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and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

GIBBERELLINS (GA4, GA7)

Volume 3 – B.5 (PPP) – Novagib

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B.5. METHODS OF ANALYSIS

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.5.1. METHODS USED FOR THE GENERATION OF PRE-AUTHORISATION DATA

B.5.1.1. Analysis of the plant protection product

B.5.1.1.1. Methods for the determination of the active substance and/or variant in the plant protection product

Validated analytical methods for the determination of the active substance in the plant protection product are provided below in accordance with Commission Regulation (EU) No 284/2013. The methods have been previously peer-reviewed at member state level in a registration report (zRMS: The Netherlands, July 2014).

Previous evaluation:	This study was evaluated in the RR (KIIIA1 5.2.1/01)
Data point addressed:	KCP 5.1.1/01
Author(s) (year):	Parsons A.H. (2006)
Title:	HPLC Determination of Gibberellins GA4 and GA7 in Technical Material and Formulations
Laboratory report / project number:	J 15061
Testing facility:	G C Laboratories Ltd.
Published:	No
Test guideline used:	PSD Guidelines for the Validation of Analytical Methods for Pesticides (PRD 2400) Commission Directive 96/46/EC SANCO/3030/99 rev. 4 Annex II (part A, section 4) and Annex III (part A, section 5) of Commission Directive 91/414
Deviations:	None
GLP:	Yes
EU agreed endpoint:	No

Materials and methods
Method: M564
Test material: Novagib
Lot/Batch No.: 2309CA
Purity: Determined in the study, ca. 1.02 % w/w (GA4)
Stability of test compound: Not reported

Principle of the method

The method was validated for the determination of GA4 in Novagib formulation at concentrations equivalent to 0.375 to 0.625 g/L (75 to 125% nominal). Following the addition of valerophenone internal standard and perchloric acid and filtration (where necessary), concentrations of GA4 were determined by high performance liquid chromatography with ultraviolet (HPLC-UV) at 210 nm.

Linearity

The linearity of the detector was demonstrated by duplicate determinations of five calibration solutions of GA4 prepared in methanol over the range 0.25 to 0.75 g/L, equivalent to 50 to 150% nominal amount of GA4. A

calibration plot was provided with values given for the intercept (-0.0005) and slope (0.0765). The coefficient of determination was 0.9998.

Specificity

No interfering peaks occurred at the retention times of the analyte GA4 in the blank formulation or internal standard solution. No interfering peaks occurred at the retention time of the internal standard, valerophenone, in sample solutions of the Novagib formulation. Example chromatograms of all control samples and the sample solutions were provided.

Samples of GA4 technical material and Novagib formulation were further analysed by high performance liquid chromatography coupled with a diode array detector (HPLC-DAD, 190 to 400 nm). Analyte identity was confirmed by spectra and retention time matching with the GA4 analytical standard.

The purities of GA4 peaks in the Novagib formulation and analytical standard were confirmed by recording UV spectra using HPLC/DAD at seven different points across each of the chromatographic peaks visible at the GA4 retention time and statistical analysis by the instrument software. A strong correlation was observed between he spectra indicating GA4 peak purity and the lack of co-eluting peaks.

Accuracy

Three recovery samples were prepared where known amounts of GA4 technical were added to portions of a blank Novagib formulation (without GA4) at nominal weights of ±25%, equivalent to 0.375, 0.50 and 0.625 g/L. Individual and mean recoveries for GA4 were within the nominal acceptance range 97 to 103% for active substances present at levels between 1 and 10% in a formulation (SANCO/3030/99 rev. 4) and therefore considered acceptable.

Recovery findings

A summary of the recovery results for GA4 in the test samples is presented in the table below.

Table 5.1.1.1-01: Recovery results of GA4 in Novagib formulation

Analyte	Fortification level (g GA4/L)	Nominal GA4 concentration vs. Novagib formulation (%)	Individual recoveries (%)	Number of analysis (n)	Mean recovery (%)
GA4	0.375	75	101.9	1	-
	0.50	100	99.8	1	-
	0.625	125	100.1	1	-
	Overall	-	-	3	100.6

Repeatability

Five replicate samples of technical material and the Novagib formulation were prepared at concentrations across the range 75 to 125% nominal, equivalent to 0.375, 0.438, 0.50, 0.563 and 0.625 g/L. The %RSD values obtained were 0.745% for the technical material and 0.693% for the Novagib formulation, and were therefore below the %RSD calculated using the modified Horwitz equation (1.36% for the technical material and 2.67% for the Novagib formulation) and considered acceptable.

RMS/ comments and conclusion:

Accuracy determination was done at three concentrations in only one measurement. However since the SANCO/30/99 rev. 4 does not require accuracy determination, the method is fully validated for determination of the active substance GA4 in the PPP according to SANCO/3030/99 rev.4. No further data are required.

Previous evaluation	This study was evaluated in the RR (KIIIA1 5.2.1/02).
Data point addressed:	KCP 5.1.1/02
Author(s) (year):	Knowles R.J. (2010)
Title:	Validation of GC Laboratories Ltd Method M652/A "HPLC Determination of Impurities in Technical Gibberellin GA4/GA7 and Determination of GA7 in Technical Gibberellin GA4/GA7 and Formulations" for the Determination of GA7 in the Novagib Formulation
Laboratory report / project number:	J17950
Testing facility:	G C Laboratories Ltd.
Published:	No
Test guideline used:	SANCO/3030/99 rev. 4 Annex II (part A, section 4) and Annex III (part A, section 5) of Commission Directive 91/414
Deviations:	None
GLP:	Yes
EU agreed endpoint:	No

Materials and methods

Method: M652/A
Test material: Novagib (9:1)
Lot/Batch No.: 1401437008
Purity: Determined in the study, ca. 0.0274 % w/w (GA7)
Stability of test compound: Not reported

Test material: Novagib (2:1)
Lot/Batch No.: 1401441002
Purity: Determined in the study, ca. 0.272 % w/w (GA7)
Stability of test compound: Not reported

Principle of the method

Samples of Novagib 9:1 and 2:1 (ca. 25 g for 2:1 or 50 g for 9:1) were weighed into volumetric flasks (100 mL). An aliquot (10 mL) of internal standard solution (n-butyl paraben in methanol) was added followed by methanol (20 mL), acetonitrile (25 mL) and water (20 mL). Test solutions are placed in an ultrasonic bath or five minuted. Solutions are made to volume with water. Concentrations of GA4/7 were determined by HPLC and UV detection at 206 nm.

The RR states:

"The products Novagib (9:1) and Novagib (2:1) were used in this study. These products are identical to Novagib".

Linearity

The linearity of the detector was demonstrated by determinations of five concentrations of standard calibration solutions of GA7 in internal standard:methanol:acetonitrile:deionised water solution over the range 26.5 to 1259 µg/mL, equivalent to 50 to 2500 µg/g. A calibration plot was provided with values given for the slope (0.00619) and intercept (0.01698). The coefficient of determination was 0.99997.

Specificity

No peak interferences occurred at the retention times of GA7 in the blank formulation (propylene glycol) or internal standard solution. No peak interferences occurred at the retention times of the internal standard, n-butyl paraben, in the blank formulation or GA7 standard solution. Example chromatograms of all control samples and the sample solutions were provided. Analyte identity was confirmed by UV spectra (collected using HPLC-DAD) and retention time matching with the GA7 analytical standard.

The purities of the GA7 peak in the Novagib formulations and analytical standard were confirmed by collecting UV spectra using HPLC-DAD at several different points across each of the peaks eluting at the GA7 retention time and statistical analysis by the instrument software. A strong correlation was obtained between the spectra indicated GA7 peak purity and confirming the lack of co-eluting peaks.

Accuracy

Two sets of five recovery samples were prepared where known amounts of GA7 analytical standard were added to blank Novagib formulation (without GA7). One set of recovery samples were for the Novagib 9:1 formulation, with solutions prepared using 3.1 to 9.5 mg GA7. The other set of samples were for the Novagib 2:1 formulation, with solutions prepared using 15.3 to 44.3 mg GA7. The overall mean recoveries for GA7 in the formulations (100.1 and 100.4) were within the range 97 to 103% for active substances present at levels between 1 and 10% in a formulation (SANCO/3030/99 rev. 4) and were therefore considered acceptable.

Repeatability

Five replicate samples of Novagib 9:1 and 2:1 formulations were prepared taking sample weights of between 12 and 38 g for 9:1 and 6 and 19 g for 2:1. The %RSD values obtained (9:1 formulation: 0.40%, 2:1 formulation: 1.79%) were below the %RSD calculated using the modified Horwitz equation (9:1 formulation: 4.6%, 2:1 formulation: 3.26%), therefore are considered acceptable.

RMS/Co-RMS comments and conclusion:

The method is fully validated for determination of the active substance GA7 in the PPP according to SANCO/3030/99 rev.4. No further data are required.

B.5.1.1.2. Methods for determination of relevant impurities identified in the technical material or which may be formed during manufacture of the plant protection product or from degradation of the plant protection product during storage

No relevant impurities have been identified in technical GA4/7 as manufactured. No relevant impurities are formed during manufacture of the plant protection product Novagib or form upon storage.

B.5.1.1.3 Methods for the determination of relevant co-formulants or components of co-formulants, where required by the national competent authorities

No relevant co-formulants or components of co-formulants are present in the plant protection product Novagib.

B.5.1.2. Methods for the determination of residues

B.5.1.2.1. Methods In soil, water, sediment, air and any additional matrices used in support of environmental fate studies

Methods for the determination of residues of the representative product Novagib are equivalent to those provided for the active substance. Therefore, methods supplied and conclusions given for the active substance are applicable here also.

For methods supporting the determination of residues of the active substance GA4/7 in environmental fate studies, refer to Vol.3 Section B.5 for active substance.

B.5.1.2.2. Methods in soil, water and any additional matrices used in support of efficacy studies

No new efficacy studies have been submitted for the representative product or active substance as part of the active substance renewal of GA4/7. Therefore, methods in soil, water and any additional matrices used in support of efficacy studies are not required.

B.5.1.2.3. Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

Methods for the determination of residues of the representative product Novagib are equivalent to those provided for the active substance. Therefore, methods supplied and conclusions given for the active substance are applicable here also.

For methods supporting the determination of residues of the active substance GA4/7 in toxicological studies, refer to Vol.3 Section B.5 for active substance.

B.5.1.2.4. Methods in body fluids, air, and any additional matrices used in support of operator, worker, resident and bystander exposure studies

Methods for the determination of residues of the representative product Novagib are equivalent to those provided for the active substance. Therefore, methods supplied and conclusions given for the active substance are applicable here also.

For methods supporting the determination of residues of the active substance GA4/7 in exposure studies, refer to Vol.3 Section B.5 for active substance.

B.5.1.2.5. Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies

Methods for the determination of residues of the representative product Novagib are equivalent to those provided for the active substance. Therefore, methods supplied and conclusions given for the active substance are applicable here also.

For methods supporting the determination of residues of the active substance GA4/7 in residues studies, refer to Vol.3 Section B.5 for active substance.

B.5.1.2.6. Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

Pre-registration methods that have been used in support of ecotoxicological studies are presented below in accordance with Commission Regulation (EU) No 284/2013. These methods have not been considered previously at EU level.

Previous evaluation:	None
Data point addressed:	KCP 5.1.2/01
Author(s) (year):	[redacted] (2010)
Title:	Novagib: Acute Toxicity to Rainbow Trout (*Oncorhynchus Mykiss*)
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	OECD 203 Method C.1
Deviations:	None
GLP:	Yes
EU agreed endpoint:	No

Materials and methods
Method: Verification of test item concentration, appendix to report 0673/0013
Test material: Novagib
Lot/Batch No.: 1401437008
Purity: 10.3 g/L (GA4/7)
Stability of test compound: The test item was shown to be stable in the aqueous test medium

Principle of the method

Dechlorinated tap water was fortified with Novagib in solutions of methanol and dechlorinated tap water (50:50, v/v) at a concentration of GA4/7 equivalent to 100 mg/L. Samples were diluted with methanol prior to analysis by HPLC-MS/MS to determine GA4/7 contents.

Recovery findings

A summary of the recovery results for GA4/7 in dechlorinated tap water is presented in the table below.

Table 5.1.2.6-01: Recovery results for GA4/7 in dechlorinated water analysed during the method validation

Analyte	Fortification level (mg/L)	Individual Recoveries (%)	Number of Analysis (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
GA4/7	100	N/R	5	99	0.83	99-101

NR – Not reported

Linearity

The linearity of the detector was confirmed using single determinations of nine calibration solutions of Novagib (in methanol:dechlorinated tap water, 50:50 v/v) prepared over the range 1.1 to 81 mg/L of GA4/7. A representative calibration curve was provided in the report and values given for slope (12267) and intercept (-249.67). The coefficient of determination was 0.9999.

Specificity

Representative chromatograms of control samples, analytical standards at the lowest calibration level and samples fortified at the LOQ (100 mg/L) were provided. No interferences were observed ≥30% of the LOQ at the retention times of GA4/7 in the chromatograms of three control samples. LC-MS/MS as a detection technique can be considered to be highly specific and therefore a confirmatory technique is not required.

Accuracy and repeatability

Five samples of aqueous test media were fortified with Novagib (in methanol:dechlorinated tap water, 50:50 v/v) at concentrations of GA4/7 equivalent to 100 mg/L. Although the accuracy of the method was only reported at one fortification level, the overall mean recovery for GA4/7 in the matrix was within the range 70 to 120% and the %RSD value was 0.83 which is <20%, therefore the method is still considered to be accurate and precise.

Limit of quantification

The LOQ for GA4/7 in aqueous media was reported to be 0.42 mg/L, calculated as the mean of GA4 and GA7 sample concentrations that gave peak equivalents ten times the baseline noise. However, the lowest fortification level validated was 100 mg/L. Therefore, the LOQ according to SANCO/3029/99 rev. 4 is 100 mg/L.

Conclusion

The method was successfully validated for the determination of GA4/7 in deionised water in accordance with SANCO/3029/99 rev. 4.

RMS/Co-RMS comments and conclusion:

The method was validated for determination of GA4/7 in deionised water according to SANCO/3029/99 rev.4. No further data required.

Previous evaluation:	None
Data point addressed:	KCP 5.1.2/02
Author(s) (year):	Goodband T.J., Mullee D.M. (2010a)
Title:	Novagib: Acute Toxicity to *Daphnia Magna*
Laboratory report / project number:	0673/0014
Testing facility:	Harlan Laboratories Ltd.
Published:	No

Test guideline used:	OECD 202 Commission Regulation (EC) No. 440/2008 Method C.2
Deviations:	None
GLP:	Yes
EU agreed point:	No

Materials and methods
Method: Verification of test item concentration, appendix to report 0673/0014
Test material: Novagib
Lot/Batch No.: 1401437008
Purity: 10.3 g/L (GA4/7)
Stability of test compound: The active ingredients were shown to be stable in the aqueous test medium

Principle of the method

Reconstituted water was fortified with solutions of Novagib in methanol and reconstituted water (50:50, v/v), at a concentration of GA4/7 equivalent to 100 mg/L. Solutions were diluted with methanol prior to analysis by LC-MS/MS to determine GA4/7 content using an external standard.

Recovery findings

A summary of the recovery results for GA4/7 in reconstituted water is presented in the table below.

Table 5.1.2.6-02: Recovery results of GA4/7 in the test item during method validation

Analyte	Fortification level (mg/L)	Individual recoveries (%)	Number of analysis (n)	Mean recovery (%)	RSD (%)	Recovery Range (%)
GA4/7	100	N/R	5	102	1.1	100-103

NR – Not reported

Linearity

The linearity of the detector was confirmed using duplicate determinations of seven calibration solutions of Novagib (in methanol and reconstituted water) prepared over the range 1 to 76 mg/L of GA4/7. A representative linear calibration curve was provided with values for intercept (5142.6) and slope (11473) given. The coefficient of determination was 0.9996.

Specificity

Representative chromatograms of control samples, analytical standards at the lowest calibration level and samples fortified at the LOQ were provided. No interferences were observed ≥30% of the LOQ at the retention times of GA4/7 in the chromatograms of five control samples. LC-MS/MS as a detection technique can be considered to be highly specific and therefore a confirmatory technique is not required.

Accuracy and repeatability

Five samples of aqueous test media were fortified with Novagib (in methanol and reconstituted water) at concentrations of GA4/7 equivalent to 100 mg/L. Although the accuracy of the method was only reported at one fortification level, the overall mean recovery for GA4/7 in the matrix was within the range 70 to 120% and the %RSD value was ≤20%, therefore the method is still considered to be sufficiently accurate and precise.

Limit of quantification

The LOQ for GA4/7 in aqueous media was reported to be 0.44 mg/L, calculated as the sample concentration that gave a peak equivalent to ten times the baseline noise. However, the lowest fortification level validated was 100 mg/L. Therefore, the LOQ according to SANCO/3029/99 rev. 4 is 100 mg/L.

RMS/Co-RMS comments and conclusion:

The method was validated for determination of GA4/7 in aqueous test media according to SANCO/3029/99 rev.4. No further data required.

Previous evaluation:	None
Data point addressed:	KCP 5.1.2/03
Author(s) (year):	Vryenhoef H., Mullee D.M. (2010)
Title:	Novagib: Algal Growth Inhibition Test
Laboratory report / project number:	0673/0015
Testing facility:	Harlan Laboratories Ltd.
Published:	No
Test guideline used:	OECD 201 Commission Regulation (EC) No. 440/2008 Method C.3
Deviations:	None
GLP:	Yes
EU agreed point:	No

Materials and methods

Method: Verification of test item concentration, appendix to report 0673/0015
Test material: Novagib
Lot/Batch No.: 1401437008
Purity: 10.3 g/L (GA4/7)
Stability of test compound: The active ingredients were shown to be stable in the aqueous test medium

Principle of the method

Culture test medium was fortified with solutions of Novagib in culture medium and methanol (50:50, v/v) at concentrations of GA4/7 equivalent to 1.0, 10 and 100 mg/L. Amounts of GA4/7 were determined by LC-MS/MS using an external standard.

Recovery findings

A summary of the recovery results for GA4/7 in the culture medium is presented in the table below.

Table 5.1.2.6-03: Recovery results for GA4/7 in culture medium

Analyte	Fortification Level (mg/L)	Individual Recoveries (%)	Number of Analysis (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
GA4/7	1.0	N/R	5	88	3.0	84-90
	10	N/R	5	95	2.4	92-98
	100	N/R	5	99	0.7	99-100
	Overall	-	15	94	4.8	84-100
	1.0 plus algae	86	1	86	-	86
	100 plus algae	101	1	101	-	101

N/R – Not reported

Linearity

The linearity of the detector was confirmed using single determinations of 10 concentrations of calibration solutions of Novagib (in culture medium and methanol) prepared over the range 0.42 to 79 mg/L of GA4/7. A representative linear calibration curve was provided with values for the slope (12272) and intercept (2870.1) given. The coefficient of determination was 0.9993.

Specificity

Representative chromatograms of control samples, analytical standards at the lowest calibration level and samples fortified at the LOQ (1.0 mg/L) have been provided. No interferences were observed at or above the LOQ at the retention times of GA4/7 in the chromatograms of five control samples. LC-MS/MS as a detection technique can be considered to be highly specific.

Accuracy

Quantitative samples of culture medium were fortified with Novagib (in culture medium and methanol) at concentrations of GA4/7 equivalent to 1.0, 10 and 100 mg/L. Five samples were analysed per fortification level. The %RSD for GA4/7 recoveries were ≤20% for all fortification levels and therefore are considered acceptable. Two further recovery samples at concentrations of 1.0 and 100 mg/L were also analysed following addition of algal cells to test solutions to assess the effects of algae on GA4/7 recoveries. The presence of algal cells showed no effect on GA4/7 recoveries.

Repeatability

Mean recoveries at each fortification level and overall were within the range 70 to 120%, therefore are considered acceptable.

Limit of quantification

The method LOQ for GA4/7 in culture medium was reported to be 0.70 mg/L, calculated as the sample concentration that gave a peak equivalent to ten times the baseline noise. However, the lowest fortification level validated was 1.0 mg/L. Therefore, the LOQ according to SANCO/3029/99 rev. 4 is 1.0 mg/L.

RMS/Co-RMS comments and conclusion:

The method was validated for determination of GA4/7 in culture medium according to SANCO/3029/99 rev.4. No further data required.

Previous evaluation:	None
Data point addressed:	KCP 5.1.2/04
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
Published:	No
Test guideline used:	OECD 221 SANCO 3029/99 rev.4
Deviations:	None
GLP:	Yes
EU agreed endpoint:	No

Materials and methods

Method: Verification of GA4 concentration, used in report 120508FM

Test material: Novagib

Lot/Batch No.: 1021437001

Purity: 9.98 g/L (GA4/7)

Stability of test compound: Not reported

Principle of the method

Swedish Standard (SIS) medium was fortified with 50 and 100 mg/L of Novagib, equivalent to GA4 concentrations of 0.432 and 0.864 mg/L. Acetonitrile (10%) was added to the fortified solutions to stabilise the sample. Amounts of GA4 were determined by ultra-high performance liquid chromatography coupled with tandem mass spectrometric detection (UHPLC-MS/MS) using an external standard. GA4 was used to represent combined GA4/7 due to the low content of GA7 in the test item <0.1% and poor sensitivity within the UHPLC-MS/MS system.

Recovery findings

A summary of the recovery results for GA4 in SIS medium is presented in the table below.

Table 5.1.2.6-04: Recovery results for GA4 in SIS medium

Analyte	Fortification Level (mg GA4/L)	Individual Recoveries (%)	Number of Analysis (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
GA4	0.433	97, 94, 91, 103, 99	5	97	4.8	91-103
	0.859	92, 102, 102, 96, 103	5	99	4.8	92-103
	Overall	-	10	98	4.7	91-103

Linearity

The detector response was demonstrated to be quadratic using single determinations of 7 calibration solutions of GA4 standard (in methanol:water:acetonitrile solution) prepared over the range 0.25 to 3.00 mg/mL. A representative calibration curve was provided in the report along with the equation of the line. The coefficients of determinations were ≥0.992.

Specificity

Representative chromatograms of control samples, analytical standards at the lowest calibration level and samples fortified at the LOQ (0.433 mg/L) have been provided. No interferences were observed ≥30% of the LOQ at the retention times of GA4 in the chromatograms of two control samples. LC-MS/MS as a detection technique can be considered to be highly specific. The method measured two MS/MS transitions: 331.3 → 269.2 m/*z* (quantification) and 331.2 → 213.2 m/*z* (confirmation).

Accuracy

Quantitative samples of SIS medium were fortified with 50 and 100 mg/L of Novagib, equivalent to 0.432 and 0.864 mg/L of GA4 (or 0.433 and 0.859 mg/L taking weighing of the test item into account). Five samples were analysed per fortification level. Individual and overall %RSD values for GA4 recoveries were ≤20%, therefore are considered acceptable.

Repeatability

Mean recoveries at each fortification level and overall were within the range 70 to 120%, therefore are acceptable.

Limit of quantification

The method LOQ for GA4 in SIS medium was reported to be 0.25 mg/L. However, the lowest fortification level validated was 0.433 mg/L. Therefore, the LOQ according to SANCO/3029/99 rev. 4 is 0.433 mg/L.

RMS/Co-RMS comments and conclusion:

The method was validated for determination of GA4 in SIS medium according to SANCO/3029/99 rev.4.. No further data required.

Previous evaluation:	None
Data point addressed:	KCP 5.1.2/05
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:	Institut für Biologische Analytik und Consulting IBACON GmbH
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, 22-Jul-2013 and revision of 22-Jul-2013 Draft, 02-Dec-2013 OECD 219 (2013) OECD 239 (Draft)
Deviations:	None*
GLP:	Yes
EU agreed endpoint:	No

*Deviations to the method were noted in the report. These deviations did not affect the analytical method.

Materials and methods

Method: Verification of test item concentrations, described in report 90251215, raw data archived under project number 90251215A.
Test material: Novagib
Lot/Batch No.: 1021437010
Purity: 9.92 g/L (GA4/7)
Stability of test compound: Not reported

Principle of the method

Samples of test water were fortified with Novagib at concentrations of 80 and 120 mg/L. Samples were homogenised (by mechanical shaking or ultrasound treatment for one minute), dilution with acetonitrile and analysed for GA4/7 contents by HPLC-UV at 206 nm.

Recovery findings

A summary of the recovery results for GA4/7 in aqueous medium is presented in the table below.

Table 5.1.2.6-04: Recovery results for GA4/7 in aqueous medium

Analyte	Fortification Level (mg/L)	Individual Recoveries (%)	Number of Analysis (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
GA4/7	80	95, 94, 94, 94, 95	5	94	0.6	94-95
	120	92, 93, 93, 95, 96	5	94	1.8	92-96
	Overall	-	10	94	1.3	92-96

Linearity

The linearity of the detector was confirmed using single determinations of eight calibration solutions of Novagib (in acetonitrile and test water, 1:1 v/v) over the range 25 to 80 mg/L. A linear calibration curve was provided with values given for the slope (548) and intercept (-3965). The coefficient of determination was 0.9999.

Specificity

Labelled chromatograms of control samples, analytical standards at the lowest calibrated level and samples fortified at the LOQ (80 mg/L) were provided. No interference of total peak area for the target analyte (GA4/7) were found in any of the control samples.

Accuracy

Quantitative samples of test water were fortified with Novagib at 80 and 120 mg/L. Five samples were analysed per fortification level. Individual and overall %RSD values for GA4 recoveries were ≤20%, therefore are considered acceptable.

Repeatability

Mean recoveries at each fortification level and overall for GA4 were within the range 70 to 120% and therefore considered to be acceptable.

Limit of quantification

The method LOQ was reported to be 80 mg/L, equivalent to the lowest validated fortification level.

RMS/Co-RMS comments and conclusion:

The method was validated for determination of GA4/7 in the aqueous medium according to SANCO/3029/99 rev.4. No further data required.

B.5.1.2.7. Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

Methods for the determination of residues of the representative product Novagib are equivalent to those provided for the active substance. Therefore, methods supplied and conclusions given for the active substance are applicable here also.

For methods supporting the determination of residues of the active substance, GA4/7 in physico-chemical studies, refer to Vol.3 Section B.5 for the active substance.

B.5.2. METHODS FOR POST-APPROVAL CONTROL AND MONITORING PURPOSES

B.5.2.1. Methods for the determination of residues in or on plants, plant products, processed food commodities, food and feed of plant and animal origin

Methods for the determination of residues of the representative product Novagib will be equivalent to those provided for the active substance. Therefore, post-authorisation control and monitoring methods supplied and conclusions given for the active substance are applicable here also.

For post-monitoring methods supporting the determination of residues of the active substance GA4/7 in or on plants, plant products, processed food commodities, food and feed of plant and animal origin, refer to Vol.3, part B.5 for the active substance.

B.5.2.2. Methods for the determination of residues in body fluids and tissues

No studies have been submitted for the active substance GA4/7 or representative product Novagib as part of active substance renewal of GA4/7. Methods are not required as GA4/7 is presented as a low-risk, non-toxic active substance that is a naturally occurring with detected levels found to be close to background (EFSA Journal 2012; 10(1); 2502).

Regulation (EC) No. 1107/2009 concerning the placing of plant protection products on the market describes the criteria for approval of active substances as low-risk (Section 5, Annex II). This criterion has been amended in the guidance document SANTE/11953/2015 rev.5 (in draft, 22 March 2016). In accordance with the criteria outlined in SANTE/11953/2015 rev.5 (in draft, 22 March 2016), GA4/7 is considered low risk as the representative uses do not require specific risk mitigation measures (i.e. measures deduced as a result of a risk assessment that must be applied to ensure safe use and are not a generalised precautionary approach). Furthermore, GA4/7 is not explosive nor classified as acutely toxic (Cat. 1, 2 or 3), a skin corrosive (Cat. 1A, 1B or 1C), a skin sensitiser (Cat. 1), mutagenic (Cat. 1A, 1B or 2), carcinogenic (Cat. 1A, 1B or 2), toxic to reproduction (Cat. 1A, 1B or 2), a Specific Target Organ Toxicant (Cat. 1 or 2) or causes serious damage to the eyes (Cat. 1). GA4/7 is not expected to display neurotoxic or immunotoxic effects and there is no evidence for GA4/7 as an endocrine disrupter. GA4/7 is neither persistent in soil/water-sediment nor expected to be bio-accumulative or leach to groundwater through soil. GA4/7 is also not classified as toxic to aquatic life (Cat. 1) and risk mitigation measures were not required to protect the environment based on the outcome of the ecotoxicological risk assessment.

B.5.2.3. Methods for the determination of residues in soil

Methods for the determination of residues of the representative product Novagib will be equivalent to those provided for the active substance. Therefore, post-authorisation control and monitoring methods supplied and conclusions given for the active substance are applicable here also.

For post-monitoring methods supporting the determination of residues of the active substance GA4/7 in soil, refer to Vol.3, part B.5 for the active substance.

B.5.2.4. Methods for the determination of residues in water

Methods for the determination of residues of the representative product Novagib will be equivalent to those provided for the active substance. Therefore, post-authorisation control and monitoring methods supplied and conclusions given for the active substance are applicable here also.

For post-monitoring methods supporting the determination of residues of the active substance GA4/7 in water, refer to Vol.3, part B.5 for the active substance.

B.5.2.5. Methods for the determination of residues in air, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible

Methods for the determination of residues of the representative product Novagib will be equivalent to those provided for the active substance. Therefore, post-authorisation control and monitoring methods supplied and conclusions given for the active substance are applicable here also.

For post-monitoring methods supporting the determination of residues of the active substance GA4/7 in air, refer to Vol.3, part B.5 for the active substance.

B.5.3. REFERENCES RELIED ON

By Data point

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
KCP 5.1.1/01	Parsons, A.H.	2006	HPLC Determination of Gibberellins GA4 and GA7 in Technical Material and Formulation Report No. J 15061 G C Laboratories Ltd., Analytical Chemistry Centre, Stotfold, Hitchin, UK GLP Unpublished	N	N	-	Fine Agrochemicals Ltd.	In RR: KIIIA1 5.2.1/01
KCP 5.1.1/02	Knowles, R.J.	2010	Validation of GC Laboratories Ltd Method M652/A "HPLC Determination of Impurities in Technical Gibberellin GA4/GA7 and Determination of GA7 in Technical Gibberellin GA4/GA7 and Formulations" for the Determination of GA7 in the Novagib Formulation Report No. J17950 G C Laboratories Ltd., Analytical Chemistry Centre, Stotfold, Hitchin GLP Unpublished	N	N	-	Fine Agrochemicals Ltd.	In RR: KIIIA1 5.2.1/02

CP 5.1.2/01	[redacted]	2010a	Novagib: Acute Toxicity Study To Rainbow Trout (*Oncorhynchus mykiss*) [redacted] GLP Unpublished	Y	Y	New study submitted for the purpose of renewal	Fine Agrochemicals Ltd.	-
CP 5.1.2/02	Goodband, T.J. and Mullee, D.M.	2010 b	Novagib: Acute Toxicity Study To *Daphnia Magna* Report No. 0673/0014 Harlan Laboratories Ltd., Shardlow, UK GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Fine Agrochemicals Ltd.	-
CP 5.1.2/03	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.	-
CP 5.1.2/04	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.	-
CP 5.1.2/05	Hermes, H. and Wydra, V.	2014	Toxicity of Novagib to the Aquatic Plant *Myriophyllum Spicatum* in a Static Growth Inhibition Limit Test with a Prior Rooting Phase	N	Y	New study submitted for the purpose of	Fine Agrochemicals Ltd.	-

			Report No. 90251215 IBACON GmbH, Rossdorf, Germany GLP Unpublished			renewal		