329/2002) on soil microbial activity up to a maximum tested concentration of 0.13 mg a.s./kg soil, after 28 days. As the maximum tested concentration is much higher than the maximum initial PEC from the proposed use of Novagib, an acceptable risk to soil microbial activity is concluded. Furthermore, gibberellins GA4/GA7 are naturally produced by bacteria and fungi (CA 8.9/01) and given that the degradation rates of GA4 and GA7 in soil are high (DRAR Vol.3 CA B.8.1.1), it is considered that there will be no significant carry over of residues between applications and that there will be no long-term risk to microbial activity. Data on effects of representative formulation, Novagib, were also available, but the study was poorly designed and the results are not reliable for use in risk assessment.

An acceptable risk to soil microorganisms is concluded following the proposed use of Novagib in apples and pears, without the need for specific risk mitigation measures.

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

Summary of screening data

a) Previous evaluation (2005-2011)

The summary of screening data was not submitted for the first inclusion of gibberellins GA4/GA7. Under EU Reg. 283/2013 screening data shall not be used for active substances with plant growth regulatory activity. Gibberellins GA4/GA7 is a plant growth regulator; therefore, toxicity studies on non-target plants are required (see B.9.11.2).

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No new data are available.

Testing on non-target plants

a) Previous evaluation (2005-2011)

Formulation toxicity data on non-target plants was not submitted for the first inclusion of gibberellins GA4/GA7. A low risk to non-target terrestrial plants was concluded on the basis of a quantitative argument concerning the non-toxic mode-of-action and natural occurrence of gibberellins GA4/GA7 in many terrestrial plants.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

Toxicity data for the representative formulation, Novagib, were not previously submitted for the Annex I evaluation of GA4/GA7. A new study assessing the effects of Novagib on vegetative vigour is available and submitted here to support the renewal of GA4/GA7. The study is summarised below (CP 10.6.2/01) and referenced in DRAR Vol.2. No data is available regarding the toxicity of formulated product Novagib on seedling emergence of non-target terrestrial plants. A study of the effects of gibberellins GA4/GA7 on the seedling emergence and early development of seedlings of non-target terrestrial plant species has been performed with the technical active substance and is summarised in DRAR Vol.3 CA B.9.6.2. The RMS considers that a study with technical active substance is sufficient to address the risk of formulated product to seedling emergence of non-target terrestrial plants.

Data point addressed:	CP 10.6.2/01
Author(s) (year):	Fiebig, S. (2017)
Title:	Novagib GA4/GA7 (FAL460) Terrestrial Plant Test: Vegetative Vigour Test
Laboratory report / project number:	170411FO / TNW17709
Testing facility:	Noack Laboratorien GmbH, Sarstedt, Germany
Published:	No
Test guideline used:	OECD 227 (July 2006)
Deviations:	The relative humidity was occasionally <45%, but did not influence plant growth.
GLP:	Yes
Endpoint:	21 d ER_{50} (shoot height) = 34.8 g a.s./ha for lettuce

Executive summary

The phytotoxicity of the test item Novagib GA4/GA7 (FAL460) to ten terrestrial plant species was established in 21-day toxicity tests according to OECD 227 (2006) and to GLP. Test plants comprised four monocotyledons (wheat, corn, perennial ryegrass, onion) and six dicotyledons (sugar beet, rape, cabbage, lettuce, tomato, soybean). The test item was applied at the following nominal application rates: 31.3, 62.5, 125, 250 and 500 g a.s./ha (with an additional rate of 15.6 g a.s./ha tested for rape), with the exception of the test with lettuce, which tested rates of 2.56, 6.4, 16, 40 and 100 g a.s./ha. Potential toxic effects of the test item were assessed on day 7, 14 and 21 by visual observations (phytotoxic effects and number of dead plants) and on day 21 by determination of shoot height and shoot fresh weight. Analytical recovery in the stock solutions was between 88 and 111% of nominal concentrations for GA4 and GA7, respectively, indicating the correct preparation of the spray solutions. Test item application led to increased plant growth in five of the plants tested. Lettuce was the most sensitive species tested. The ER_{50} for shoot height (promoted growth) for lettuce was calculated to be 34.8 g a.s./ha (95% CI: 27.4 – 43.6 g a.s./ha). The ER_{50} for shoot fresh weight (biomass inhibition) for lettuce was >100 g a.s./ha, the highest rate tested. The $NOEL_{shoot\ height}$ and $NOEL_{shoot\ fresh\ weight}$ values for lettuce were 6.4 and 40 g a.s./ha, respectively. The tests were considered valid as results of the controls for all plant species met the required validity criteria.

I MATERIALS AND METHODS

A MATERIALS

Test item:	Novagib GA4/GA7 (FAL460)
Batch No.:	102437K602
Active ingredient:	GA4/GA7 (Gibberellins A 4/7 Technical)
CAS No. (a.i.):	468-44-0/ 510-75-8
Contents:	10.24 g a.i./L
Density:	1.0382 g/mL
Expiry date:	30th November 2018
Description:	Clear liquid

Test organisms:		
	Monocotyledonae:	
	Triticum aestivum (wheat), Julius[1]	Poaceae
	Zea mays (corn), Ronaldinio[2]	Poaceae
	Lolium perenne (perennial ryegrass), Temprano[3]	Poaceae
	Allium cepa (onion), Exhibition[4]	Liliaceae
	Dicotyledonae:	
	Beta vulgaris (sugar beet), Finola KWS[2]	Amaranthaceae
	Brassica napus (rape), Sherlock[2]	Brassicaceae
	Brassica oleraceae (cabbage), NOZOMI Precision[4]	Brassicaceae
	Lactuca sativa (lettuce), Mafalda[4]	Asteraceae
	Lycopersicon esculentum (tomato), Harzfeuer, F1[4]	Solanaceae
	Glycine max (soybean), Obelix[5]	Fabaceae
	Superscript numbers relate to origin	

Seed origin:

[1] KWS Lochow GmbH, Ferdinand-von-Lochow-Str. 5, 29303 Bergen, Germany
[2] KWS Saat SE, Grimsehlstraße 31, 37574 Einbeck, Germany
[3] Samenshop24.de, Kirchdorfer Str. 177, 26605 Aurich, Germany
[4] Hild Samen GmbH, Postfach 1161, 71666 Marbach, Germany
[5] Delley Samen und Pflanzen AG (DSP), Le Château, CH-1567 Delley

Test substrate:	2:1 mixture of natural soil LUFA 2.2 (batch number: Sp2.20717 and Sp2.22317, sandy loam (USDA classification) and quartz sand (12a)
Grain size:	≤2 mm
Clay (<0.002 mm):	6.2% / 8.0%
Silt (0.002 – 0.063 mm):	9.3% / 8.2%
Sand (0.063-2 mm):	84.5% / 83.9%
Carbon content:	1.17% / 1.18%
pH:	6.40 ± 0.05 / 6.08 ± 0.02
Origin:	Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer (LUFA), Obere Langgasse 40, 67346 Speyer, Germany Dörentrup Quarz GmbH Co. KG, An der Sandgrube 1, 31089 Duingen, Germany
Test conditions	
Temperature:	17.2 – 25.7 °C
Light intensity:	5754 ± 636 lux
Photoperiod:	16 h light; 8 h darkness
Relative humidity:	41.9 – 94.9%
Watering/fertilisation:	During the test each replicate was bottom watered with the appropriate amount of water and fertiliser as needed. Fertiliser: Hakaphos® soft Spezial 16+8+22(+3) (0.5‰) Origin: Grünes Landhaus, St. Godehard-Str. 23, 31139 Hildesheim, Germany

B STUDY DESIGN

Prior to the start of the experiment seeds were sown in a sowing container. After homogeneous and sufficient emergence (≥70%), assessed by visual observation) and complete opening of the cotyledons, the plants were transplanted into the test containers (plastic standard flower pots with a diameter of ca. 12 cm and a surface area of 113 cm^2). During the cultivation the plants were bottom watered and fertilised as necessary. The cultivation period depended on the rate of growth and the test was started when the plants had reached a 2-4 true leaf stage (BBCH 12-14). Three plants were potted per test container to allow uniform plant growth and avoid overcrowding and shading of plants. Eight replicates per application rate and control were tested in a randomised block design, with the exception of rape at 500 g a.s./ha in which seven replicates were tested. The test was conducted in a climatic hall.

Based on the results of a range-finding test, the following application rates were tested. The treatments were applied once to each plant species at the beginning of the study at a water volume of 500 L/ha.

Wheat, corn, perennial ryegrass, onion, sugar beet, rape, cabbage, tomato, soybean:

31.3, 62.5, 125, 250, 500 g a.s./ha

Rape:

15.6, 31.3, 62.5, 125, 250, 500 g a.s./ha

Lettuce:

2.56, 6.4, 16, 40 and 100 g a.s./ha

In each case a control group with 500 L water/ha was also tested.

The test solutions of the highest application rates were individually prepared in tap water by directly weighing. Further application rates were prepared by dilution out of these solutions. The application apparatus of the test facility is constructed like a fixed field sprayer under which a conveyor belt transported the test containers containing the test medium and plants. Before application, the apparatus was adjusted and calibrated to guarantee the required volume of spray solution. For calibration dry glass plates (10 x 10 cm) were weighed and reweighed once they had received the target application spray rate. When five consecutive actual weights were equal to the nominal value ±10%, application to the test containers was started.

C OBSERVATIONS

During the observation period the plants were observed on day 7, 14 and 21 for visual phytotoxic effects and number of dead plants. The rating of the treated plants was conducted in relation to the untreated control plants. Observations included all variations, either inhibitory or stimulatory, between the treated replicates and the

untreated controls. Such variations were phytotoxic symptoms (e.g. chlorosis, necrosis, wilting), formative effects of growth and development rates.
At the end of the study (21 days), the shoot height and shoot fresh weight were measured after cutting the plants.
The room temperature and relative humidity were recorded continuously throughout the test with a datalogger. The illumination is determined twice per year.
The concentrations of the active ingredients of the test item Novagib GA4/GA7 (FAL460) were confirmed by analytical verification of the spray solutions (highest application rates and control) using UPLC-MS/MS.

II RESULTS AND DISCUSSION

A FINDINGS

Treatment related visual phytotoxic effects at test end (21 days) are summarised in the table below.

Table 9.11.2- 1 : Summary of treatment related visual phytotoxic effects following exposure to Novagib GA4/GA7

Species	Main observed visual effects*	Appearance at application rates
Wheat	None	-
Corn	Increased growth	≥125 g a.s./ha
Perennial ryegrass	None	-
Onion	None	-
Sugar beet	None	-
Rape	None	-
Cabbage	Increased growth	≥125 g a.s./ha
Lettuce	Increased growth Wilting	≥16 g a.s./ha 100 g a.s./ha
Tomato	Increased growth Necrosis	≥62.5 g a.s./ha 500 g a.s./ha
Soybean	Increased growth	≥31.5 g a.s./ha

* Effects were considered as main effect when >2 replicates were influenced or a mortality >10% occurred.

A summary of no-observed effect level (NOEL), lowest observed effect level (LOEL) and ER_x values for effects on shoot height are summarised in the table below.

Table 9.11.2- 2 : Summary of effects on shoot height following exposure to Novagib GA4/GA7

Species	NOEL (g a.s./ha)	LOEL (g a.s./ha)	ER_{25} (g a.s./ha) (95% confidence range)	ER_{50} (g a.s./ha) (95% confidence range)
Wheat	500	>500	>500	>500
Corn	125	250	>500	>500
Perennial ryegrass	500	>500	>500	>500
Onion	250	500	>500	>500
Sugar beet	500	>500	>500	>500
Rape	62.5	125	>500	>500
Cabbage	31.3	62.5	>500	>500
Lettuce	**6.4**	**16**	**12.3 (9.11-16.6)**	**34.8 (27.4-43.6)**
Tomato	31.3	62.5	92.5 (51.2-188)	>500
Soybean	31.3	62.5	>500	>500

Species in bold is most sensitive species based on observed effects
Where confidence range is not given, it was not determinable

A summary of no-observed effect level (NOEL), lowest observed effect level (LOEL) and ER_x values for effects on shoot fresh weight are summarised in the table below.

Table 9.11.2- 3 : Summary of effects on shoot fresh weight following exposure to Novagib GA4/GA7

Species	**NOEL (g a.s./ha)**	**LOEL (g a.s./ha)**	**ER_{25} (g a.s./ha) (95% confidence range)**	**ER_{50} (g a.s./ha) (95% confidence range)**
Wheat	500	>500	>500	>500
Corn	500	>500	>500	>500
Perennial ryegrass	500	>500	>500	>500
Onion	500	>500	>500	>500
Sugar beet	500	>500	>500	>500
Rape	500	>500	>500	>500
Cabbage	500	>500	>500	>500
Lettuce	**40**	**100**	**61.0 (40.2-88.0)**	**>100**
Tomato	250	500	443 (358->500)	>500
Soybean	62.5	125	130 (87.9-186)	452 (332->500)

Species in bold is most sensitive species based on observed effects
Where confidence range is not given, it was not determinable

B VALIDITY

The test was considered valid as the following validity criteria were met:
- Seedling emergence was at least 70%
- Control plants did not exhibit visible phytotoxic effects and the mean growth and morphology were within the normal variation for the particular plant species
- Mean survival of control plants was at least 90% at the end of the test
- 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.

III CONCLUSIONS

The phytotoxicity of the test item Novagib GA4/GA7 (FAL460) to ten terrestrial plant species was established in 21-day toxicity tests according to OECD 227 (2006) and to GLP. Test plants comprised four monocotyledons (wheat, corn, perennial ryegrass, onion) and six dicotyledons (sugar beet, rape, cabbage, lettuce, tomato, soybean). Potential toxic effects of the test item were assessed on day 7, 14 and 21 by visual observations (phytotoxic effects and number of dead plants) and on day 21 by determination of shoot height and shoot fresh weight. Test item application led to increased plant growth in five of the plants tested. Lettuce was the most sensitive species tested. The ER_{50} for shoot height (promoted growth) for lettuce was calculated to be 34.8 g a.s./ha (95% CI: 27.4 – 43.6 g a.s./ha). The ER_{50} for shoot fresh weight (biomass inhibition) for lettuce was >100 g a.s./ha, the highest rate tested. The $NOEL_{shoot\ height}$ and $NOEL_{shoot\ fresh\ weight}$ values for lettuce were 6.4 and 40 g a.s./ha, respectively. The tests were considered valid as results of the controls for all plant species met the required validity criteria.

RMS comments and conclusion:

The study was conducted according to test guideline OECD TG 227 Terrestrial Plant Vegetative Vigour Test. The OECD TG 227 assesses the potential effects of tested substance on plants following deposition of the test

substance on the leaves and above-ground portions of plants. Endpoints obtained in the study are effective concentration EC_x or an effective application rate ER_x (e.g. EC_{25}, ER_{25}, EC_{50}, ER_{50}) for the most sensitive parameter(s) of interest. Also, the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) can be calculated in this test.

Validity: According to the OECD 227 test guideline in order for the test to be considered valid, the following performance criteria must be met:

- the seedling emergence is at least 70 % **(in test: yes, condition fulfilled)**

and in the controls:

- the plants do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that particular species **(in test: controls exhibited no phytotoxic effects, condition fulfilled.)**

- the mean plant survival is at least 90 % for the duration of the study **(in test: control plant survival is 100% for all species, condition fulfilled)**

- environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source **(in test: yes, condition fulfilled)**

The humidity during the test was 41.9 – 94.9% which is outside of the recommended range 45 – 95%. The RMS considers this to be a minor deviation that does not affect the validity of the result.

Acceptability of the analytical methods used in the test:

Endpoints: The NOEL for *Lactuca sativa* (lettuce) (the most sensitive species tested) was determined to be 6.4 g a.s./ha, based on shoot height. The ER_{50} for *Lactuca sativa* (lettuce) was determined to be 34.8 g a.s./ha based on shoot height.

Conclusion of the RMS: The terrestrial plant vegetative vigour test is considered valid.

Extended laboratory studies on non-target plants

An acceptable risk to non-target plants was concluded at the first-tier risk assessment and further data is therefore not considered necessary.

Semi-field and field tests on non-target plants

An acceptable risk to non-target plants was concluded at the first-tier risk assessment and further data is therefore not considered necessary.

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

A summary of the toxicity data for terrestrial non-target higher plants is provided below in Table 9.12- 1.

Table 9.12- 1 : Endpoints and effect values relevant for the risk assessment of terrestrial non-target higher plants

Species	Substance	Exposure system	Results	Reference
10 species, including *Lactuca sativa* (lettuce)	GA4/GA7	21 d Seedling emergence	[1)] ER_{50} emergence > 222 g a.s./ha **[2)] ER_{50} plant weight > 222 g a.s./ha**	CA 8.6.2/01
Lactuca sativa (Lettuce)	Novagib	21 d Vegetative vigour	[1)] ER_{50} plant weight > 100 g a.s./ha **[2)] ER_{50} plant height = 34.8 g a.s./ha**	CP 10.6.2/01

Endpoints in bold are used in the risk assessment

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.

Table 9.12- 2 : Assessment of the risk for non-target plants due to the use of Novagib in apples and pears

Intended use	Apples and pears			
Active substance/product	GA4/GA7/ Novagib			
Application rate (g/ha)	1 x 12 BBCH 55-75			
Test species	**ER_{50} (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
10 species (seedling emergence)	>222	15.73*	1.89	>117
Lettuce (vegetative vigour)	34.8	15.73*	1.89	18.4

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
*Drift rate for late fruit crops at 3 m (Rautmann et al. 2001)

The TER values are above the trigger of 5 for the most sensitive species tested in the seedling emergence and vegetative vigour (*Lactuca sativa*) studies. An acceptable risk to non-target plants is concluded at the first tier following the proposed use of Novagib in apples and pears, without the need for specific risk mitigation measures. No further data are considered necessary.

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

a) Previous evaluation (2005-2011)

Further studies regarding the effects on other terrestrial organisms were not submitted for the first inclusion of gibberellins GA4/GA7. The RMS confirms that no other group of terrestrial organisms are considered to be at risk. No further data are required.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

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

The risks to non-target terrestrial organisms are considered to be adequately addressed under the preceding headings of this Vol.3 CP, Section B.9 document.

Additional risk assessments for other specific groups of terrestrial organisms are not considered to be necessary in view of the proposed use of Novagib in apples and pears and have therefore not been performed.

B.9.15. REFERENCES RELIED ON

The literature search was performed and is considered adequate. The process of public literature search is described in document Volume 3 B9 (AS) in chapter B.9.11. Detailed public literature review is reported in 2 documents in KCA Section 9. The search process is documented in all details according the Guidance of EFSA, Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009, EFSA Journal 2011;9(2):2092. For the section of ecotoxicology no relevant studies were identified.

The formulated product Novagib was one of three representative formulations for the first inclusion of gibberellins GA4/GA7 on Annex I. The representative formulations for the first inclusion of gibberellins GA4/GA7 on Annex I were Regulex 10 SG, a soluble granule formulation containing 10% w/w of gibberellins GA4/GA7, Novagib and Gibb Plus, both of which are a soluble concentrate formulation containing 10 g/L GA4/GA7. The current representative formulation for re-registration is Novagib, a soluble concentrate formulation containing 10 g/L GA4/GA7. There were three studies on aquatic species with gibberellins GA4/GA7 formulation 10g/L SL submitted for the first inclusion on Annex I, however none of those studies were submitted for re-registration by the applicant. The RMS confirms that all previously submitted studies give endpoints that are higher than the newly submitted studies; therefore their results were not used for risk assessment. The RMS included the original summaries of the previously submitted studies in the section B.9.3.

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
CP 10.2.1/01	[redacted]	2010a	Novagib: Acute Toxicity To Rainbow Trout (*Oncorhynchus mykiss*) Report No. [redacted] GLP Unpublished	Y	Y	New study submitted for the purpose of renewal	Fine Agrochemicals Ltd	N
CP 10.2.1/02	Goodband, T. J. and Mullee, D. M.	2010b	Novagib: Acute Toxicity To *Daphnia magna* Report No. 0673/0014 Harlan Laboratories Ltd., Shardlow, UK GLP	N	Y	New study submitted for the purpose of renewal	Fine Agrochemicals Ltd	N

			Unpublished					
CP 10.2.1/03	Vryenhoef, H. and Mullee, D. M.	2010	Novagib: Algal Growth Inhibition Test Report No. 0673/0015 Harlan Laboratories Ltd., Shardlow, UK GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Fine Agrochemicals Ltd	N
CP 10.2.1/04	Scheerbaum, D.	2012	Novagib: Aquatic plant toxicity test, *Lemna minor*, limit-test, semi-static, 7 days Report No. 120508FM/ SLM15085 Dr. U. Noack-Laboratorien, Sarstedt, Germany GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Fine Agrochemicals Ltd	N
CP 10.2.1/05	Hermes, H. & Wydra, V.	2014	Toxicity of Novagib to the aquatic plant *Myriophyllum spicatum* in a static growth inhibition limit test with a prior rooting phase Report No. 90251215 IBACON GmbH, Rossdorf, Germany GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Fine Agrochemicals Ltd	N
CP 10.3.2.1/01	Jeker, L.	2010	Novagib: Toxicity to the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae) under Worst-Case Laboratory Conditions Report No. C94908 Harlan Laboratories Ltd., Itingen, Switzerland GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Fine Agrochemicals Ltd	N
CP 10.4.1.1/01	Goodband, T.J.	2011	Novagib: Acute toxicity to earthworms (*Eisenia foetida)* Report No. 41004741 Harlan Laboratories Ltd., Shardlow, UK	N	Y	New study submitted for the purpose of renewal	Fine Agrochemicals Ltd	N

			GLP Unpublished					
CP 10.6.2/01	Fiebig, S.	2017	Novagib GA4/7 (FAL460) Terrestrial Plant Test: Vegetative Vigour Test Report No. 170411FO / TNW17709 Noack Laboratorien GmbH, Sarstedt, Germany GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Fine Agrochemicals Ltd	N