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 (GA 4, GA7)

Volume 3 – B.7 (AS)

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

Version History

When	What
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B.7. RESIDUE DATA

Introduction

This document has been prepared to evaluate the European Gibberellins 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).
Gibberelin has been identified as a presumed low-risk active substance in the Commission working document on the AIR-IV renewal programme (SANTE-2016-10616-rev 8). The EU Gibberellin Task Force (EGTF) proposes that Gibberelin is a low risk active substance according to Regulation (EC) 1107/2009 as amended by Commission Regulation 2017/1432.

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

Previous EU assessment

The dossier to support the first inclusion of gibberellins 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 July 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

Summaries of available data and overall assessments of each sub-section, as well as the exposure assessments, generally are not included in this document. Instead these parts of the assessment are included in Vol. 1, Level 2. The reason behind this structure is to avoid repetition and facilitate revisions of the assessment. As a result, this Annex B only contains the presentation and evaluation of individual study reports on the active substance.

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 gibberellins 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.7.1. STORAGE STABILITY OF RESIDUES

a) Previous evaluation (2005-2011)

Data on the stability of residues of $GA_4/_7$ were not evaluated as part of the original EU review.

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

Two new studies are available and are summarised below.

Data point addressed:	CA 6.1.1/01
Author(s) (year):	Jean-Baptiste C, 2011
Title:	Frozen storage stability of residues of gibberellic acid, gibberellin A_4 and gibberellin A_7 in pears
Laboratory report / project number:	R A9206
Testing facility:	ANADIAG, France
Published:	No
Test guideline used:	EU Working Document 7032/VI/95 rev 5. (Appendix H) SANCO 825/00 rev7 (14 March 2004) SANCO 3029/99 rev 4 (11 July 2000)
Deviations:	n/a
GLP:	Yes

Executive summary

Samples of pears were fortified with either gibberellin A_4 (GA_4) or gibberellin A_7 (GA_7) at a concentration equivalent to 0.2 mg/kg. The samples were then stored under frozen conditions (approximately - 18°C) and analysed at 0, 1, 3, 6 and 12 month intervals.

Residues of GA_4 and GA_7 were found to be stable in pears for up to 12 months when stored frozen, with no significant decrease (>30% compared with the zero time value) over that period of time.

Materials and methods

The storage stability samples and associated procedural recovery samples were fortified with GA_4 or GA_7 standard solutions prepared in ethyl acetate using either GA_4 analytical standard (batch number 090505-CR-GIB-II, purity 95.83%) or GA_7 analytical standard (batch number 100119-CR-GIB-I, purity 94.46% or batch number 090505-CR-GIB-I, purity 97.13%).

Test commodities

The storage stability study was conducted on pears.

Study design and methods

Fortification and storage of samples

The storage stability and associated procedural recovery samples were fortified at a GA_4 or GA_7 concentration equivalent to 0.2 mg/kg.

A sufficient number of samples were prepared to enable at least duplicate analysis at each time point for each active substance. After fortification, the samples were placed in the freezer and stored at approximately -18°C.

At the appropriate time point, samples were removed and analysed. This was with the exception of three samples per active substance which were analysed immediately after fortification as zero day samples. In addition, a sufficient number of untreated control samples were stored frozen under the same conditions to enable analysis of a single control and a single procedural recovery sample per active substance at each time point.

Analytical method

Analysis of the samples was performed according to an analytical method previously developed and validated in ANADIAG report A9006.

After adding milliQ water to the pear samples and adjustment of the pH to 2, residues of GA_4 and GA_7 were extracted with ethyl acetate. After centrifugation, the resulting ethyl acetate extracts were evaporated prior to dilution with methanol and quantification by high performance liquid chromatography with tandem mass spectrometry (LC-MS/MS). Quantification of GA_4 residues was achieved using the ion transition m/z 332.9→268.9, while confirmation analysis incorporated the ion transitions m/z 332.9→286.9 and m/z 332.9→314.9. Quantification of GA_7 residues was achieved using the ion transition m/z 330.9→268.9, while confirmation analysis incorporated the ion transitions m/z 330.9→294.9 and m/z 330.9→312.9.

Results and discussion

At each time point, a single procedural recovery sample was prepared at a GA_4 or GA_7 concentration of 0.2 mg/kg and analysed alongside the corresponding storage stability and procedural control samples. Across the time points, procedural recovery values for GA_4 and GA_7 ranged from 87-110%, confirming the efficient performance of the analytical method.

The recovery of GA_4 and GA_7 from pears stored at approximately -18°C are summarised in Tables 7.1-01 and 7.1-02, respectively, below. The results presented are both corrected and uncorrected for freshly fortified recoveries.

Table 7.1-01 Freezer storage stability for GA_4 in pears

Sampling interval (nominal months)	Sampling interval (actual days)	Uncorrected recoveries (%)	Mean uncorrected recovery (%)	Procedural recovery (%)	Mean corrected recovery (%)[1]
Pears					
0	0	96, 89, 103	96	87	110
1	30	102, 97	100	109	109
3	91	103, 104	104	109	109
6	178	105, 100	103	103	103
12	359	95, 89	92	96	96

[1] Mean recovery only corrected when the procedural recovery is <100%

Table 7.1-02 Freezer storage stability for GA_7 in pears

Sampling interval (nominal months)	Sampling interval (actual days)	Uncorrected recoveries (%)	Mean uncorrected recovery (%)	Procedural recovery (%)	Mean corrected recovery (%)[1]
Pears					
0	0	104, 106, 107	106	110	106

Sampling interval (nominal months)	Sampling interval (actual days)	Uncorrected recoveries (%)	Mean uncorrected recovery (%)	Procedural recovery (%)	Mean corrected recovery (%)[1]
1	30	104, 95	100	96	104
3	91	97, 105	102	105	102
6	178	103, 107	106	107	106
12	359	103, 89	97	109	97

[1] Mean recovery only corrected when the procedural recovery is <100%

Conclusions

Residues of GA4 and GA7 were stable in pears for at least 12 months when stored frozen.

RMS comment and conclusion:

Untreated samples of pears were fortified with GA_4 and GA_7 separately at a level of 0.2 mg/kg (10 x LOQ) and frozen at -18°C. At least two parallel samples of these pears were analysed for GA_4 and GA_7 separately following storage for 0, 1, 3, 6 and 12 months. One pear sample freshly spiked with GA_4 and GA_7 separately was analysed to obtain procedural recoveries each time the stored spiked samples were analysed. Results show that GA_4 and GA_7 are stable in pears stored at -18°C for at least 12 months. The study was conducted under GLP conditions and a valid guideline (OECD 506). Therefore study results are acceptable.

Data point addressed:	CA 6.1.1/02
Author(s) (year):	Harrison C, 2010
Title:	To determine the stability of gibberellin A_4 (GA4) and gibberellin A_7 (GA7) in pome fruit apple specimens following storage at ca -18°C for 0, 1, 3, 12, 18 and 30 months.
Laboratory report / project number:	AD/6258/VB
Testing facility:	EUROFINS Agroscience Services, UK
Published:	No
Test guideline used:	EU Working Document 7032/VI/95 rev 5. (Appendix H)
Deviations:	n/a
GLP:	Yes

Executive summary

Samples of apples were fortified with GA_4/A_7 at a concentration equivalent to 0.5 mg/kg. The samples were then stored under frozen conditions (approximately - 18°C) and analysed at 0, 1, 3, 12, 18 and 30 month intervals.

Residues of GA_4/A_7 were found to be stable in apples for up to 30 months when stored frozen, with no significant decrease (>30% compared with the zero time value) over that period of time.

Materials and methods

The storage stability samples and associated procedural recovery samples were fortified with a GA_4/A_7 standard solution prepared in acetonitrile using GA_4/A_7 analytical standard (code number 12519, purity 90.6%).

Test commodities

The storage stability study was conducted on apples.

Study design and methods

Fortification and storage of samples

The storage stability and associated procedural recovery samples were fortified at a GA_4/A_7 concentration equivalent to 0.5 mg/kg.

A sufficient number of samples were prepared to enable at least triplicate analysis at each time point. After fortification, the samples were placed in the freezer and stored at approximately -18°C. At the appropriate time point, samples were removed and analysed. This was with the exception of three samples which were analysed immediately after fortification as zero day samples. In addition, a sufficient number of untreated control samples were stored frozen under the same conditions to enable analysis of a single control and a single procedural recovery sample at each time point.

Analytical method

Analysis of the samples was performed according to analytical method VBC 30011/Crops/KB/03/1, based on a method previously developed and validated in sponsor TNO study 738, Report V99.1181.

The apple samples were extracted by the addition of methanol followed by homogenisation and centrifugation, after which an aliquot of the resulting supernatant was taken and concentrated prior to dilution with formic acid solution. The resulting sample was subject to reverse phase solid phase extraction (SPE) PolarPlus C18 clean-up prior to quantification by high performance liquid chromatography and tandem mass spectrometry (LC-MS/MS). Quantification was achieved using the ion transitions m/z 331.3→225.1, 331.1→243.2 and 331.3→287.4 for GA_4 and ion transition m/z 329.4→223.2 for GA_7.

Results and discussion

At each time point, a single procedural recovery sample was prepared at a GA_4/A_7 concentration of 0.5 mg/kg and analysed alongside the corresponding storage stability and procedural control samples. Across the time points, procedural recovery values for GA_4/A_7 ranged from 79-125%, confirming the efficient performance of the analytical method.

The recovery of GA_4/A_7 from apples stored at approximately -18°C are summarised in Table 7.1-03, below. The results presented are both corrected and uncorrected for freshly fortified recoveries.

Table 7.1-03 Freezer storage stability for GA_4/A_7 in apples

Sampling interval (nominal months)	Sampling interval (actual days)	Uncorrected recoveries (%)	Mean uncorrected recovery (%)	Procedural recovery (%)	Mean corrected recovery (%)[1]
Apples					
0	0	103, 99, 106	103	101	103
1	42	72, 78, 75	75	79	95
3	74	122, 127, 116	122	125	122
12	369	118, 133, 93	115	109	115
18	553	111, 110, 102	108	124	108

Sampling interval (nominal months)	Sampling interval (actual days)	Uncorrected recoveries (%)	Mean uncorrected recovery (%)	Procedural recovery (%)	Mean corrected recovery (%)[1]
30	921	75, 80, 85	80	85	94

[1] Mean recovery only corrected when the procedural recovery is <100%

Conclusions

Residues of GA4 and GA7 were stable in apples for at least 30 months when stored frozen.

RMS comment and conclusion:

Untreated samples of apples were fortified with $GA_4 + GA_7$ at a level of 0.5 mg/kg (10 x LOQ) and frozen at -18°C. Three parallel samples of these pears were analysed for GA_4 and GA_7 following storage for 0, 1, 3, 6, 12, 18 and 30 months. One apple sample freshly spiked with $GA_4 + GA_7$ was analysed to obtain procedural recoveries each time the stored spiked samples were analysed. Results show that $GA_4 + GA_7$ mixture is stable in apples stored at -18°C for at least 30 months. The study was conducted under GLP conditions and a valid guideline (OECD 506). Therefore study results are acceptable.

Overall conclusions for Storage stability of Residues

Acceptable storage stability data are available that demonstrate residues of $GA_4/_7$ are stable in crops representative of pome fruit for up to 30 months.

RMS comment and conclusion:

According to valid guideline (OECD 506) if uses are sought in just one of the five commodity categories (for instance high water content category), then residue freezer storage stability data beyond one representative commodity in that category will be needed. Therefore storage stability of GA_4/GA_7 is proven for at least 30 months in high water content category (where pome fruit is included).

Stability of residues in sample extracts

RMS comment and conclusion:

Stability in sample extracts is proven with recoveries obtained during validation of analytical methods and procedural recoveries obtained during measurement of residues.

B.7.2. METABOLISM, DISTRIBUTION AND EXPRESSION OF RESIDUES

B.7.2.1. Plants

a) Previous evaluation (2005-2011)

Information on the metabolism of gibberellins $GA_4/_7$ was evaluated as part of the original EU review for $GA_4/_7$ and is available in the EU DAR. This information is considered appropriate for the current assessment to support renewal of $GA_4/_7$ and no new studies are submitted.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. No new evaluation has been performed. The conclusion has not been changed.
Data point addressed:	CA 6.2.1/01 (II A 6.1.1/01 in original DAR)
Author(s) (year):	Goodyear A. (2005)
Title:	The Metabolism of Gibberellins GA_4/A_7 in Plants.
Laboratory report / project number:	22-1-02.PMET
Testing facility:	Technology Sciences (Europe) Limited, Knaresborough, UK
Published:	No
Test guideline used:	Not applicable, summary of published academic research
Deviations:	n/a
GLP:	No
EU Agreed Endpoint:	Gibberellins are a group of naturally occurring diterpenoid acids that function as plant growth regulators by influencing a range of developmental processes in higher plants including stem elongation, germination, dormancy, flowering, sex expression, enzyme induction and leaf an fruit senescence. Specific plant metabolism studies were not conducted since the metabolism of gibberellins in plants is widely documented in literature accessible in the public domain.

RMS comment and conclusion:

In this study representative literature reference (scientific paper) showed that GA_4 and GA_7 are widespread in plants including many common varieties of fruits, vegetables and cereals.

In this study also representative literature references (scientific papers) containing information on the metabolism of gibberellins in plants have been summarised and it can be concluded that the metabolism of gibberellins GA_4 in plants primarily involves conjugation with glucose to form 3-O-glucosides and glucosyl esters. Hydroxylation at the 13-C position also occurs and results in the formation of GA_1 from which the GA_4-13-O-glucoside is then subsequently formed. Hydroxylation at the 2-carbon position was also reported giving rise to the gibberellins GA_8 and GA_{34}, with subsequent glucose conjugation possible. Gibberellin GA_2 was also tentatively observed as a further hydroxylation product. Similarly from GA_7 metabolites like GA_7-3-O-glucoside are formed.

Fact that GA_4 and GA_7 are naturally present in plants and that metabolism of GA_4 and GA_7 is accessible in literature, no further studies are required.

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

No new studies were submitted.

B.7.2.2. Poultry

a) Previous evaluation (2005-2011)

No data were submitted or evaluated as part of the original EU review for $GA_{4/7}$.

Gibberellins occur naturally in a wide range of plants and it would not be possible to distinguish naturally occurring $GA_{4/7}$ in plants from that resulting from plant protection uses. Information in published literature

indicates that there is no significant difference between naturally occurring levels and the levels found in supervised residue trials supporting the use of $GA_4/_7$ on pome fruit.
Furthermore, pome fruit and their processed products are not considered to be potential feed items for poultry (OECD Guidance Document on Overview of Residue Chemistry Studies, 2009). Therefore metabolism studies in poultry are not required.

RMS comment and conclusion:

Studies on metabolism and distribution of GA_4/GA_7 in poultry are not necessary, since it is not possible to distinguish GA_4/GA_7 in products of animal origin, resulting from consumption of naturally occurring residues, from those resulting from the use of plant growth regulators. Moreover pome fruit is not a feeding stuff for poultry. Therefore metabolism data in poultry are not necessary.

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

No new studies were submitted.

B.7.2.3. Lactating ruminants

a) Previous evaluation (2005-2011)

No data were submitted or evaluated as part of the original EU review for $GA_4/_7$.

Gibberellins occur naturally in a wide range of plants and it would not be possible to distinguish naturally occurring $GA_4/_7$ in plants from that resulting from plant protection uses. Information in published literature indicates that there is no significant difference between naturally occurring levels and the levels found in supervised residue trials supporting the use of $GA_4/_7$ on pome fruit.

Apple pomace is considered a potential animal feed item for ruminants in the EU. Residues of $GA_4/_7$ in apples were below the LOQ of 0.05 mg/kg in all supervised residue trials (see B 7.3.). Therefore, residues of $GA_4/_7$ in the diet of lactating ruminants will not exceed 0.004 mg/kg bw/day and metabolism studies are not required.

RMS comment and conclusion:

Studies on metabolism and distribution of GA_4/GA_7 in ruminants are not necessary, since it is not possible to distinguish GA_4/GA_7 in products of animal origin, resulting from consumption of naturally occurring residues, from those resulting from the use of plant growth regulators. Moreover residues of $GA_4/_7$ in apples, the only feeding stuff for animals in this assessment, were below the LOQ of 0.05 mg/kg. Therefore the trigger value of 0.004 mg/kg bw/day in the diet of lactating ruminants is not exceeded, meaning that metabolism data in ruminants are not necessary.

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

No new studies were submitted.

B.7.2.4. Pigs

a) Previous evaluation (2005-2011)

No data were submitted or evaluated as part of the original EU review for $GA_4/_7$.

Gibberellins occur naturally in a wide range of plants and it would not be possible to distinguish naturally occurring $GA_4/_7$ in plants from that resulting from plant protection uses. Information in published literature indicates that there is no significant difference between naturally occurring levels and the levels found in supervised residue trials supporting the use of $GA_4/_7$ on pome fruit.
Furthermore, pome fruit and their processed products are not considered to be potential animal feed items for swine (OECD Guidance Document on Overview of Residue Chemistry Studies, 2009). Therefore metabolism studies in pig are not required.

RMS comment and conclusion:

Studies on metabolism and distribution of GA_4/GA_7 in pigs are not necessary, since it is not possible to distinguish GA_4/GA_7 in products of animal origin, resulting from consumption of naturally occurring residues, from those resulting from the use of plant growth regulators. Moreover pome fruit is not a feeding stuff for pigs. Therefore metabolism data in pigs are not necessary.

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

No new studies were submitted.

B.7.2.5. Fish

a) Previous evaluation (2005-2011)

Gibberellins occur naturally in a wide range of plants and it would not be possible to distinguish naturally occurring $GA_4/_7$ in plants from that resulting from plant protection uses. Information in published literature indicates that there is no significant difference between naturally occurring levels and the levels found in supervised residue trials supporting the use of $GA_4/_7$ on pome fruit.

Furthermore, pome fruit and their processed products are not considered to be potential feed items for fish (SANCO/11187/2013 rev. 3). Therefore metabolism studies in fish are not required.

RMS comment and conclusion:

Studies on metabolism and distribution of GA_4/GA_7 in fish are not necessary, since it is not possible to distinguish GA_4/GA_7 in products of animal origin, resulting from consumption of naturally occurring residues, from those resulting from the use of plant growth regulators. Moreover pome fruit is not a feeding stuff for fish. Therefore metabolism data in fish are not necessary.

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

No new studies were submitted.

B.7.3. MAGNITUDE OF RESIDUE TRIALS IN PLANTS

B.7.3.1. Treated apples

A summary of the EU GAP for apples is given in Table B.7.3.1-01.

Table B.7.3.1-01: Critical GAP on apples for $GA_{4/7}$ in the EU

Crop	EU residues zone	Application					PHI (days)
		Max. no.	Interval (days)	Max. rate (kg a.s./ha)	Spray Volume (L/ha)	Growth stage (BBCH)	
Apples	North and south	4	7	0.005	300-1000	69 to 74	n/a

a) Previous evaluation (2005-2011)

Residue trials conducted on apples were evaluated as part of the original EU review for $GA_{4/7}$ and are available in the EU DAR. Two studies were evaluated. These studies are considered appropriate for the current assessment to support renewal of $GA_{4/7}$, and no new studies are submitted. Full details of the study are provided in the EU DAR; however the trial summary results are presented in Table B.7.3.1-02 for information.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. No new evaluation has been performed.
Data point addressed:	CA 6.3.1/01 (II A 6.3.1/01 in original DAR)
Author(s) (year):	Harrison C. (2005a).
Title:	VBC 30011: Residue levels in apples and pears from trials carried out in Northern France, Southern France, Italy and Northern Spain during 2002.
Laboratory report / project number:	AF/6256/VB
Testing facility:	Agrisearch UK, Ltd
Published:	No
Test guideline used:	EU Working Document 1607/VI/97 rev. 2
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	Residues of GA_4/GA_7 in apples and pears were <0.05 mg/kg at normal harvest. See summary in Table 6.3.1-02.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. No new evaluation has been performed.
Data point addressed:	CA 6.3.1/02 (II A 6.3.1/02 in original DAR)
Author(s) (year):	Harrison C. (2005b).
Title:	VBC 30011: Residue levels in apples and pears from trials carried out in Northern France, Southern France, Italy and Northern Spain during 2003.
Laboratory report / project number:	AF/6989/VB
Testing facility:	Agrisearch UK, Ltd
Published:	No
Test guideline used:	EU Working Document 1607/VI/97 rev. 2
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	Residues of GA_4/GA_7 in apples and pears were <0.05 mg/kg at normal harvest. See summary in Table 6.3.1-02.

Executive summary

Supervised crop residue trials were carried out in northern and southern Europe on apples, using SL and SG formulations containing gibberellins $GA_{4/7}$ at concentrations of 10 g a.s./L and 100 g a.s/kg, respectively. Treatments were applied during or immediately after flowering, which is consistent with the recommended timing. Trials were conducted at a higher rate per application (14-17 g a.s/ha) than the critical EU GAP (5 g a.s/ha). In all trials, residues of gibberellins $GA_{4/7}$ measured in whole apples were below the LOQ of 0.05 mg/kg.

Apple samples were stored frozen (*ca* -18°C) on the day of harvest and extracted within 27 months for the 2002 trials and within 12 months for the 2003 trials. Acceptable data demonstrating the stability of residues of $GA_{4/7}$ in apples is available for a period of up to 30 months, therefore the data are considered fully supported.

A total of eight acceptable trials on apples (2 in NEU and 6 x SEU) are available. In all the trials residues of $GA_{4/7}$ were below the LOQ of 0.05 mg/kg, including those trials that were overdosed in relation to the supported EU GAP. As apples are considered major crops in both the northern and southern residues regions, generally a minimum of eight trials on each crop per region are required to supported a use. However according to EU guidance a reduced trials data set is acceptable when residues are predicted to be below the limit of quantification, and two residue trials confirm this to be the case.

Therefore there are sufficient acceptable trials available, given that residues were all < LOQ in trials where the application rate was higher than the proposed GAP to support a use on apples in both northern and southern EU. The GAP on apples is supported by the available data.

Materials and Methods

Apples are major crops in northern and southern Europe, therefore generally a minimum of eight trials are required from each zone, conducted across at least two growing seasons.

Two trials on apples were conducted in northern Europe, all conducted in northern France. A further six trials on apples were conducted in southern Europe, comprising three trials in southern France, two trials in Italy and one trial in Spain. Each trial consisted of three plots, one to be used as the treated plot using a 100 g/kg SG

formulation, one to be used as the treated plot using a 10 g/L SL formulation and the remaining untreated plot to be used to generate control samples.

At each apple trial, four applications were made to the treated plots, between growth stages BBCH 65-74, at rates ranging from 0.014-0.017 kg a.s./ha and with intervals ranging from 10-12 days. Samples of apples were harvested from the untreated plot and both treated plots on one occasion 103-131 days post-last application, the harvest being conducted at growth stage BBCH 87 which corresponds to normal commercial harvest.

Following harvest, the apple samples were stored frozen at approximately - 18°C for up to 790 days prior to analysis. The storage period for the residue samples fell within the known period of freezer storage stability for GA_4 and GA_7 in apples of 30 months.

The apple samples were analysed for residues of GA_4 and GA_7 using analytical method VBC 30011/CROPS/KB/03/1 previously developed and validated in study/report V99.1181.

Samples of apples were extracted by the addition of methanol followed by homogenisation and centrifugation, after which an aliquot of the resulting supernatant was taken and concentrated prior to dilution with formic acid solution. The resulting sample was subject to reverse phase solid phase extraction (SPE) PolarPlus C18 clean-up prior to quantification by high performance liquid chromatography and tandem mass spectrometry (LC-MS/MS). Quantification was achieved using the ion transitions m/z 331.3→225.1, 331.1→243.2 and 331.3→287.4 for GA4 and ion transition m/z 329.4→223.2 for GA7.

Results

A summary of the residue data is presented in Table B.7.3.1-02.

Residues of $GA_{4/7}$ were determined to be < 0.05 mg/kg (< LOQ) for treated apples collected from each trial, at each harvest interval. In addition, no residues of $GA_{4/7}$ were detected in the corresponding control samples.

Procedural recovery values ranged from 63-112% (mean 88%) and were considered sufficient to demonstrate efficient performance of the analytical method.

Table B.7.3.1-02 **Summary of residues trial data previously reviewed for $GA_{4/7}$ in apples in the EU**

Trial No./ Location/ EU zone/ Year (a)	Commodity/ Variety (a)	Date of 1.Sowing or planting 2.Flowering 3. Harvest (b)	Formulation	Application rate per treatment			Dates of treatment or no. of treatments and last date (c)	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days) (d)	Details on trial (e)
				g a.s./ ha	Water (l/ha)	g a.s./hl				$GA_{4/7}$		
AF/6256/VB/1 45160, Rouvres, France NORTHERN EU 2002	Apples/ Jonagored	1. 1996 2. – 3. 2002	100 g/kg SG	15.2 14.8 14.5 13.6	1013 987 968 904	1.5 1.5 1.5 1.5	09 Apr 02 19 Apr 02 29 Apr 02 09 May 02	BBCH 65 BBCH 67 BBCH 69-71 BBCH 72	Fruit	<0.05	131	Data point CA 6.3.1/01. Sampling date: 17 Sep 02 Storage time before analysis: 766 days
			10 g/l SL	14.5 14.8 14.8 14.6	968 987 987 974	1.5 1.5 1.5 1.5	09 Apr 02 19 Apr 02 29 Apr 02 09 May 02	BBCH 65 BBCH 67 BBCH 69-71 BBCH 72	Fruit	<0.05	131	
AF/6256/VB/3 82200, Meauzac France * SOUTHERN EU 2002	Apples/ Golden	1. 1986 2. – 3. 2002	100 g/kg SG	15.6 15.5 15.5 15.5	1042 997 1033 1031	1.5 1.5 1.5 1.5	02 Apr 02 12 Apr 02 22 Apr 02 02 May 02	BBCH 65 BBCH 67 BBCH 69-71 BBCH 71-72	Fruit	<0.05	125	Data point CA 6.3.1/01. Sampling date: 04 Sep 02 Storage time before analysis: 779 days
			10 g/l SL	15.9 15.0 15.9 15.5	1058 1000 1058 1033	1.5 1.5 1.5 1.5	02 Apr 02 12 Apr 02 22 Apr 02 02 May 02	BBCH 65 BBCH 67 BBCH 69-71 BBCH 71-72	Fruit	<0.05	125	

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Formulation	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Details on trial
				g a.s./ ha	Water (l/ha)	g a.s./hl				$GA_{4/7}$		
	(a)	(b)					(c)				(d)	(e)
AF/6256/VB/4 82700, Meauzac France * SOUTHERN EU 2002	Apples/ Golden	1. 1992 2. – 3. 2002	100 g/kg SG	15.0 15.6 15.1 15.9	1000 1040 1004 1063	1.5 1.5 1.5 1.5	02 Apr 02 12 Apr 02 22 Apr 02 02 May 02	BBCH 65 BBCH 67 BBCH 69-71 BBCH 71-72	Fruit	<0.05	117	Data point CA 6.3.1/01. Sampling date: 27 Aug 02 Storage time before analysis: 790 days
			10 g/l SL	15.3 15.7 15.2 15.7	1020 1044 1012 1048	1.5 1.5 1.5 1.5	02 Apr 02 12 Apr 02 22 Apr 02 02 May 02	BBCH 65 BBCH 67 BBCH 69-71 BBCH 71-72	Fruit	<0.05	117	
AF/6256/VB/5 40061, Emilia Romagna Italy SOUTHERN EU 2002	Apples/ Golden	1. 1992 2. – 3. 2002	100 g/kg SG	14.3 15.7 14.6 13.6	952 1046 970 904	1.5 1.5 1.5 1.5	04 Apr 02 14 Apr 02 24 Apr 02 06 May 02	BBCH 65 BBCH 67-69 BBCH 69 BBCH 71-72	Fruit	<0.05	126	Data point CA 6.3.1/01 Sampling date: 09 Sep 02 Storage time before analysis: 777 days
			10 g/l SL	13.6 16.5 14.0 15.8	904 1101 933 1053	1.5 1.5 1.5 1.5	04 Apr 02 14 Apr 02 24 Apr 02 06 May 02	BBCH 65 BBCH 67-69 BBCH 69 BBCH 71-72	Fruit	<0.05	126	

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Formulation	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Details on trial
				g a.s./ ha	Water (l/ha)	g a.s./hl				$GA_{4/7}$		
	(a)	(b)					(c)				(d)	(e)
AF/6989/VB/2 Maine- et-Loire France NORTHERN EU 2003	Apples/ Gala	1. 1989 2. – 3. 2003	100 g/kg SG	15.9 14.3 15.7 15.2	1060 950 1047 1013	1.5 1.5 1.5 1.5	15 Apr 03 25 Apr 03 05 May 03 15 May 03	BBCH 65 BBCH 67 BBCH 71 BBCH 71-72	Fruit	<0.05	104	Data point CA 6.3.1/02 Sampling date: 27 Aug 03 Storage time before analysis: 338 days
			10 g/l SL	15.5 15.6 16.0 15.4	1033 1040 1067 1027	1.5 1.5 1.5 1.5	15 Apr 03 25 Apr 03 05 May 03 15 May 03	BBCH 65 BBCH 67 BBCH 71 BBCH 71-72	Fruit	<0.05	104	
AF/6989/VB/4 Rhone France SOUTHERN EU 2003	Apples/ Golden	1. 1990 2. – 3. 2003	100 g/kg SG	15.1 14.7 15.0 15.0	1006 981 1000 1000	1.5 1.5 1.5 1.5	17 Apr 03 27 Apr 03 07 May 03 17 May 03	BBCH 65 BBCH 67 BBCH 73 BBCH 72	Fruit	<0.05	110	Data point CA 6.3.1/02 Sampling date: 04 Sep 03 Storage time before analysis: 330 days
			10 g/l SL	15.0 14.7 14.8 15.2	1000 981 988 1012	1.5 1.5 1.5 1.5	17 Apr 03 27 Apr 03 07 May 03 17 May 03	BBCH 65 BBCH 67 BBCH 73 BBCH 72	Fruit	<0.05	110	

Trial No./ Location/ EU zone/ Year (a)	Commodity/ Variety (a)	Date of 1.Sowing or planting 2.Flowering 3. Harvest (b)	Formulation	Application rate per treatment			Dates of treatment or no. of treatments and last date (c)	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days) (d)	Details on trial (e)
				g a.s./ ha	Water (l/ha)	g a.s./hl				$GA_{4/7}$		
AF/6989/VB/5 Emilia Romagna Italy SOUTHERN EU 2003	Apples/ Nero red	1. 1985 2. – 3. 2003	100 g/kg SG	15.7 14.9 13.8 16.3	1048 991 917 1088	1.5 1.5 1.5 1.5	28 Apr 03 08 May 03 18 May 03 28 May 03	BBCH 65-67 BBCH 69 BBCH 71-72 BBCH 72-74	Fruit	<0.05	125	Data point CA 6.3.1/02 Sampling date: 30 Sep 03 Storage time before analysis: 304 days
			10 g/l SL	15.6 13.8 14.6 14.5	1042 921 976 965	1.5 1.5 1.5 1.5	28 Apr 03 08 May 03 18 May 03 28 May 03	BBCH 65-67 BBCH 69 BBCH 71-72 BBCH 72-74	Fruit	<0.05	125	
AF/6989/VB/6 Aragon Spain SOUTHERN EU 2003	Apples/ Golden	1. 1995 2. – 3. 2003	100 g/kg SG	15.2 15.6 14.7 14.9	1010 1039 980 990	1.5 1.5 1.5 1.5	22 Apr 03 02 May 03 12 May 03 22 May 03	BBCH 65 BBCH 67 BBCH 71 BBCH 72	Fruit	<0.05	103	Data point CA 6.3.1/02 Sampling date: 02 Sep 03 Storage time before analysis: 332 days
			10 g/l SL	15.3 16.6 15.0 14.9	1020 1108 1000 990	1.5 1.5 1.5 1.5	22 Apr 03 02 May 03 12 May 03 22 May 03	BBCH 65 BBCH 67 BBCH 71 BBCH 72	Fruit	<0.05	103	

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)

(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Conclusions

Following up to four applications of either VBC30011 or Regulex to apples between growth stage BBCH 65-74, at rates ranging from 0.014-0.017 kg a.s./ha and with intervals varying from 10-12 days, residues of $GA_{4/7}$ were < 0.05 mg/kg in fruit collected 103-131 days post the last application, corresponding to normal commercial harvest.

RMS comment and conclusion:

The stability of analysed apples is covered by stability data.

All trials were conducted at GAP 4-times at excessive application rate (14-17 g a.s./ha instead of 5 g a.s./ha), latest treatment at BBCH 72-74, therefore they can be considered as covering the proposed GAP.

Residues of $GA_{4/7}$ were < 0.05 mg/kg in apples in all 6 field trials in Southern Europe and in both trials in Northern Europe.

Table 7.3.1-03: Summary of procedural recoveries for $GA_{4/7}$ in apples/pears in study AF/6256/VB (conducted in 2002)

Fortification level (mg/kg)	**Recovery (%)**
0.05	103
0.05	112
Mean recovery (%)	108
RSD (%)	5.9

Table 7.3.1-04: Summary of procedural recoveries for $GA_{4/7}$ in apples in study AF/6989/VB (conducted in 2003)

Fortification level (mg/kg)	**Recovery (%)**
0.01	94
0.1	63
Mean recovery (%)	79
RSD (%)	27.9

Mean procedural recoveries (70-110%) and RSD (≤ 20 %) were acceptable according to SANCO/3029/99 rev. 4 in study AF/6256/VB. In study AF/6989/VB one procedural recovery at 0.1 mg/kg was too low (63%), which also resulted in too high RSD (27.9%). According to Document SANTE/11813/2017 (Guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed) acceptable recovery during routine analyses is 60-140%. We think that recovery 63 % had no significant impact on determination of residues in field trials. Therefore all trials conducted in 2002 and 2003 are acceptable.

Trials marked with * conducted in 2002 are located in the same region and are considered as one trial.

All trials were conducted under GLP conditions and a valid guideline (OECD 509; OECD 66/164; SANCO 7525/VI/95 rev. 10.3).

For the reasons listed above 7 trials are acceptable: 2 on North of Europe and 5 on South of Europe. According to SANCO 7525/VI/95 rev. 10.3 when the residues of an active substance are foreseen to be below the LOQ and at least two trials confirm this then no further trials are normally necessary. Therefore proposed use on apples can be approved in Northern and Southern Europe.

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

No new studies were submitted.

B.7.3.2. Untreated apples

a) Previous evaluation (2005-2011)

(Ref: Hedden P; 2003; as reported in EU DAR and addenda, Hungary, 2011).

Gibberellins (GAs) are a family of naturally occurring plant hormones which are widespread in plants and fungi. Gibberellins are ubiquitous in higher plants and are usually most abundant in growing tissues, where they are present at concentrations of 0.00001 to 0.1 mg/kg mg/kg fresh weight, depending on tissue, species and nature of the GA. Roots also produce gibberellins, which are normally present at lower concentrations than in shoots. Reproductive tissues have generally higher gibberellins content than vegetative organs, with particularly large amounts in anthers and pollen, and in developing seeds. Concentrations greater than 10 mg/kg fresh weight have been found in the endosperm and/or immature cotyledons of some species of plants.

RMS comment and conclusion:

The above mentioned reference was listed among references evaluated in DAR in ecotoxicology section. This reference is a scientific paper, which among others describes natural occurrence of Gibberellins in plants. The conclusion of applicant is correct (Gibberellins are the most abundant in growing tissues and seeds) except the fact that maximum concentration of Gibberellins in growing tissues is 10^{-8} g/g fresh weight which is equal to 0.01 mg/kg fresh weight and not 0.1 mg/kg fresh weight.

(Ref: MacMillan J; 2002; *as reported in EU DAR and addenda, Hungary, 2011).*

The most widespread gibberellin in plants is GA_1, although its 13-deoxy analogue, GA_4 is usually also present, but at lower concentrations. In some species, such as Arabidopsis thaliana and members of the Cucurbitaceae, GA_4 is the predominant form and indeed has more growth-stimulating activity than GA_1 in these species. The occurrence of Gibberellins, including GA_4 and GA_7 in vascular plants, fungi and bacteria has been researched and listed by MacMillan.

The reference above (MacMillan, 2002) was cited in the EU DAR (Hungary 2011), however is presented below in more detail:

Data point addressed:	CA 6.3.2/02
Author(s) (year):	MacMillan, J. (2002)
Title:	Occurrence of Gibberellins in Vascular Plants, Fungi, and Bacteria Journal of Plant Growth Regulation, 20, 387-442
Published:	Yes

Summary

This review paper provides a compilation of the occurrence of GAs (GA_1 to GA_{126}) in vascular plants, fungi and bacteria, together with the type of tissue in which the identification was made.

The study summary lists only the findings presented for GA_3, GA_4 and GA_7.

GA3 has been found in 45 plant species, across 15 different families, often in immature or mature seeds, as well as in roots, shoots, leaves, fruits, pollen and silk. GA3 has also been found in 1 fungus and 6 bacteria species.
GA4 has been found in 54 plant species, across 29 different families, including in seeds, leaves, shoots, buds, fruits and pollen. GA4 has also been found in 7 fungi and 3 bacteria species.
GA7 has been found in 14 plant species, across 9 different families, including in seeds, leaves, shoots and pollen, as well as in 1 fungus species.

I MATERIALS AND METHODS

The natural occurrence of GAs in plants was initially established by their isolation, mainly from immature seeds in which the amounts are high. Since the early 1970s their identification from natural sources has relied mainly upon combined gas chromatography-mass spectrometry (GC-MS) data from purified, derivatised extracts and a comparison with reference GC-MS data. In this review paper the following criteria for the identification of the GAs have been applied:
1. Only publications in refereed journals are cited
2. Identification from normal (tall) phenotypes is given preference
3. Preliminary communications are not cited where a full paper has been published
4. GAs identified after hydrolysis of plant extracts are not included
5. Some papers, even though they are contained in refereed journals, have not been cited on the grounds that they are judged to contain insufficient data upon which to justify claimed identification.

II RESULTS AND DISCUSSION

The data presented in this review paper for GA_1 to GA_{126} show the widespread natural occurrence of GAs (128 plants, 7 fungi, and 7 bacteria). Summaries of the natural occurrence of GA_3, GA_4 and GA_7 are presented in the tables below.
The results show that GA_3 has been found in 45 plant species, across 15 different families, often in immature or mature seeds, as well as in roots, shoots, leaves, fruits, pollen and silk. GA_3 has also been found in 1 fungus and 6 bacteria species.
The results show that GA_4 has been found in 54 plant species, across 29 different families, including in seeds, leaves, shoots, buds, fruits and pollen. GA_4 has also been found in 7 fungi and 3 bacteria species.
The results show that GA_7 has been found in 14 plant species, across 9 different families, including in seeds, leaves, shoots and pollen, as well as in 1 fungus species.

III FINDINGS

Table 7.3.2-01: List of tissues of vascular plants, fungi and bacteria in which GA_3 has been found

Plant	Tissue	Common name of plant	Plant family
Abelmoschus esculentus	immature seed	Okra	Malvaceae
Althaea rosea	shoot apices	Common hollyhock	Malvaceae
Arabidopsis thaliana	seeds	Thale cress	Brassicaceae
Avena sativa	inflorescences	Oat	Poaceae
Brassica napus	stems, immature siliques	Oilseed rape	Brassicaceae
Calystegia soldanella	immature seeds	Bindweed	Convolvulaceae
Camellia sinensis	endosperm	Tea plant	Theaceae
Carica papaya	fruits	Papaya	Caricaceae
Carthamus tinctorius	stems	Safflower	Asteraceae
Citrus sinensis	fruitlets	Blood orange	Rutaceae
Cucumis melo	mature seeds	Muskmelon	Cucurbitaceae

Plant	Tissue	Common name of plant	Plant family
Cucumis sativus	mature seeds	Cucumber	Cucurbitaceae
Dalbergia dolichopetela	germinating seed		
Fragaria x ananassa Duch.	immature fruit- day neutral, leaves- day neutral, leaf exudates- short day	Strawberry	Rosaceae
Hordeum vulgare	germinating grain, leaf sheaths, developing grain, shoots	Barley	Poaceae
Ipomoea batatas	immature seeds	Sweet potato	Convolvulaceae
Ipomoea reptans	immature seeds	Water spinach	Convolvulaceae
Lactuca sativa	shoots	Lettuce	Asteraceae
Lolium temulentum	leaves	Darnel	Poaceae
Lycopersicon esculentum	cultured roots, leaves and shoot tips, unpollinated ovaries	Tomato	Solanaceae
Lupinus albus	seeds	Lupin	Fabaceae
Malus domestica	immature seeds	Apple	Rosaceae
Mangifera indica	leaves	Mango	Anacardiaceae
Marah macrocarpus	endosperm, embryo	Wild cucumber	Cucurbitaceae
Pennisetum glaucum	shoots	Millet	Poaceae
Pharbitis purpurea	immature seeds	Common morning glory	Convolvulaceae
Pharbitis tricolor	immature seeds	Mexican morning glory	Convolvulaceae
Phaseolus coccineus	immature seeds	Runner bean	Fabaceae
Phaseolus lunatus	stems, root nodules	Butter bean	Fabaceae
Picea abies	shoots	Norway spruce	Pinaceae
Picea sitchensis	shoots	Sitka spruce	Pinaceae
Pinus attenuata	pollen	Knobcone pine	Pinaceae
Pinus sylvestris	stem and needles	Scots pine	Pinaceae
Pisum sativum	pods, ovules, pollinated ovaries	Pea	Fabaceae
Prunus avium	fruitlets, germinating seeds, apices ex 10-wk and mature plants	Wild cherry	Rosaceae
Prunus cerasus	immature seeds	Sour cherry	Rosaceae
Prunus persica	immature seeds	Peach	Rosaceae
Pseudotsuga menziesii	shoots	Douglas fir	Pinaceae
Saccarum spp	leaves, shoot apical meristem	Sugarcane	Poaceae
Secale cereale	plants	Rye	Poaceae
Sechium edule	endosperm, embryo, testa	Chayote	Cucurbitaceae
Triticum aestivum	leaves, roots, stems, shoots, expanding internodes, young ears	Common wheat	Poaceae
Vigna unguiculata/ sinensis	leaves, petioles, epicotyls, stems	Cowpea	Fabaceae
Vitis vinifera	seeds, seeded berries	Common grapevine	Vitaceae
Zea mays	shoots, silk	Maize	Poaceae
Fungi			
Gibberella fujikuroi			
Bacteria			
Acetobacter diazotropicus			

Plant	Tissue	Common name of plant	Plant family
Azospirillum lipoferum			
Azospirillum brasilense			
Bacillus licheniformis			
Bacillus pumilus			
Herbospirillum seropedicae			

Table 7.3.2-02: List of tissues of vascular plants, fungi and bacteria in which GA_4 has been found

Plant	Tissue	Common name of plant	Plant family
Abelmoschus esculentus	immature seed	Okra	Malvaceae
Allium cepa	leaf sheaths	Onion	Amaryllidaceae
Alstroemeria hybrid	leaves	Lily	Alstroemeriaceae
Anemia phyllitidis	sporophytes	Fern	Anemiaceae
Arabidopsis thaliana	shoots, seeds	Thale cress	Brassicaceae
Aralia cordata	basal buds	Spikenard	Araliaceae
Begonia x cheimantha	leaves	Christmas begonia	Begoniaceae
Brassica napus	immature siliques	Oilseed rape	Brassicaceae
Calystegia soldanella	immature seeds	Bindweed	Convolvulaceae
Cibotium glaucum	sporophytes	Tree fern	Cibotiaceae
Citrus reticulata	developing fruit	Mandarin orange	Rutaceae
Citrus sinensis	immature fruit	Blood orange	Rutaceae
Citrus unshiu	young fruit, developing fruit	Tangerine	Rutaceae
Cucumis melo	mature seeds	Muskmelon	Cucurbitaceae
Cucumis sativus	mature seeds	Cucumber	Cucurbitaceae
Cucurbita maxima	endosperm, embryo	Squash	Cucurbitaceae
Cyathea australis	sporophytes	Rough tree fern	Cyatheaceae
Dalbergia dolichopetela	germinating seed		
Daucus carota	somatic cell embryo cultures	Wild carrot	Apiaceae
Dicksonia antarctica	sporophytes	Soft tree fern	Dicksoniaceae
Dioscorea opposita	dormant bulbils	Yam	Dioscoreaceae
Eucalyptus globulus	cambial region	Tasmanian bluegum	Myrtaceae
Helianthus annuus	seeds	Sunflower	Asteraceae
Hordeum vulgare	developing grain	Barley	Poaceae
Juglans regia	pollinated and unpollinated ovaries	Common walnut	Juglandaceae
Lilium elegans	bulbs	Lily	Liliaceae
Lupinus albus	seeds	Lupin	Fabaceae
Lycopersicon esculentum	leaves and shoot tips	Tomato	Solanaceae
Malus domestica	immature seeds, developing seeds	Apple	Rosaceae
Marah macrocarpus	endosperm, embryo	Wild cucumber	Cucurbitaceae
Matthiola incana	shoots and flower buds	Stock	Brassicaceae
Ornithogalum thyroides	inflorescences	Chinkerinchee	Asparagaceae
Orobanche minor	arial parts	Common broomrape	Orobanchaceae
Oryza sativa	ears, spikelets	Rice	Poaceae
Phaseolus coccineus	dark-grown seedlings, light-	Runner bean	Fabaceae

Plant	Tissue	Common name of plant	Plant family
	grown seedlings, cotyledonary embryo, suspensor		
Phaseolus vulgaris	immature seeds	Common bean	Fabaceae
Picea abies	shoots	Norway spruce	Pinaceae
Picea sitchensis	shoots	Sitka spruce	Pinaceae
Pimpinella anisum	somatic cell embryo cultures	Aniseed	Apiaceae
Pinus attenuata	pollen	Knobcone pine	Pinaceae
Pinus sylvestris	stem and needles	Scots pine	Pinaceae
Pisum sativum	internodes, fertilized ovules	Pea	Fabaceae
Pseudotsuga menziesii	shoots	Douglas fir	Pinaceae
Raphanus sativus	leaves, stem	Radish	Brassicaceae
Rumex acetosa	shoots	Sorrel	Polygonaceae
Rumex palustris	shoots	Marsh dock	Polygonaceae
Saccharum spp	apical meristem	Sugarcane	Poaceae
Sechium edule	endosperm, embryo	Chayote	Cucurbitaceae
Spinacia olerocea	shoots	Spinach	Amaranthaceae
Trifolium repens	arial parts	White clover	Fabaceae
Triticum aestivum	leaves and stems, expanding internode, shoots	Common wheat	Poaceae
Vigna unguiculata/ sinensis	hypocotyls	Cowpea	Fabaceae
Vitis vinifera x V.rupestris	somatic embryos	Grapevine	Vitaceae
Zea mays	shoots	Maize	Poaceae
Fungi			
Gibberella fujikuroi			
Phaeospheria sp. L489			
Sphaceloma bidentis			
Sphaceloma manihiticola			
Sphaceloma menthea			
Sphaceloma perseae			
Sphaceloma rhois			
Bacteria			
Bacillus licheniformis			
Bacillus pumilus			
Rhizobium phaseoli			

Table 7.3.2-03: List of tissues of vascular plants, fungi and bacteria in which GA_7 has been found

Plant	Tissue	Common name of plant	Plant family
Calystigia soldanella	seeds	Bindweed	Convolvulaceae
Daucus carota	somatic cell embryo cultures	Wild carrot	Apiaceae
Malus domestica	immature seeds, developing seeds	Apple	Rosaceae
Marah macrocarpus	endosperm, embryos	Wild cucumber	Cucurbitaceae
Ornithogalum thyroides	inflorescences	Chinkerinchee	Asparagaceae
Picea abies	shoots	Norway spruce	Pinaceae
Pimpinella anisum	somatic cell embryo cultures	Aniseed	Apiaceae
Pinus attenuata	pollen	Knobcone pine	Pinaceae
Pisum sativum	fertilized ovules	Pea	Fabaceae
Pseudotsuga menziesii	shoots	Douglas fir	Pinaceae
Sechium edule	endosperm, embryos, testa	Chayote	Cucurbitaceae
Spinacia oleracea	shoots	Spinach	Amaranthaceae
Triticum aestivum	leaves and stems	Common wheat	Poaceae
Zea mays	shoots	Maize	Poaceae
Fungi			
Gibberella fujikuroi			
Bacteria			
None listed			

IV CONCLUSIONS

The data presented in this review paper for GA_1 to GA_{126} show the widespread natural occurrence of GAs in plants, fungi and bacteria (reported here in 128 plants, 7 fungi, and 7 bacteria).

GA_3 has been found in 45 plant species, across 15 different families, often in immature or mature seeds, as well as in roots, shoots, leaves, fruits, pollen and silk. GA_3 has also been found in 1 fungus and 6 bacteria species.

GA_4 has been found in 54 plant species, across 29 different families, including in seeds, leaves, shoots, buds, fruits and pollen. GA4 has also been found in 7 fungi and 3 bacteria species.

GA_7 has been found in 14 plant species, across 9 different families, including in seeds, leaves, shoots and pollen, as well as in 1 fungus species.

RMS comment and conclusion:

The above mentioned reference was mentioned in text, but not listed among references evaluated in DAR. This reference is a review scientific paper presenting that the Gibberellins GA_1 to GA_{126} was identified in various tissues of numerous plants in fungus and in bacterium, which confirms their natural presence. GA_3 has been identified in 45 plant species, 1 fungus and 6 bacteria species. In plants GA_3 was found mainly in seeds, roots, shoots, leaves, fruits, pollen and silk. GA_4 has been identified in 54 plant species, 7 fungus and 3 bacteria species. In plants GA_4 was found mainly in seeds, leaves, shoots, buds, fruits and pollen. GA_7 has been identified in 14 plant species and 1 fungus species. In plants GA_7 was found mainly in seeds, leaves, shoots and pollen.

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

A review of published literature, focussing on the natural background levels in apples has been conducted and is presented below:

Data point addressed:	CA 6.3.2/03
Author(s) (year):	Stephan M., Bangerth F., Schneider G.; 1999
Title:	Quantification of endogenous gibberellins in exudates from fruits of *Malus domestica*. Plant Growth Regulation 28: 55-58, 1999
Published:	Yes – This reference was briefly reported in the EU DAR (Hungary, 2011) but not in detail.

Summary

A study was conducted to identify and quantify gibberellins in developing apple fruits from an orchard in Germany. Samples of fruits from four different varieties of apples were excised and placed pedicel end down in agar gel before being incubated in the dark at 20°C for 20 hours. The fruits were then removed and the agar plates containing the exudate were extracted.

Plates were lyophilised, powdered by pestle and mortar and then triple extracted at 4°C in 80% methanol contain 1mM BHT. Deuterium labelled standards of relevant gibberellins were used as internal standards for the procedure. The extracts were purified by anion exchange HPLC, concentrated and re-dissolved in methanol before analysis by LC-MS (ESI mode) monitoring for two ions per gibberellin.

The results are presented in terms of ng gibberellin per fruit and are summarised below:

Variety	**Sampling time (WAFB)[1]**	**Gibberellins (ng/fruit)**					
		GA_1	**GA_3**	**GA_4**	**GA_7**	**GA_{20}**	**GA_{34}**
Elstar	4	0.2	0.7	0.1	-	0.5	0.2
Spencer Seedless	6.5	0.4	3.5	14.4	-	0.5	-
Golden Delicious	5	0.2	2.8	21.3	0.8	0.7	0.1
Jonica	6	0.1	2.7	33.3	n.d[2]	0.2	0.3
Jonica	7	0.1	2.8	20.1	n.d[2]	0.4	0.3

[1] WAFB: Weeks after full bloom
[2] n.d: not determined

Levels of GA_4 found were in the range of 0.1 – 33.3 ng/fruit and levels of GA_7 were found up to 0.8 ng/fruit. GA_4 was the most abundant gibberellin found, followed by GA_3. Levels varied depending on variety. It should be noted that the levels reported are those were found in exudates rather than the total level in the apple fruit. It could be expected therefore that levels in the fruit themselves could be higher.

RMS comment and conclusion:

This reference is a scientific paper presenting that untreated apples contained GA_4 up to level 33.3 ng/fruit, which is equivalent to 0.00017 mg/kg when assumption is made that the weight of fruit is 200 g. Content of GA_7 is up to 0.8 ng/fruit, which is equivalent to 0.000004 mg/kg when assumption is made that the weight of fruit is

200 g. We can conclude that natural background of $GA_{4/7}$ in apples measured in this paper is less than LOQ of method used for measurement of $GA_{4/7}$ in treated apples (0.05 mg/kg).

Conclusion on natural background levels

There is evidence of the widespread natural occurrence of GAs in plants, fungi and bacteria across many different species and also within different plant parts of the same species.

Information in published literature indicates that naturally occurring gibberellins in untreated pome fruits can be found at levels up to 0.06 mg/kg at various stages of fruit development. It can be expected that naturally occurring levels in fruit will vary depending on such parameters as the stage of development, variety and part of the plant analysed as borne out by literature previously considered in the EU review that indicated that the highest levels will be found in apple seeds.

Residues of $GA_{4/7}$ in apples and pears at harvest maturity from supervised residue trials conducted in support of the use of $GA_{4/7}$ are also low (< LOQ) meaning there is evidence that there is no significant difference between naturally occurring levels and levels arising from the use of $GA_{4/7}$ as a plant protection product.

RMS comment and conclusion:

GA_4 has been identified in 54 plant species, 7 fungi and 3 bacteria species. In plants GA_4 was found mainly in seeds, leaves, shoots, buds, fruits and pollen.

GA_7 has been identified in 14 plant species and 1 fungus species. In plants GA_7 was found mainly in seeds, leaves, shoots and pollen.

In scientific papers natural background concentration of GA_4 in apples was up to 0.00017 mg/kg and natural background concentration of GA_7 up to 0.000004 mg/kg.

We can conclude that the use of $GA_{4/7}$ as a plant protection product results in residue levels similar to the natural levels in plants.

B.7.3.3. Treated pears

A summary of the EU GAP for pears is given in Table B.7.3.3-01.

Table B.7.3.3-01: Critical GAP on pears for $GA_{4/7}$ in the EU

Crop	EU residues zone	Application					PHI (days)
		Max. no.	Interval (days)	Max. rate (kg a.s./ha)	Spray Volume (L/ha)	Growth stage (BBCH)	
Pears	North and south	2	3	0.006	300-1000	62 to 69	n/a
Pears	North and south	1	n/a	0.012	300-1000	62 to 69	n/a

a) Previous evaluation (2005-2011)

Residue trials conducted on pears were evaluated as part of the original EU review for $GA_{4/7}$ and are available in the EU DAR. Two studies were evaluated. These studies are considered appropriate for the current assessment to

support renewal of $GA_{4/7}$, and no new studies are submitted. Full details of the study are provided in the EU DAR; however the trial summary results are presented in Table B.7.3.3-02 for information.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. No new evaluation has been performed.
Data point addressed:	CA 6.3.1/01 (II A 6.3.1/01 in original DAR)
Author(s) (year):	Harrison C. (2005a).
Title:	VBC 30011: Residue levels in apples and pears from trials carried out in Northern France, Southern France, Italy and Northern Spain during 2002.
Laboratory report / project number:	AF/6256/VB
Testing facility:	Agrisearch UK, Ltd
Published:	No
Test guideline used:	EU Working Document 1607/VI/97 rev. 2
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	Residues of GA_4/GA_7 in apples and pears were <0.05 mg/kg at normal harvest. See summary in Table 6.3.3-02.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. No new evaluation has been performed.
Data point addressed:	CA 6.3.1/02 (II A 6.3.1/02 in original DAR)
Author(s) (year):	Harrison C. (2005b).
Title:	VBC 30011: Residue levels in apples and pears from trials carried out in Northern France, Southern France, Italy and Northern Spain during 2003.
Laboratory report / project number:	AF/6989/VB
Testing facility:	Agrisearch UK, Ltd
Published:	No
Test guideline used:	EU Working Document 1607/VI/97 rev. 2
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	Residues of GA_4/GA_7 in apples and pears were <0.05 mg/kg at normal harvest. See summary in Table 6.3.3-02.

Executive summary

Supervised crop residue trials were carried out in northern and southern Europe on pears, using SL and SG formulations containing gibberellins $GA_{4/7}$ at concentrations of 10 g a.s./L and 100 g a.s/kg, respectively. Treatments were applied during or immediately after flowering, which is consistent with the recommended timing. Trials were conducted at a higher rate per application (14-16 g a.s/ha) than the critical EU GAP (2 x 6 g

a.s/ha or 1 x 12 g a.s./ha). In all trials, residues of gibberellins $GA_{4/7}$ measured in whole pears were below the LOQ of 0.05 mg/kg.

Pear samples were stored frozen (*ca* -18°C) on the day of harvest and extracted within 27 months for the 2002 trials and within 12 months for the 2003 trials. Acceptable data demonstrating the stability of residues of $GA_{4/7}$ in pears is available for a period of up to 30 months, therefore the data are considered fully supported.

A total of four acceptable trials on pear (2 x NEU and 2 x S EU) are available. In all the trials residues of $GA_{4/7}$ were below the LOQ of 0.05 mg/kg, including those trials that were overdosed in relation to the supported EU GAP. As pears are considered major crops in both the northern and southern residues regions, generally a minimum of eight trials on each crop per region are required to support a use. However according to EU guidance a reduced trials data set is acceptable when residues are predicted to be below the limit of quantification, and two residue trials confirm this to be the case.

Therefore there are sufficient acceptable trials available, given that residues were all < LOQ in trials where the application rate was higher than the proposed GAP to support a use on pears in both northern and southern EU. The GAP on pears is supported by the available data.

Materials and Methods

Pears are both major crops in northern and southern Europe, therefore generally a minimum of eight trials are required from each zone, conducted across at least two growing seasons.

Two trials on pears were conducted in northern Europe, all conducted in northern France. A further two trials on pears were conducted in southern Europe, comprising one trial in southern France and one trial in Spain. Each trial consisted of three plots, one to be used as the treated plot using a 100 g/kg SG formulation, one to be used as the treated plot using a 10 g/L SL formulation and the remaining untreated plot to be used to generate control samples.

At each pear trial, two applications were made to the treated plots, between growth stages BBCH 62-68, at rates ranging from 0.014-0.016 kg a.s./ha and with intervals ranging from 3-4 days. Samples of pears were harvested from the untreated plot and both treated plots on one occasion 143-152 days post-last application, the harvest being conducted at growth stage BBCH 87-89 which corresponds to normal commercial harvest.

Following harvest, the pear samples were stored frozen at approximately - 18°C for up to 798 days prior to analysis. The storage period for the residue samples fell within the known period of freezer storage stability for GA_4 and GA_7 in pears of 30 months.

The pear samples were analysed for residues of GA_4 and GA_7 using analytical method VBC 30011/CROPS/KB/03/1 previously developed and validated in study/report V99.1181.

Samples of pears were extracted by the addition of methanol followed by homogenisation and centrifugation, after which an aliquot of the resulting supernatant was taken and concentrated prior to dilution with formic acid solution. The resulting sample was subject to reverse phase solid phase extraction (SPE) PolarPlus C18 clean-up prior to quantification by high performance liquid chromatography and tandem mass spectrometry (LC-MS/MS). Quantification was achieved using the ion transitions m/z 331.3→225.1, 331.1→243.2 and 331.3→287.4 for GA4 and ion transition m/z 329.4→223.2 for GA7.

Results

A summary of the residue data is presented in Table B.7.3.3-02.

Residues of $GA_{4/7}$ were determined to be < 0.05 mg/kg (< LOQ) for treated pears collected from each trial, at each harvest interval. In addition, no residues of $GA_{4/7}$ were detected in the corresponding control samples.

Procedural recovery values ranged from 63-112% (mean 88%) and were considered sufficient to demonstrate efficient performance of the analytical method.

Table B.7.3.3-02 **Summary of residues trial data previously reviewed for $GA_{4/7}$ in pears in the EU**

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Formulation	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Details on trial
				g a.s./ ha	Water (l/ha)	g a.s./hl				GA4/7		
	(a)	(b)					(c)				(d)	(e)
AF/6256/VB/2 45160, Rouvres, France NORTHERN EU 2002	Pears/ Doyenne de Comice	1. 1996 2. – 3. 2002	100 g/kg SG	14.8 14.6	984 976	1.5 1.5	29 Mar 02 02 Apr 02	BBCH 62 BBCH 68	Fruit	<0.05	148	Data point CA 6.3.1/01 Sampling date: 28 Aug 02 Storage time before analysis: 789 days
			10 g/l SL	15.0 15.1	1000 1008	1.5 1.5	29 Mar 02 02 Apr 02	BBCH 62 BBCH 68	Fruit	<0.05	148	
AF/6256/VB/6 50280, Zaragoza Spain SOUTHERN EU 2002	Pears/ Conference	1. 1999 2. – 3. 2002	100 g/kg SG	15.1 15.6	1007 1039	1.5 1.5	25 Mar 02 28 Mar 02	BBCH 65 BBCH 65	Fruit	<0.05	144	Data point CA 6.3.1/01 Sampling date: 19 Aug 02 Storage time before analysis: 798 days
			10 g/l SL	15.7 15.8	1046 1053	1.5 1.5	25 Mar 02 28 Mar 02	BBCH 65 BBCH 65	Fruit	<0.05	144	

Trial No./ Location/ EU zone/ Year	Commodity/ Variety	Date of 1.Sowing or planting 2.Flowering 3. Harvest	Formulation	Application rate per treatment			Dates of treatment or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed	Residues (mg/kg)	PHI (days)	Details on trial
				g a.s./ ha	Water (l/ha)	g a.s./hl				GA4/7		
	(a)	(b)					(c)				(d)	(e)
AF/6989/VB/1 Loiret, France NORTHERN EU 2003	Pears/ Doyenne de Comice	1. 1996 2. – 3. 2003	100 g/kg SG	14.4 15.4	960 1029	1.5 1.5	01 Apr 03 04 Apr 03	BBCH 63 BBCH 64-65	Fruit	<0.05	152	Data point CA 6.3.1/02 Sampling date: 03 Sep 03 Storage time before analysis: 330 days
			10 g/l SL	14.5 16.1	968 1074	1.5 1.5	01 Apr 03 04 Apr 03	BBCH 63 BBCH 64-65	Fruit	<0.05	152	
AF/6989/VB/3 Tarn et Garrone France SOUTHERN EU 2003	Pears/ Comice	1. 1990 2. – 3. 2003	100 g/kg SG	16.1 14.2	1070 946	1.5 1.5	31 Mar 03 04 Apr 03	BBCH 63 BBCH 65	Fruit	<0.05	143	Data point CA 6.3.1/02 Sampling date: 25 Aug 03 Storage time before analysis: 339 days
			10 g/l SL	15.0 15.1	998 1008	1.5 1.5	31 Mar 03 04 Apr 03	BBCH 63 BBCH 65	Fruit	<0.05	143	

(a) According to CODEX Classification / Guide
(b) Only if relevant
(c) Year must be indicated
(d) Days after last application (Label pre-harvest interval, PHI, underline)

(e) Remarks may include: Climatic conditions; Reference to analytical method and information which metabolites are included

Conclusions

Following up to two applications of either VBC30011 or Regulex to pears between growth stage BBCH 62-68, at rates ranging from 0.014-0.016 kg a.s./ha and with intervals varying from 3-4 days, residues of $GA_{4/7}$ were < 0.05 mg/kg in fruit collected 143-152 days post the last application, corresponding to normal commercial harvest.

RMS comment and conclusion:

The stability of analysed pears is covered by stability data.

All trials were conducted at GAP 2-times at excessive application rate (14-16 g a.s./ha instead of 6 g a.s./ha or 12 g a.s./ha), latest treatment at BBCH 64-68, therefore they can be considered as covering the proposed GAP.

Residues of $GA_{4/7}$ were < 0.05 mg/kg in pears in both field trials in Southern Europe and in both trials in Northern Europe.

Table 7.3.1-03: Summary of procedural recoveries for $GA_{4/7}$ in apples/pears in study AF/6256/VB (conducted in 2002)

Fortification level (mg/kg)	**Recovery (%)**
0.05	103
0.05	112
Mean recovery (%)	108
RSD (%)	5.9

Table 7.3.1-04: Summary of procedural recoveries for $GA_{4/7}$ in pears in study AF/6989/VB (conducted in 2003)

Fortification level (mg/kg)	**Recovery (%)**
0.01	76
0.1	82
Mean recovery (%)	79
RSD (%)	5.4

Mean procedural recoveries (70-110%) and RSD (≤ 20 %) were acceptable according to SANCO/3029/99 rev. 4 in both studies. Therefore all trials conducted in 2002 and 2003 are acceptable.

All trials were conducted under GLP conditions and a valid guideline (OECD 509; OECD 66/164; SANCO 7525/VI/95 rev. 10.3).

For the reasons listed above all 4 trials are acceptable: 2 on North of Europe and 2 on South of Europe. According to SANCO 7525/VI/95 rev. 10.3 when the residues of an active substance are foreseen to be below the LOQ and at least two trials confirm this then no further trials are normally necessary. Therefore proposed use on pears can be approved in Northern and Southern Europe.

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

No new studies were submitted.

B.7.3.4. Untreated pears

a) Previous evaluation (2005-2011)

See B.7.3.2 Untreated apples – previous evaluation. Facts reported in this section include untreated pears.

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

A review of published literature, focussing on the natural background levels in pears has been conducted and is presented below:

Data point addressed:	CA 6.3.4/01
Author(s) (year):	Zhang C., Tateishi N., Tananbe K.; 2010
Title:	Pollen density on the stigma affects endogenous gibberellin metabolism, seed and fruit set, and fruit quality in *Pyrus pyrifolia.* Journal of Experimental Botany, Vol.61. No. 15, pp 4291-4302, 2010.
Published:	Yes.

Summary

As part of a broader study investigating the relationship between pollen density and gametophyte competition in *Pyrus Pyrifolia,* samples of pollen and immature fruits (fruitlets) from pear trees in Japan were analyses for their gibberellin content.

Samples of immature fruits from the Gold Nijisseiki variety of pears were taken at 0, 1, 4, 12, 24 and 72 hours after hand pollination with pollen. Samples of pollen from the same pear variety were collected and placed on petri dishes containing a growth medium of agar and sucrose and were incubated at 25°C. Petri dishes were taken at intervals up to 48 hours after incubation for analysis.

Samples of fruitlets or growth medium contain pollen were extracts overnight in 80% aqueous methanol containing butyl hydroxytoluene (BHT). Deuterium labelled standards of relevant gibberellins were used as internal standards for the procedure. The extracts were filtered, concentrated, partitioned against hexane and then acidified before partitioning against ethyl acetate. Further clean-up of extracts was conducted using C_{18} SPE cartridges, followed by a separation HPLC stage to collect fractions containing the individual gibberellins. Final determination was by GC-MS of the methylated (derivatised) fractions, monitoring two ions per gibberellin. Concentration of gibberellins was expressed as ng/g fresh weight for fruitlets or ng/g pollen grains.

The results are presented in graphical form rather than as tabulated data meaning that exact concentrations are not reported. However from the graphs it can be concluded that levels of total gibberellins in immature fruits ranged from approximately 5 ng/g to 60 ng/g fresh weight with levels of GA_4 specifically ranging from approximately <1 to 12 ng/g fresh weight.

Levels of total gibberellin in pollen ranged from approximately 160 – 320 ng/g with levels of GA_4 specifically ranging from approximately 4 to 14 ng/g.

RMS comment and conclusion:

This reference is a scientific paper presenting that untreated immature pears contained GAs up to level 0.06 mg/kg and GA_4 up to level 0.012 mg/kg. We can conclude that the highest natural background of GAs is slightly above LOQ from residue trials in pears (0.05 mg/kg) and the highest natural background of GA_4 is below LOQ from residue trials in pears (0.05 mg/kg).

Conclusion on natural background levels

There is evidence of the widespread natural occurrence of GAs in plants, fungi and bacteria across many different species and also within different plant parts of the same species.

Information in published literature indicates that naturally occurring gibberellins in untreated pome fruits can be found at levels up to 0.06 mg/kg at various stages of fruit development. It can be expected that naturally occurring levels in fruit will vary depending on such parameters as the stage of development, variety and part of the plant analysed as borne out by literature previously considered in the EU review that indicated that the highest levels will be found in apple seeds.

Residues of $GA_{4/7}$ in apples and pears at harvest maturity from supervised residue trials conducted in support of the use of $GA_{4/7}$ are also low (< LOQ) meaning there is evidence that there is no significant difference between naturally occurring levels and levels arising from the use of $GA_{4/7}$ as a plant protection product.

RMS comment and conclusion:

GA_4 has been identified in 54 plant species, 7 fungi and 3 bacteria species. In plants GA_4 was found mainly in seeds, leaves, shoots, buds, fruits and pollen.

GA_7 has been identified in 14 plant species and 1 fungus species. In plants GA_7 was found mainly in seeds, leaves, shoots and pollen.

In scientific papers natural background concentration of GAs in pears was up to 0.06 mg/kg.

We can conclude that the use of $GA_{4/7}$ as a plant protection product results in residue levels similar to the natural levels in plants.

B.7.4. FEEDING STUDIES

B.7.4.1. Poultry

a) Previous evaluation (2005-2011)

No data were submitted or evaluated as part of the original EU review for $GA_{4/7}$.

RMS comment and conclusion:

No studies were required. Studies were considered not relevant.

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

No new studies were submitted.

Gibberellins occur naturally in a wide range of plants and it would not be possible to distinguish naturally occurring $GA_{4/7}$ in plants from that resulting from plant protection uses. Information in published literature indicates that there is no significant difference between naturally occurring levels and the levels found in supervised residue trials supporting the use of $GA_{4/7}$ on pome fruit. Therefore any exposure of livestock to

residues of $GA_{4/7}$ from plant protection uses could not be distinguished from consumption of naturally occurring residues.

Furthermore, pome fruit and their processed products are not considered to be potential feed items for poultry (OECD Guidance Document on Overview of Residue Chemistry Studies, 2009).

Therefore feeding studies are not required.

RMS comment and conclusion:

$GA_{4/7}$ occurs naturally in apples and pears at similar level than in treated apples and pears therefore it would be impossible to distinguish between natural background and $GA_{4/7}$ originating from treatment with PPP. Moreover apples and pears are not feed to poultry. Therefore studies are not required.

B.7.4.2. Ruminants

a) Previous evaluation (2005-2011)

No data were submitted or evaluated as part of the original EU review for GA_3.

RMS comment and conclusion:

No studies were required. Studies were considered not relevant.

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

No new studies were submitted.

Gibberellins occur naturally in a wide range of plants and it would not be possible to distinguish naturally occurring $GA_{4/7}$ in plants from that resulting from plant protection uses. Information in published literature indicates that there is no significant difference between naturally occurring levels and the levels found in supervised residue trials supporting the use of $GA_{4/7}$ on pome fruit. Therefore any exposure of livestock to residues of $GA_{4/7}$ from plant protection uses could not be distinguished from consumption of naturally occurring residues.

Apple pomace is considered a potential animal feed item for ruminants in the EU, however residues of $GA_{4/7}$ in apples were below the LOQ of 0.05 mg/kg in all supervised residue trials conducted according to the GAP (see section B.7.3.1). Therefore, residues of $GA_{4/7}$ in the diet of lactating ruminants will not exceed 0.004 mg/kg bw/day.

Therefore feeding studies are not required.

RMS comment and conclusion:

$GA_{4/7}$ occurs naturally in apples and pears at similar level than in treated apples and pears therefore it would be impossible to distinguish between natural background and $GA_{4/7}$ originating from treatment with PPP. Moreover apples are feed to animals, but since their residues in field trials were <0.05 mg/kg, the trigger value of 0.004 mg/kg bw/day is not exceeded. Therefore studies are not required.

B.7.4.3. Pigs

a) Previous evaluation (2005-2011)

No data were submitted or evaluated as part of the original EU review for GA_3.

RMS comment and conclusion:

No studies were required. Studies were considered not relevant.

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

No new studies were submitted.

Gibberellins occur naturally in a wide range of plants and it would not be possible to distinguish naturally occurring $GA_{4/7}$ in plants from that resulting from plant protection uses. Information in published literature indicates that there is no significant difference between naturally occurring levels and the levels found in supervised residue trials supporting the use of $GA_{4/7}$ on pome fruit. Therefore any exposure of livestock to residues of $GA_{4/7}$ from plant protection uses could not be distinguished from consumption of naturally occurring residues.

Furthermore, pome fruit and their processed products are not considered to be potential feed items for pigs (OECD Guidance Document on Overview of Residue Chemistry Studies, 2009).

Therefore feeding studies are not required.

$GA_{4/7}$ occurs naturally in apples and pears at similar level than in treated apples and pears therefore it would be impossible to distinguish between natural background and $GA_{4/7}$ originating from treatment with PPP. Moreover apples and pears are not feed to animals. Therefore studies are not required.

B.7.4.4. Fish

a) Previous evaluation (2005-2011)

No data were submitted or evaluated as part of the original EU review for GA_3.

RMS comment and conclusion:

No studies were required.

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

Gibberellins occur naturally in a wide range of plants and it would not be possible to distinguish naturally occurring $GA_{4/7}$ in plants from that resulting from plant protection uses. Information in published literature indicates that there is no significant difference between naturally occurring levels and the levels found in supervised residue trials supporting the use of $GA_{4/7}$ on pome fruit. Therefore any exposure of livestock to residues of $GA_{4/7}$ from plant protection uses could not be distinguished from consumption of naturally occurring residues.

Furthermore, pome fruit and their processed products are not considered to be potential feed items for fish (SANCO/11187/2013 rev. 3). Therefore feeding studies in fish are not required.

RMS comment and conclusion:

$GA_{4/7}$ occurs naturally in apples and pears at similar level than in treated apples and pears therefore it would be impossible to distinguish between natural background and $GA_{4/7}$ originating from treatment with PPP. Moreover apples and pears are not feed to fish. Therefore studies are not required.

B.7.5. EFFECTS OF PROCESSING

B.7.5.1. Nature of the residue

a) Previous evaluation (2005-2011)

No data were submitted or evaluated as part of the original EU review for GA_3.

RMS comment and conclusion:

No studies were required. Studies were considered not relevant.

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

No new studies were submitted.

Gibberellins occur naturally in a wide range of plants and it would not be possible to distinguish naturally occurring $GA_{4/7}$ in plants from that resulting from plant protection uses.

Residues in apples and pears from trials conducted according to the representative GAP did not give rise to detectable residues of $GA_{4/7}$ (<0.05 mg/kg), as shown in section B.7.3. Information in published literature indicates that there is no significant difference between naturally occurring levels and the levels found in supervised residue trials supporting the use of $GA_{4/7}$ on pome fruit. Therefore data to address the nature of the residue are not required.

RMS comment and conclusion:

Residues in treated apples and pears were <LOQ (<0.05 mg/kg). Therefore studies are not required.

B.7.5.2. Distribution of the residue in peel and pulp

a) Previous evaluation (2005-2011)

No data were submitted or evaluated as part of the original EU review for $GA_{4/7}$.

RMS comment and conclusion:

No studies were required. Studies were considered not relevant.

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

No new studies were submitted.

No data are required as the representative crops (apple and pear) do not have an inedible peel.

RMS comment and conclusion:

Since apples and pears have edible peel no studies are required.

B.7.5.3. Magnitude of residues in processed commodities

a) Previous evaluation (2005-2011)

No data were submitted or evaluated as part of the original EU review for GA_3.

RMS comment and conclusion:

No studies were required. Studies were considered not relevant.

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

No new studies were submitted.

Gibberellins occur naturally in a wide range of plants and it would not be possible to distinguish naturally occurring $GA_{4/7}$ in plants from that resulting from plant protection uses.

Residues in apples and pears from trials conducted according to the representative GAP did not give rise to detectable residues of $GA_{4/7}$ (<0.05 mg/kg), as shown in section B.7.3. Information in published literature indicates that there is no significant difference between naturally occurring levels and the levels found in supervised residue trials supporting the use of $GA_{4/7}$ on pome fruit. Therefore data to address the magnitude of residues are not required.

RMS comment and conclusion:

Residues in treated apples and pears were <LOQ (<0.05 mg/kg). Therefore studies are not required.

B.7.6. RESIDUES IN SUCCEEDING OR ROTATIONAL CROPS

B.7.6.1. Metabolism in rotational crops

a) Previous evaluation (2005-2011)

Studies were considered not relevant.

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

No new studies were submitted.

Orchards are established permanent crops; therefore consideration of residues in rotational crops is not relevant.

RMS comment and conclusion:

No studies were submitted or required since orchards are permanent crops.

B.7.6.2. Magnitude of residues in rotational crops

a) Previous evaluation (2005-2011)

Studies were considered not relevant.

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

No new studies were submitted.

Orchards are established permanent crops; therefore consideration of residues in rotational crops is not relevant.

RMS comment and conclusion:

No studies were submitted or required since orchards are permanent crops.

B.7.7. OTHER STUDIES

B.7.7.1. Effect on the residue level in pollen and bee products

a) Previous evaluation (2005-2011)

No studies were submitted or required.

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

The potential of residues in pollen and bee products for human consumption resulting from residues taken up by honeybees from crops at blossom should be considered.

$GA_{4/7}$ is used on pome fruit and is applied to apple between the growth stages BBCH 69 to 74 and to pear between the growth stages of BBCH 62 to 69 i.e. during flowering; therefore a more detailed consideration is required.

According to the EFSA Guidance Document on the Risk Assessment of Plant Protection Products on Bees (EFSA Journal 2013; 1(7):3295) both pollen and nectar from pome fruit are attractive to bees and could be foraged.

Gibberellins occur naturally in a wide range of plants and it would not be possible to distinguish naturally occurring $GA_{4/7}$ in plants from that resulting from plant protection uses. It is known that higher levels of gibberellins are naturally found in reproductive tissues such as anthers and pollen and data from published literature indicates that naturally occurring gibberellins (total) can be found in pollen at levels up to 320 ng/g (Zhang et al., 2010). Therefore naturally occurring gibberellins are already available for uptake by honeybees

and could be transferred into bee products, leading to naturally occurring levels in bee products for human consumption.

A published paper that considered levels of phytohormones in honey is presented below:

Data point addressed:	CA 6.7.1/01
Author(s) (year):	Wang Q, et al; 2017
Title:	Comprehensive Profiling of Phytohormones in Honey by Sequential Liquid−Liquid Extraction Coupled with Liquid Chromatography−Mass Spectrometry. J Agric. and Food Chemistry, 65,575-585, 2017
Published:	Yes

Summary

As part of a wider study quantifying a range of phytohormones in honey samples, levels of 8 gibberellins were determined in 17 honey samples taken either as raw "mono-floral" samples from beekeepers or from commercial outlets in China.

Honey samples were subjected to sequential liquid/liquid extraction and analysed by LC-MS/MS.

The results for the most commonly detected gibberellins are summarised below:

Honey sample	**Gibberellins (µg/kg)**				
	GA_1	**GA_4**	**GA_{15}**	**GA_{24}**	**GA_{34}**
Robonia Raw 1	-	0.4108	0.0066	0.0080	0.0176
Robonia Raw 2	-	0.5227	0.0040	0.0236	0.0110
Robonia Raw 3	-	0.4751	0.0087	0.0087	0.0306
Robonia Raw 4	-	0.4787	0.0111	0.0239	0.0190
Robonia Raw 5	-	0.5175	0.0179	0.0086	0.0353
Rape Raw 1	-	0.5069	0.0097	0.0118	0.1399
Rape Raw 2	-	0.3928	0.0037	0.0245	0.1246
Rape Raw 3	-	0.4714	0.0149	0.0316	0.1842
Rape Raw 4	-	0.4743	0.0184	0.0287	0.2309
Rape Raw 5	-	0.4927	0.0087	0.0072	0.1334
Milk Vetch Raw 1	-	0.5822	0.0160	0.0092	0.0816
Milk Vetch Raw 2	-	0.4688	0.0124	0.0168	0.0900
Milk Vetch Raw 3	-	0.4117	0.0094	0.0279	0.1027
Milk Vetch Raw 4	-	0.4690	0.0204	0.0225	0.1733
Commercial 1 (Roobina)	0.0708	0.0671	nd	0.0086	0.0081
Commercial 2 (Medlar)	0.1797	0.2184	0.0148	0.0081	0.0656
Commercial 3 (Date)	nd	0.0533	nd	0.0049	0.0203

nd = not detected (< 0.007 µg/kg)

Levels of gibberellins in honey samples were generally low (<0.001 mg/kg). Some gibberellins such as GA_4, GA_{24} and GA_{34} were detected in all samples. No GA_7 was detected.

Although the published paper considered samples of honey from China and it is not known if the crops that were foraged by bees were subject to treatment with gibberellins at any point; it can be seen that levels of gibberellins in honey are generally very low; indicating that transfer from pollen to honey is limited.

It is likely therefore that any exposure of honey bees to naturally occurring $GA_{4/7}$ would not be significantly impacted by the use of $GA_{4/7}$ as a plant protection product, and that the transfer of any gibberellins naturally present from pollen into honey would be minimal.

RMS comment and conclusion:

This reference is a scientific paper. Determination of $GA_{4/7}$ was performed with LC-MS/MS achieving LD (limit of detection) 0.0000147 mg/kg and LOQ 0.000049 mg/kg for GA_4 and LD (limit of detection) 0.0000185 mg/kg and LOQ 0.0000616 mg/kg for GA_7. In 17 honey samples from China GA_7 was not detected and content of GA_4 was up to 0.0005822 mg/kg; indicating that transfer from pollen (where $GA_{4/7}$ is naturally present) to honey is minimal and limited.

B.7.8. REFERENCES RELIED ON

Literature search

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

Two searches were undertaken for relevant literature in the public domain on the active substance gibberellins ($GA_{4/7}$ and relevant synonyms). The initial search was undertaken in April 2016, and a supplementary search was also carried out in November 2017 following the 1 year extension to the submission deadline for the renewal dossier.

Both searches were conducted in accordance with:

- Commission Implementing Regulation (EU) No 844/2012, as referred in Article 8(5) of Regulation (EC) No 1107/2009 and,

- the EFSA document; 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.

The search strategies for both were based on a single concept search. For details regarding the search strategy, relevance criteria applied and the results obtained, please see the Literature Review Report (KCA9).

The selection process in each search resulted in three categories of publication:

- Publications which meet the relevance criteria and are assessed to be reliable which are addressed at the appropriate data points in the relevant MCA & MCP Sections of the dossier.

- Publications which meet the relevance criteria but are assessed to be non-reliable are referenced and a justification for not meeting the reliability criteria provided in Section 6 of the Literature Review Report (KCA 9).

- Publications not meeting the relevance criteria are referenced in Section 6 of the Literature Review Report (KCA 9). .

Results of Initial Search (April & May 2016)

In the April 2016 search 1,157 summary records were retrieved from bibliographic databases and were screened by expert reviewers and grouped into two categories according to their likely relevance after rapid assessment of titles and, when available, abstracts:

1. Obviously not relevant: 1,126 summary records.

These summary records (titles and/or abstracts) did not contain specific information relevant to the criteria specified in Table 1 of the KCA 9 report.

2. Not excluded after rapid assessment: 31 summary records were classified as potentially relevant and thus were assessed in detail, a full assessment of the full-text documents.

3. Following assessment 30 of the full text documents were excluded from the dossier.

4. Following assessment 3 of the full text documents were included in the dossier. However, 2 of these were EFSA Conclusions and therefore have not been included in this report. 1 paper was considered relevant and included in the dossier.

After discussion with the client, it was decided that the next phase of searching should use more specific Residues and Toxicology/Human Health nested search terms only.

In the updated search (May 2016), 418 summary records were retrieved from bibliographic databases and were screened by expert reviewers and grouped into two categories according to their likely relevance after rapid assessment of titles and, when available, abstracts:

1. Obviously not relevant: 399 summary records. These summary records (titles and/or abstracts) did not contain specific information relevant to the criteria specified in Table 1.
2. Not excluded after rapid assessment: 19 summary records were classified as potentially relevant and thus were assessed in detail, a full assessment of the full-text documents.
3. Following assessment 17 of the full text documents were excluded from the dossier.
4. Following assessment 1 the full text documents were considered to be of interest but as these were EFSA Conclusions they are not specifically listed as references in the dossier.

It was concluded that 1 of the 418 summary records were relevant. Full details can be found in the Literature Review Reports (KCA 9 & KCP 11) and the relevant papers are included in dossier under the appropriate the KCA & KCP data points.

Results of Top Up Search (November 2017)

In summary, in the November 2017 search 1,728 summary records were retrieved from bibliographic databases and were screened by expert reviewers and grouped into two categories according to their likely relevance after rapid assessment of titles and, when available, abstracts:

1. Obviously not relevant: 1,695 summary records. These summary records (titles and/or abstracts) did not contain specific information relevant to the criteria specified in Table 1.
2. Not excluded after rapid assessment: 33 summary records were classified as potentially relevant and thus were assessed in detail, a full assessment of the full-text documents.
3. Following assessment 31 of the full text documents were excluded from the dossier.
4. Following assessment 2 of the full text documents were included in the dossier.

It was concluded that 2 of the 1,728 summary records were relevant. Full details can be found in the Literature Review Reports (KCA 9 & KCP 11) and the relevant papers are included in dossier under the appropriate the KCA & KCP data points.

RMS comments and conclusion:

The literature search was performed for both GA_3 and $GA_{4/7}$ at once since the applicant expected that there would be overlap of relevant papers. Only searches in bibliographic databases were undertaken. The public literature search process is documented according to 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. The first public literature search was performed on April 2016 and update on May 2016 due to the finding of too few references for Residues and Toxicology/Human Health. An additional search was carried out in November 2017 due to the extension of the submission date for the renewal dossier. The search period is in line with the requirements of the Commission Implementing Regulation (EU) No 844/2012, as referred in Article 8(5) of Regulation (EC) No 1107/2009.

The search strategies were based on a single concept search. The search was performed combining the terms gibberellic acid or GA_3 or gibberellin or $GA_{4/7}$ or using the belonging CAS Registry numbers and applying them

to each of the search terms listed by scientific area (Physical Chemistry, Residues, Toxicology, Environmental fate, Ecotoxicology) the "AND" operator. The summary record retrieved was reported for all the scientific area and searched databases together.

The selected Relevance Criteria for the data requirements regarding the Residues are:
1. Well-defined test material (including its purity and impurity profile).
2. Any information on residues in crops relevant to the EU.
3. Well described methodology and results.

In this renewal 5 scientific contributions were used: 4 scientific papers were published in journals with impact factor 0.9-4.8 in year of publication. One scientific contribution was insert from the encyclopedia. In one paper apples was grown in Germany, in the second paper pears was grown in Japan and in the third paper honey was collected in China. The criteria of defined analytical methods for determination of $GA_{4/7}$ were met in all 4 scientific papers. Regardless of pears and honey production location, which was outside the Europe, the data from papers were accepted. No significant changes in natural background level of $GA_{4/7}$ are expected because of production location. The RMS is of the opinion that the applicant literature search is acceptable.

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**
CA 6.1.1/01	Jean-Baptiste C.	2011	Frozen Storage Stability of Residues of Gibberellic acid, Gibberellin A_4 and Gibberellin A_7 in Pears. ANADIAG, France Report No: R A9206 GLP Unpublished	N	Y	New study submitted for the purpose of renewal	GC	-
CA 6.1.1/02	Harrison C.	2010	To determine the stability of gibberellin A_4 (GA_4) and gibberellin A_7 (GA_7) in pome fruit apple specimens following	N	Y	New study submitted for the purpose of renewal	VBC	-

			storage at ca -18°C for 0, 1, 3, 12, 18 and 30 months. EUROFINS Agroscience services, UK Report No: AD/6258/VB GLP Unpublished					
CA 6.2.1/01	Goodyear A.	2005	The Metabolism of Gibberellins GA_4/A_7 in Plants. TSGE, Knaresboroug, UK 22-1-02.PMET Non GLP Un published	N	N	-	-	II A 6.2.1/01 in original DAR
CA 6.3.1/01 and CA 6.3.3/01	Harrison C., Oxspring S.	2005a	VBC 30011: Residue levels in apples and pears from trials carried out in Northern France, Southern France, Italy and Northern Spain during 2002. Agrisearch UK, Ltd Report No: AF/6256/VB GLP Unpublished	N	N	-	VBC	II A 6.3.1/01 in original DAR
CA 6.3.1/02 and CA 6.3.3/02	Harrison C., Oxspring S.	2005b	VBC 30011: Residue levels in apples and pears from trials carried out in Northern	N	N	-	VBC	II A 6.3.1/02 in original DAR

			France, Southern France, Italy and Northern Spain during 2003. Agrisearch UK, Ltd Report No: AF/6989/VB GLP Unpublished					
CA 6.3.2/01, CA 6.3.4	Hedden P.	2003	Regulators of Growth: Gibberellins. In Encyclopaedia of Applied Plant Sciences (Thomas, B., Murphy D.J., Murray B.G., eds) pp. 1011-1019, Academic Press. Non GLP Published	N	N	-	Published data	IIA 8.0 in original DAR
CA 6.3.2/02 CA 6.3.4	MacMillan J.	2002	Occurrence of Gibberellins in Vascular Plants, Fungi and Bacteria. IACR Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol, BS41 9AF.	N	N	-	Published data	IIA 1.0 in original DAR

			Non GLP Published					

CA 6.3.2/03	Stephan M., Bangerth F., Schneider G.	1999	Quantification of endogenous gibberellins in exudates from fruit from *Malus domestica*. J. Plant Growth Regulation 28: 55-58, 1999 Non GLP Published	N	N	-	Published data	IIA 6.2.1/02 in original DAR
CA 6.3.4/01	Zhang C., Tateishi N., Tananbe K.	2010	Pollen density on the stigma affects endogenous gibberellin metabolism, seed and fruit set, and fruit quality in *Pyrus pyrifolia.* J. of Experimental Botany, Vol 61. No. 15 4291-4302, 2010. Non GLP Published	N	N	-	Published data	
CA 6.7.1/01	Wang Q., Cai W.J., Yu L., Ding J., Feng Y.Q.	2017	Comprehensive Profiling of Phytohormones in Honey by Sequential Liquid−Liquid Extraction Coupled with Liquid Chromatography−Mass Spectrometry. J Agric. and Food Chemistry,	N	N	-	Published data	

			65,575-585, 2017 Non GLP Published					