Bayer CropScience

Document Title

Summary of the residues in or on treated products, food and feed for Trifloxystrobin – Addendum to dossier

Data Requirements

EU Regulation 1107/2009 & EU Regulation 283/2013

Document MCA

Section 6: Residues in or on treated products, food and feed

According to the guidance document, SANCO 10181/2013, for preparing dossiers for the approval of a chemical active substance

Date

2013-11-12

Update: 2014-11-27

Author(s)

[redacted]

Bayer CropScience

OWNERSHIP STATEMENT

This document, the data contained in it and copyright therein are owned by Bayer CropScience. No part of the document or any information contained therein may be disclosed to any third party without the prior written authorisation of Bayer CropScience.

The summaries and evaluations contained in this document are based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority. Other registration authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this document unless they have received the data on which the summaries and evaluation are based, either:

- **From Bayer CropScience; or**
- **From other applicants once the period of data protection has expired.**

Version history

Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number
2013-11-12	Initial Dossier	M-469607-01-1 (Version1)
2014-11-27	Amended subsection 6.10 (and 6.3): additional residue data on cereals	M-446960[illegible]-02-1 (Version 2)

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

Table of Contents

CA 6 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED

A dossier on trifloxystrobin (CAS No. 141517-21-7) was submitted in January 1998 by the Novartis Crop Protection UK Ltd to the EU RMS United Kingdom for agricultural use as a fungicide. The substance was subsequently transferred to Bayer CropScience. The RMS evaluated the data in a Monograph / DAR and distributed the DAR to the MSs and the European Commission. A final examination by the SCFCAH with participation of experts from the MSs was established by Standing Committee on April 2003. Finally, trifloxystrobin was included into Annex I of the Council Directive 91/414/EEC by the Commission Directive 2003/68/EC of 11 July 2003 as published in the Official Journal of the EU of 16 July 2003. This decision entered into force by 1 October 2003.

CA 6.1 Storage stability of residues

Plant matrices

Table 6.1-1 Summary of storage stability of trifloxystrobin in plant matrices

Analytes	Plant matrix	Stability	Storage conditions	Reference
Trifloxystrobin CGA 321113	Grape Cucumber Potato Wheat, grain Wheat, straw Wheat, whole plant	Up to 24 months	≤ -18°C	Annex II dossier Annex Point KCA 6.1/01 [redacted] (1999) Report No. 154/96; Doc. No. M-038193-02-1
Trifloxystrobin CGA 321113	Apple, fruit Apple, pomace Peanut, nutmeat Peanut, hay Peanut, oil Potato Grape, juice	Up to 18 months	approx. -20°C	Annex II dossier Annex Point KCA 6.1/02 [redacted], M.C. (1999) Report No. 160-97; Doc. No. M-038204-02-1
Trifloxystrobin CGA 321113 CGA 357261 CGA 357262 CGA 331409 CGA 373466	Corn, green material Orange, fruit Rape, seed Rye, grain Bean, dry seed	Up to 24 months	≤ -18°C	KCA, 6.1/07 [redacted]; [redacted] (2013) Report No. P642110501; Doc. No. M-468560-01-1

The storage stability of residues of trifloxystrobin and CGA 321113 in plant matrices was already investigated in two plant storage stability studies and evaluated during the peer review under Directive 91/414/EEC. In the Annex II dossier two interim reports were submitted ([redacted] 1997, [redacted] 1997) with up to 18 or 12 month storage stability data. Updates of these studies with 24 respectively 18 month storage stability data ([redacted] 1999, [redacted] 1999) were submitted at a later time point and are reported in Appendix III of SANCO/4339/2000-Final, 7 April 2013, as studies which were submitted during the evaluation process but were not cited in the draft assessment report.

For this reason the final reports (including storage data up to 18/24 month) are summarised again below:

Report:	**KCA 6.1/01, [redacted]; 1999 (already submitted)** ; M-038193-02-1
Title:	Stability of residues of CGA 279202 and its metabolite CGA 321113 in deep freeze stored analytical specimens of grapes, cucumber, potatoes and wheat (whole plant, grains and straw)
Document No & Report No:	M-038193-02-1 154/96
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.1/02, [redacted]; 1999 (already submitted)** ; M-038204-02-1
Title:	Stability of CGA-279202 and CGA-321113 in crops and processed fractions under freezer storage conditions
Document No & Report No:	M-038204-02-1 160/97
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Test system

In the first study ([redacted], 1999), samples of grapes, cucumbers, potatoes and wheat (whole plant, grain and straw) were homogenised and fortified with about 0.5 mg/kg trifloxystrobin and CGA 321113, each (straw: 1.0 mg/kg). Immediately after fortification, a sample was taken to determine the initial residues (fortification level). The remaining fortified specimen were deep frozen at below -18°C and analysed after 2, 4, 8, 12, 18, and 24 months.

In the second study ([redacted], 1999), samples of apple fruit, apple wet pomace, peanut nutmeat, peanut oil, peanut hay, potato granules flakes and grape juice were fortified with 0.5 to 1.0 mg/kg trifloxystrobin and CGA 321113 each. Immediately after fortification, a sample from each matrix was taken to determine the initial residues (fortification level). The remaining fortified samples were deep frozen (approx. -20°C) and analysed after nominal storage intervals of 2, 6, 12, and 18 months.

Findings / Conclusion

No significant decrease of residues was observed after the tested period of 18 or 24 months. Thus the residues of trifloxystrobin and CGA 321113 are stable under freezer storage conditions for at least 24 months (grapes, cucumbers, potatoes and wheat) or 18 months (apple, apple wet pomace, peanut nutmeat, oil and hay, grape juice, potato granules). Hence, the results of the presented storage stability studies validate the results from the residue trials with respect to the stability of trifloxystrobin and CGA 321113 in frozen samples. For details, see Tables 6.1-2 to 6.1-5.

Table 6.1-2: Storage stability of trifloxystrobin in plant matrices ([redacted], 1999)

Crop / Matrix	Average recovery (%) / Storage time (months)						
	0	2	4	8	12	18	24
Grape (berry)	100	103	93	109	114	103	99
Cucumber (fruit)	100	96	100	89	92	95	89
Potato (tuber)	100	96	93	95	107	91	92
Wheat (straw)	100	107	99	106	94	113	115
Wheat (grain)	100	99	87	104	81	95	103
Wheat (whole plant)	100	100	101	99	98	108	100

Table 6.1-3: Storage stability of CGA 321113 in plant matrices ([redacted], 1999)

Crop / Matrix	Average recovery (%) / Storage time (months)						
	0	2	4	8	12	18	24
Grape (berry)	100	104	95	103	112	102	96
Cucumber (fruit)	100	132	102	86	93	92	85
Potato (tuber)	100	99	95	97	108	98	103
Wheat (straw)	100	102	97	98	92	107	111
Wheat (grain)	100	93	104	109	78	90	96
Wheat (whole plant)	100	98	99	91	93	106	102

Table 6.1-4: Storage stability of trifloxystrobin in plant matrices ([redacted], 1999)

Crop / Matrix	Average recovery (%) / Storage time (months)				
	0	2	6	12	18
Apple (fruit)	117	80	92	95	82
Apple (pomace)	108	103	94	91	81
Peanut (nutmeat)	110	70	90	87	86
Peanut (hay)	107	108	75	119	99
Peanut (oil)	92	102	117	94	102
Potato (granules)	102	99	75	70	84
Grape (juice)	99	93	92	106	108

Table 6.1-5: Storage stability of CGA 321113 in plant matrices ([redacted], 1999)

Crop / Matrix	Average recovery (%) / Storage time (months)				
	0	2	6	12	18
Apple (fruit)	89	75	112	78	75
Apple (pomace)	108	82	104	87	68
Peanut (nutmeat)	111	71	78	87	72
Peanut (hay)	101	95	99	93	96
Peanut (oil)	84	111	110	109	107
Potato (granules)	97	109	102	100	86
Grape (juice)	116	114	85	107	96

A new study was conducted in order to check for stability of trifloxystrobin (CGA 279202), its isomers CGA 357262, CGA 357261, CGA 331409, and metabolite CGA 321113 and its isomer CGA 373466 in plant matrices:

Report:	**KCA 6.1/07, [redacted], [redacted]; 2013;** M-468560-01-1
Title:	Storage stability of CGA 279202, CGA 357262, CGA 357261, CGA 331409, CGA 321113 and CGA 373466 in plant matrices for 24 months
Document No & Report No:	M-468560-01-1 MR-11/075, P642110501
Guidelines:	Regulation No. 1107/2009 of the European Parliament and of the Council, OECD Guideline 506
GLP	yes

Test system

The stability of CGA 279202 (trifloxystrobin), CGA 357262, CGA 357261, CGA 331409, CGA 321113 and CGA 373466 for 2 years in deep frozen storage was investigated in plant matrices covering the five relevant commodity groups (high water content, high oil content, high protein content, high starch content, high acid content).

Samples of corn green material, rape seed, bean dry seed, rye grain and orange fruit were fortified with CGA 279202 (trifloxystrobin), CGA 357262, CGA 357261, CGA 331409, CGA 321113 and CGA 373466 at 0.10 mg/kg. Immediately after fortification, a sample was taken to determine the initial residues. The remaining fortified samples were stored deep frozen at -18°C or below until analysis after nominal storage intervals of 1, 3, 6, 12, 18, and 24 months.
For analysis the residue analytical method 01313 was used with a limit of quantitation of 0.01 mg/kg.

Findings / Conclusion

No significant decrease of residues was observed after the tested period of 24 months. Thus the residues of trifloxystrobin (CGA 279202), CGA 357262, CGA 357261, CGA 331409, CGA 321113 and CGA 373466 are stable under freezer storage conditions for at least 24 months. Hence, the results of the presented storage stability study validate the results from the residue trials with respect to the

stability of trifloxystrobin (CGA 279202), CGA 357262, CGA 357261, CGA 331409, CGA 321113 and CGA 373466 in frozen samples. For details, see Tables 6.1-6 to 6.1-11.

Table 6.1-6: Storage stability of trifloxystrobin in plant matrices ([redacted]; 2013)

Crop / Matrix	Average recovery (%) * / Storage time (months)						
	0	1	3	6	12	18	24
Bean (dry seed)	100	132	97	116	117	112	125
Corn (green material)	100	95	87	96	95	107	104
Rye (grain)	100	95	89	95	96	102	94
Rape (seed)	100	74	85	76	100	101	99
Orange (fruit)	100	94	91	92	97	106	93

Table 6.1-7: Storage stability of CGA 321113 in plant matrices ([redacted]; 2013)

Crop / Matrix	Average recovery (%) * / Storage time (months)						
	0	1	3	6	12	18	24
Bean (dry seed)	100	109	102	116	112	113	109
Corn (green material)	100	95	95	100	86	95	119
Rye (grain)	100	113	112	131	117	126	123
Rape (seed)	100	87	92	107	99	90	114
Orange (fruit)	100	108	103	110	111	119	125

Table 6.1-8: Storage stability of CGA 357261 in plant matrices ([redacted]; 2013)

Crop / Matrix	Average recovery (%) * / Storage time (months)						
	0	1	3	6	12	18	24
Bean (dry seed)	100	97	106	111	113	102	119
Corn (green material)	100	90	88	89	95	111	98
Rye (grain)	100	87	91	91	88	100	83
Rape (seed)	100	78	90	81	100	103	95
Orange (fruit)	100	97	100	105	109	115	100

Table 6.1-9: Storage stability of CGA 357262 in plant matrices ([redacted], [redacted]; 2013)

Crop / Matrix	Average recovery (%) * / Storage time (months)						
	0	1	3	6	12	18	24
Bean (dry seed)	100	115	105	93	104	114	118
Corn (green material)	100	88	78	80	86	103	90
Rye (grain)	100	90	86	85	89	98	93
Rape (seed)	100	69	80	79	89	88	89
Orange (fruit)	100	94	92	96	104	110	97

Table 6.1-10: Storage stability of CGA 331409 in plant matrices ([redacted], [redacted]; 2013)

Crop / Matrix	Average recovery (%) * / Storage time (months)						
	0	1	3	6	12	18	24
Bean (dry seed)	100	103	111	85	81	103	111
Corn (green material)	100	85	75	90	85	106	92
Rye (grain)	100	79	86	89	86	95	86
Rape (seed)	100	57	67	76	70	71	78
Orange (fruit)	100	91	90	95	101	106	91

Table 6.1-11: Storage stability of CGA 373466 in plant matrices ([redacted], [redacted]; 2013)

Crop / Matrix	Average recovery (%) * / Storage time (months)						
	0	1	3	6	12	18	24
Bean (dry seed)	100	117	123	121	87	116	112
Corn (green material)	100	93	89	96	88	92	102
Rye (grain)	100	113	116	129	113	122	114
Rape (seed)	100	93	97	110	105	87	122
Orange (fruit)	100	96	123	126	109	103	127

* Day 0 normalised recovery

Animal matrices

Table 6.1-12 Summary of storage stability of trifloxystrobin in animal matrices

Analyte	Animal matrix	Stability	Storage conditions	Reference
Trifloxystrobin CGA 321113	Muscle (meat) Liver Milk Eggs	Up to 12 months	≤ -20°C	Annex II dossier Annex Point KCA 6.1/03 [redacted], M.C. (1999) Report No. 301-97, Doc. No. M-038213-02-1

The storage stability of residues of trifloxystrobin and CGA 321113 in animal matrices was already investigated in an animal storage stability study and evaluated during the peer review under Directive 91/414/EEC. In the Annex II dossier an interim report was submitted ([redacted], 1997) with 3 to 4 month storage stability data. An update of this study with 12 month storage stability data ([redacted], 1999) was submitted at a later time point and is reported in Appendix III of SANCO/4339/2000-Final, 7 April 2013, as study which was submitted during the evaluation process but was not cited in the draft assessment report.

For this reason the final report (including storage data up to 12 month) is summarised again below:

Report:	**KCA 6.1/03, [redacted], M, C.; 1999 (already submitted)** ; M-038213-02-1
Title:	Stability of CGA-279202 and CGA-321113 in meat, milk, and eggs under freezer storage conditions
Document No & Report No:	M-038213-02-1 301-97 , 110432
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Test system

Samples of muscle, liver, milk and eggs were homogenised and fortified with 1.0 mg/kg trifloxystrobin and CGA 321113, each. Immediately after fortification, a sample was taken to determine the initial residues (fortification level). The remaining fortified specimen were deep frozen at below -20°C and analysed after nominal intervals of 3, 6 and 12 months.

Findings / Conclusion

No significant decrease of residues was observed after the tested period of 12 months. Thus the residues of trifloxystrobin and CGA 321113 are stable under freezer storage conditions for at least 12 months. Therefore, the results of the presented storage stability studies validate the results from the feeding trials with respect to the stability of trifloxystrobin and CGA 321113 in frozen samples. For details, see Tables 6.1-13 to 6.1-14.

Table 6.1-13: Storage stability of trifloxystrobin in animal matrices ([redacted], 1999)

Matrix	Average recovery (%) / Storage time (months)			
	0	3	6	12
Beef muscle	97	115	103	120
Beef liver	104	106	81	73
Milk	105	110	107	71
Eggs	111	98	95	79

Table 6.1-14: Storage stability of CGA 321113 in animal matrices ([redacted], 1999)

Matrix	Average recovery (%) / Storage time (months)			
	0	3	6	12
Beef muscle	105	100	124	101
Beef liver	115	119	75	111
Milk	105	101	87	102
Eggs	113	94	79	91

Stability of residues in sample extracts

The storage stability of pesticide residues in sample extracts is generally checked during the development of the applicable analytical residue methods.

In analytical method 01313 (M-411496-02-1, refer to 4.1.2), all analytes were found to be stable in tested extracts of corn (green material), kidney bean (seed), wheat (grain), rape (seed), orange (fruit) and hop (cone, kiln dried) for at least 27 days at 4°C ± 3°C.

In analytical method 01300/M005 (M-453914-02-1, refer to 4.2), trifloxystrobin and CGA 321113 were found to be stable in tested extracts of bovine kidney, milk and poultry´s eggs for at least seven days. In addition trifloxystrobin was found to be stable in bovine fat and meat for at least 4 days and in bovine liver for at least 13 days. CGA 321113 was found to be stable in bovine liver and fat for at least 4 days, while it declined about 40% in bovine meat within four days.

Additionally, during residue analyses on regular sample sets, the analytical performance of the methods must be checked with concurrent recoveries on each sample set. Therefore the relevant information on the stability in the final or any intermediate step can be derived from the fortification experiments performed during method validation. Every analytical batch does contain at least one concurrent recovery which is handled and stored in parallel to the residue samples. If the recoveries in the fortified samples are within acceptable ranges, stability is considered to be sufficient.

CA 6.2 Metabolism, distribution and expression of residues

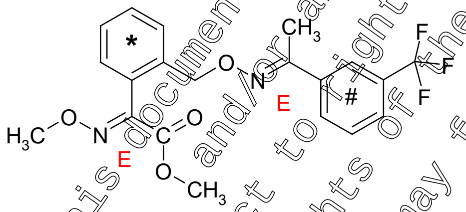
CA 6.2.1 Plants

Summary of plant metabolism in the DAR of April 2000

The original dossier contained plant metabolism studies with trifloxystrobin on wheat, apple and cucumber using ^{14}C-radiolabelled test substance with two different label positions. As these studies have been evaluated already by the RMS and respective EU authorities they are not repeated in this supplementary dossier. Only a short concluding summary of the old studies is presented in Section 6.2.1 on “Metabolism, distribution and expression of residues” of this dossier, in addition to the new studies not contained in the original dossier.

Trifloxystrobin (methyl (*E*)-methoxyimino-{(*E*)-α-[1-(α,α,α-trifluoro-*m*-tolyl)ethylidene-aminooxy]-*o*-toly}acetate, IUPAC) is a fungicide belonging to the group of strobilurin chemical compounds. The mode of action involves inhibition of mitochondrial respiration in fungi. Technical trifloxystrobin consists of a mixture of four diastereomers with the parent substance (*EE* configuration of the two C=N double bonds) being the dominant isomer. The four isomers in the technical product have a typical composition of parent-*E/E* : *E/Z* : *Z/E* : *Z/Z* = 95.8 : 1.3 : 1.2 : 0.7.

Trifloxystrobin was ^{14}C-labelled in both of the two phenyl rings of the molecule for investigation of metabolism studies in plants and animals.



* glyoxyl-phenyl-UL-^{14}C; abbr. [14C-GP]

\# trifluoromethyl phenyl-UL-^{14}C; abbr. [14C-TP]

The radiochemical purity of the test substance in metabolism studies was usually higher than the chemical purity in the technical material.

In this part of the dossier additional plant metabolism studies are reported that were not included in the original dossier for Annex I inclusion. The metabolism of trifloxystrobin in wheat was repeated since the original metabolism studies of trifloxystrobin in wheat revealed very low residues and, therefore, only constricted structure elucidation of metabolites could be performed. Therefore, the US EPA required a new wheat metabolism study that was conducted in 2002. An additional metabolism study on sugar beet was conducted in 2000 in order to demonstrate a common metabolic pathway of trifloxystrobin in at least three different crop groups. This study was submitted to the EMS ‘The Netherlands’ in the framework of establishment of temporary MRLs in root and tuber crops. A further metabolism study in peanuts performed in 1997 was submitted to USA for registration, but not to the EU. This study was assessed by JMPR (FAO, 2004)[1]. As these studies were not part of the original submission for Annex I inclusion they are now summarised in this dossier.

[1] FAO of the UN, 2004. Trifloxystrobin. In: pesticide residues in Food – 2004. FAO Plant Production and Protection Paper, 178, 240-270.

In their recent "Reasoned opinion on the modification of the existing MRL for trifloxystrobin in beans with pods" EFSA concluded (EFSA Journal 2013;11(4):3199):

> "The metabolism of trifloxystrobin after foliar applications in primary crops has been investigated in apples, cucumbers, wheat and sugar beet and the details of the metabolism studies are given in the previously issued EFSA reasoned opinion (EFSA, 2011)[2]. A metabolism study in peanuts was assessed by the JMPR (FAO, 2004).
>
> The metabolism of trifloxystrobin (*E/E* isomer) in primary crops was complex and mainly proceeded via cis/trans isomerisation (*Z/E* isomer, *Z/Z* isomer, *E/Z* isomer) and cleavage of the methyl ester group to form the metabolite (*E,E*)–methoxyimino-{2-[1-(3-trifluoromethyl-phenyl)–ethylideneamino-oxymethyl]-phenyl}-acetic acid (CGA 321113, Metabolite M5). Trifloxystrobin was the major component of residues in all crops investigated, except peanut kernels[3]. Metabolites, including CGA 321113, were below the trigger value of 10% of TRR in all samples of wheat, apples, cucumbers, peanuts and sugar beet leaves and tops, with the exception of sugar beet roots. In sugar beet root two metabolites were at levels exceeding the trigger value: the metabolite II_{19a}[4] (Metabolite 16) accounted for 20 % of the TRR (at 0 DALA) and 15 % of TRR (at 45 DALA); the metabolite CGA 321113 accounted for 11 % of TRR (at 21 and 45 DALA).
>
> The peer review assessed the metabolism on fruits and fruiting vegetables and cereals. The conclusion was that the metabolism proceeded according to a similar pathway and that the residue definition for monitoring and risk assessment should comprise the parent compound only for these crop groups (United Kingdom, 2000)[5]. JMPR concluded that the metabolism of trifloxystrobin in peanuts was similar to the metabolism observed in wheat. The metabolism study on sugar beet was submitted in the framework of the MRL application, after the conclusion of the peer review under Council Directive 91/414/EEC (EFSA, 2009a)[6].
>
> Based on the findings from the metabolism study in root and tuber vegetables and the residue trials on leafy and root vegetables (Brussels sprouts, head cabbage, celery, leek, turnip, swedes, salsify, parsnip, parsley root), where the metabolite CGA 321113 [M5] occurred even at higher levels than parent trifloxystrobin, EFSA has recommended in previously issued reasoned opinions to consider the possible inclusion of this metabolite in a revised risk assessment residue definition for plant commodities (EFSA, 2009a, 2009b, 2012)[6].
>
> Since the residue data indicated that the metabolite CGA 321113 is not found in beans with pods, EFSA concludes that the residue definitions for enforcement and risk assessment as agreed in the peer review are provisionally applicable to the crop under consideration. The current residue definition set in Regulation (EC) No 396/2005 is identical to the residue

---

[2] EFSA 2011: EFSA Journal 2011; 9(1); 1973, 26 pp.

[3] Trifloxystrobin represented about 2 % of the TRR and an extensive formation of composed triglycerides was observed in the residues (FAO, 2004).

[4] II_{19a}: {2-[1-(2,3-dihydroxy-5-methyl-phenyl)-2-hydroxy-ethylideneamino-oxymethyl]-phenyl}-methoxy-imino acetic acid (Metabolite 16).

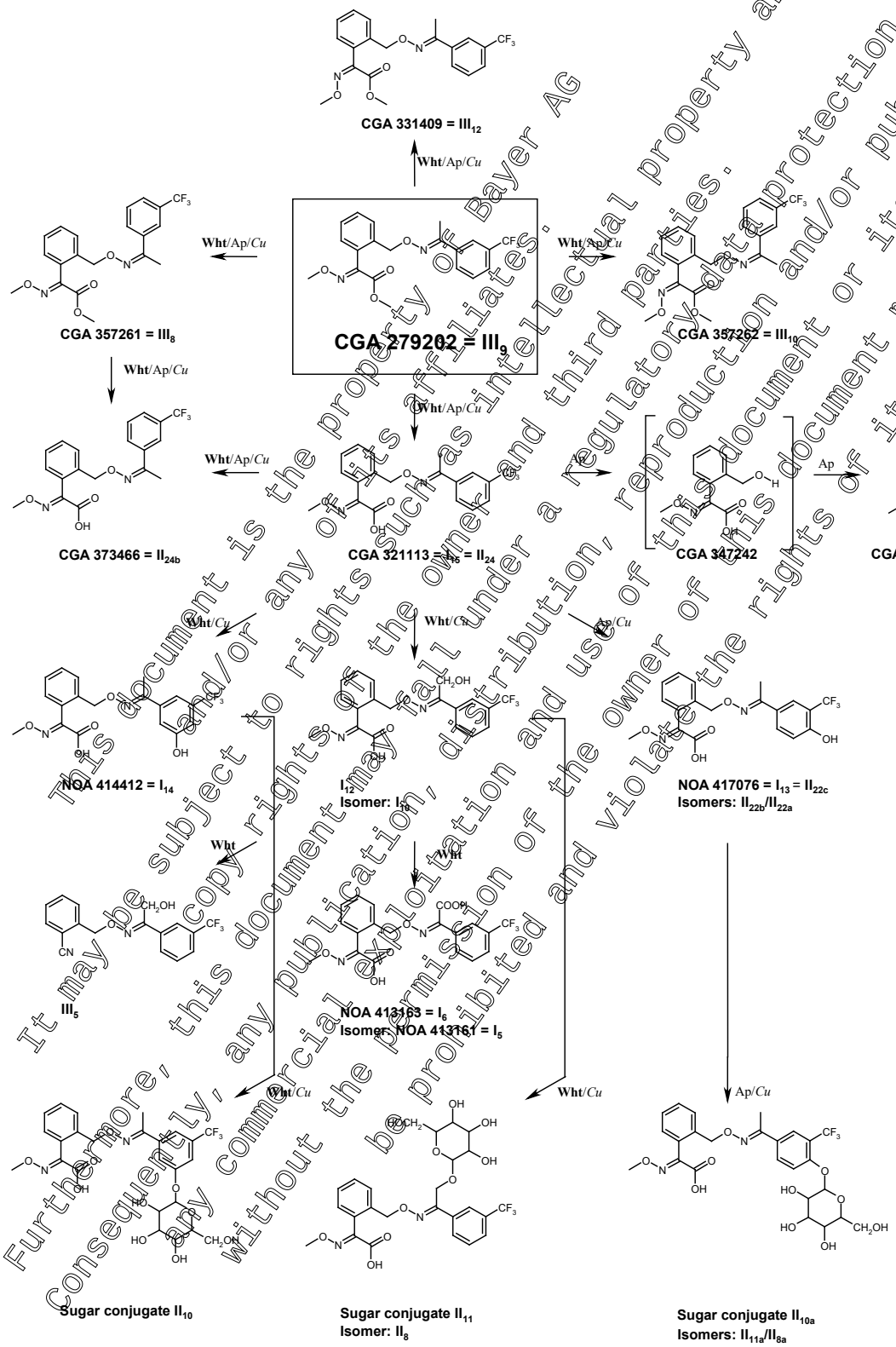
[5] DAR on the active substance trifloxystrobin prepared by the RMS the United Kingdom in the framework of Council Directive 91/414/EEC, April 2000.

[6] EFSA 2009a: EFSA Scientific Report (2009) 273, 1-27
EFSA 2009b: EFSA Scientific Report (2009) 314, 1-27
EFSA 2010: EFSA Journal 2010; 8(6)1648, 28 pp.

definition for enforcement derived in the peer review. EFSA proposes that the inclusion of the metabolite CGA 321113 in the risk assessment residue definition for plant commodities should be further discussed in the framework of Article 12 of Regulation (EC) No 396/2005."

The common metabolic pathway for trifloxystrobin in the primary crops wheat, apple fruit and cucumber fruit taken from the DAR on trifloxystrobin, Annex B.7 (2000) is shown in Figure 6.2.1- 1.

Figure 6.2.1- 1: Metabolic pathway of trifloxystrobin in plants (according to the DAR on Trifloxystrobin, Annex B.7, April 2000)



Additional plant metabolism studies with [14C-PG]- and [14C-TP]-trifloxystrobin

Wheat

The metabolism of trifloxystrobin in wheat is very complex according to the initial metabolism studies that were included into the original submission for Annex I inclusion. Using both ^{14}C-labels more than 30 metabolic fractions were detected, none of them accounting more than 7% of TRR in mature grain and straw following two spray applications of 250 g as/ha or 500 g as/ha. Additional studies applying higher efforts on identification and characterization of metabolites resulted in the identification of the main residue components and a proposal of the metabolic pathway, but still left unidentified a huge number of metabolites. Therefore, the metabolism of trifloxystrobin in wheat was re-investigated and the new studies were summarised in the following abridgements.

Report:	**KCA 6.2.1/09, [redacted], [redacted], 2002**; M-070885-01-1
Title:	Metabolism of [trifluoromethyl-phenyl-UL-^{14}C]Trifloxystrobin in Spring Wheat
Document No:	M-070885-01-1
Report No:	MR-027/02
Guidelines and data requirements:	US-EPA OPPTS 860.1300, Nature of Residues – Plants PMRA Ref.: DACO 6.3 – Plant Study EU Directive 91/414/EEC amended by the Commission Directive 96/68/EC
GLP	yes

Executive Summary

The metabolism of [trifluoromethyl-phenyl-UL-^{14}C]trifloxystrobin was investigated in spring wheat following two spray applications at single use rates of 250 g as/ha. The wheat plants were cultivated outdoors and treated at the growth stages BBCH 33 and 69. Wheat hay was sampled three and mature straw and grain 35 days after the last application. The total radioactive residues (TRR) amounted to 5.20 mg equ/kg in hay, 6.13 mg equ/kg in straw and 0.120 mg equ/kg in grain, expressed as parent equivalents.

All samples were multiple extracted with acetonitrile/water (4/1, v/v). From wheat hay 92.3% of TRR was extractable; from straw and grain 71.9% and 66.9% of TRR were extractable. An increase of the released residues was achieved by microwave support at enhanced temperature, resulting in a total portion of extracted residues of 97.9% of TRR from hay and 91.0% of TRR from straw. Diastase digestion of grain to hydrolyse the starch increased the total portion of released residues to 88.3% of TRR. In total, 80.7% of TRR (4.19 mg equ/kg) was identified in hay, 67.1% (4.11 mg equ/kg) in straw and 61.4% of TRR (0.074 mg equ/kg) in grain.

The metabolism of trifloxystrobin in wheat was very extensive as all four stereoisomers of the parent substance and at least ten free and seven conjugated metabolites could be identified. None of the isomers, except the parent substance, and metabolites exceeded 10% of TRR in each of the wheat commodities.

A significant portion of the parent *E/E*-isomer was isomerized to the *Z/E, E/Z* and *Z/Z* stereoisomers.
A main metabolic reaction revealed to be an ester hydrolysis to form the corresponding carboxylic acid. Furthermore, hydroxylation occurred at the imino-methyl group and at the trifluoromethyl phenyl ring in the *meta*-position. Significant portions of the hydroxylated metabolites were conjugated with glucose. The imino-methyl group was partly oxidised via hydroxylation to a carboxylic acid group.

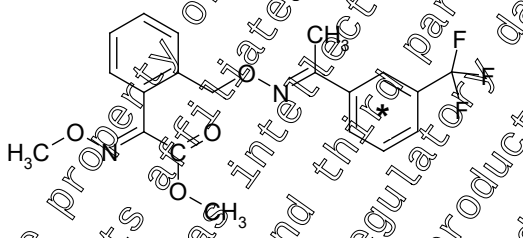
Two very minor cyano metabolites arising from the elimination of the ester/methoxyimino-group (probably via the carboxylic acid intermediate) were formed only in hay and straw.

In each wheat commodity the parent substance *E/E*-trifloxystrobin proved to be the main residue component (>10% of TRR). All other residue components appeared at levels less than 10% of TRR.

The proposed metabolic pathway of [14C-TP]trifloxystrobin in wheat is shown in Figure 6.2.1-2.

Material and Methods

Test Material

Structural formula	 * denotes the ^{14}C label
Chemical name	(*E,E*)-methoxyimino-{2-[1-(3-trifluoromethyl-phenyl)-ethylideneamino-oxymethyl]-phenyl}acetic acid methyl ester (IUPAC); (*E,E*)-α-(methoxyimino)-2-[[[1-[3-(trifluoromethyl)phenyl]ethylidene] amino]oxy]methyl]benzene acetic acid methyl ester (CAS)
Common name	Trifloxystrobin
CAS RN	141517-21-7
Empirical formula	$C_{20}H_{19}F_3N_2O_4$
Company code	CGA 279202
Molar mass (non-labelled)	408.4 g/mole
Label	[trifluoromethyl-phenyl-UL-^{14}C]Trifloxystrobin, abbr. [14C-TP]
Specific radioactivity	3.72 MBq/mg = 100.6 mCi/g = 41.1 Ci/mole
Radiochemical purity	>98% (radio-HPLC, radio-TLC)
Identity	Confirmed by HPLC/MS/MS and ^1H-NMR

Test Plants

Test plant	Spring wheat
Variety	Thasos
Study design	Outdoor study in a 1 m^2 planting container
Growth stage at application	Two spray applications at growth stages BBCH 33 and 69, spray interval: 42 days
Harvested commodities	Hay, 3 days after the last application. Mature straw and grain at growth stage BBCH 89, 35 days after the last application

Sowing of wheat grain, preparation and application of the test mixture

Main experiment

Sandy loam (72.4% sand, 22.6% silt, 5.0% clay, 1.02% organic carbon, pH 6.2 [$CaCl_2$]) was filled in an outdoor planting container with a surface of 1 m^2. Spring wheat was sown and cultivated outdoors during spring and summer 2001 at [redacted] test facility (Germany) of Bayer AG. The monthly mean temperatures ranged from 8 to 20°C, the mean sunshine periods from 99 to 242 hours/month and the natural precipitation from 22.7 to 104.7 mm/month.

Supportive experiment

An additional supportive experiment was conducted in a plant container (surface of 0.5 m^2) filled with sandy loam to generate a higher amount of metabolites. The container was located in an open vegetation hall that was surrounded by a net fence and protected against rain by a flexible glass roof. Spring wheat was sown into the container at the same time as done for the main experiment. The container was watered if required. The results of this supportive experiment are not shown in the Findings' Section.

The wheat plants in both experiments were treated by two foliar spray applications approx. 7 weeks after emergence of the plants (BBCH 33, 3rd node at least 2 cm above 2nd node) and 6 weeks later (BBCH 69, end of flowering). The single application rate was approx. 250 g as/ha. The water volume rate was approx. 800 L/ha and the mean annual use rate approx. 500 g as/ha. For both experiments radiolabelled [14C-TP]trifloxystrobin was dissolved in EC 125 blank formulation and diluted with water to yield the spray solution. This spray solution was evenly sprayed over the surface of the plant containers using a flat fan nozzle that was moved manually (outdoor main experiment) or by a computer controlled track sprayer (supportive experiment).

The stability of the test substance was confirmed by radio-HPLC before and after each spraying. Both plant containers were fertilized and treated with different pesticides as required for cultivation of healthy plants.

Harvest and processing

Three days after the second application, wheat plants in the hay growth stage were cut shortly above the soil surface. The complete hay sample was dried at room temperature for four days, cut in pieces and homogenized with liquid nitrogen using a high-speed stirrer. At maturity, 35 days after the second application, the wheat plants were cut above the soil surface. The seeds were collected by hand yielding the grain sample. The remaining ears and chaffs were combined with the straw sample. Straw was cut in pieces. Straw pieces and grain were homogenised with liquid nitrogen using a high-speed stirrer. The pulverized plant material was stored in a freezer at ≤ -20°C.

Aliquots of the homogenized plant commodities were extracted with acetonitrile/water (4/1, v/v, 4x) using a high-speed stirrer. The phases were separated by filtration. The combined extract was radioassayed, concentrated and partitioned against dichloromethane yielding an organic and an aqueous phase. The phases were separated, radioassayed, concentrated and analysed by radio-HPLC.

The remaining solids of hay and straw after the primary extraction were exhaustively extracted with acetonitrile/water (1/1, v/v) at 150°C using a microwave device. Extract and solids after extraction were radioassayed.

Enzymatic digestion of grain matrix

The remaining solids of grain after the primary extraction were hydrolysed by the starch-digesting enzyme diastase (10 days at room temperature and addition of little sodium azide to prevent microbial degradation). The hydrolysate and the filtered and air-dried solids were radioassayed. An aliquot of the diastase hydrolysate was further hydrolysed with concentrated HCl at 100°C after addition of toluene to extract less polar aglycons that exhibited some volatilization in hydrolysis experiments without toluene. The resulting aqueous and organic phases were radioassayed and analysed by radio-TLC.

Radioassaying and analysis

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). Quenching was automatically compensated using an external standard. Solid samples were firstly combusted and the formed 14CO2 absorbed in an alkaline scintillation liquid. The detection limit was set to twice the instrument background (approx. 20 cpm).

Radio-TLC was conducted on silica gel plates (Si60 F254). Following application of the residue solutions the plates were developed over a distance of 15 cm with two solvent mixtures (1) hexane : diethyl ether : tetrahydrofuran : formic acid : water (10:70:10:1:2, v:v:v:v:v) and (2) chloroform : methanol : formic acid : water (75:20:4:2, v:v:v:v). Radioactive spots were detected by a Bio-Imaging Analyzer. Co-chromatographed non-active reference standards were visualized by fluorescence extinction following excitation with mercury UV-light.

Radio-HPLC was conducted on a RP18 column (250 x 4 mm, 5 µm particle size) operated with different gradient mixtures of water and acetonitrile (both containing 1% acetic acid) at 40°C. The HPLC system was equipped with a radiomonitor with a glass scintillator (cell volume approx. 400 µL). The column recovery was examined by comparison of injected and eluted radioactivity. The eluted radioactivity amounted to 94.7 – 103.8% of the injected one (with one exemption 91.5%). The limit of quantification (LOQ) was set to a peak size of twice the detector background. The HPLC-LOQ depended on the used separation conditions and ranged between 0.001 – 0.01 mg equ/kg.

LC-MS and LC-MS/MS analyses for identification of metabolites were performed with a combination of TQS mass spectrometer connected to a HPLC system with a RP column (250 x 2 mm, particle size 5 µm) and a radiomonitor. The HPLC column was operated with gradient mixture of water and acetonitrile (both solvents containing 0.1% formic acid). Ionization was achieved by electrospray ionization. Daughter ions were generated using argon as collision gas.

Enzymatic hydrolysis of conjugated metabolites was conducted with cellulose (straw) and ß-glucosidase (grain). For 1H-NMR analysis a 300 MHz NMR spectrometer was used.

Findings

Total radioactive residues and their extractability

The total radioactive residues (TRR) were determined by summarizing the radioactivity in the primary extracts and the extracted plant matrix. TRR amounted to 5.20 mg equ/kg in wheat hay, 6.13 mg equ/kg in straw and 0.120 mg equ/kg for grain.

The portion of radioactive residues extractable with acetonitrile/water accounted for 92.3% of TRR in wheat hay, 71.9% of TRR in straw and for 66.9% of TRR in grain.

Partitioning of the extracted residues into methylene chloride resulted in the following distribution between organic/aqueous phases: 53.0/39.3% of TRR for hay, 47.8/24.1% of TRR for straw and 26.2/40.7% of TRR for grain.

An additional portion of radioactive residues could be extracted from hay: 5.6% of TRR by microwave support, from straw: 19.1% of TRR by microwave support, and from grain: 21.4% of TRR by diastase hydrolysis of the starch matrix. The non-extractable residues amounted in turn to 2.1% of TRR in hay, to 9.0% of TRR in straw and to 11.7% of TRR in grain.

The residues released from grain by diastase digestion and were hydrolysed using hydrochloric acid and partitioned between toluene and water. 10.5% of TRR portioned into the organic phase and 10.9% of TRR remained in the aqueous phase.

Residues in wheat hay, straw and grain

The composition of the radioactive residues in wheat hay, mature wheat straw and grain following two foliar treatments with [14C-TP]trifloxystrobin is presented in Table 6.2.1- 1. All four cis/trans-isomers of the parent substance and at least 10 free and 8 conjugated metabolites were observed.

The parent substance *E/E*-trifloxystrobin was the major residue component in all wheat commodities amounting to 31.1% of TRR (1.61 mg/kg) in hay, 14.3% of TRR (0.88 mg/kg) in straw and to 19.6% of TRR (0.024 mg/kg) in grain.

The identified components of the extracted radioactivity in Table 6.2.1- 1 were grouped as isomers of the parent compound and as free and conjugated (glucoside) metabolites. In total, 80.7% of TRR (4.19 mg equ/kg) was identified in hay, 67.1% (4.11 mg equ/kg) in straw and 61.4% (0.074 mg equ/kg) in grain.

Significant amounts of the parent *E,E*-isomer were transformed into the other isomers (reaction 1 in the metabolic pathway, Figure 6.2.1- 2). The proportion of parent isomers accounted for 43.5% (2.26 mg equ/kg) of the TRR in hay, 25.5% (1.56 mg equ/kg) in straw and 39.7% (0.048 mg equ/kg) in grain. As the individual isomers showed similar MS spectra they were differentiated by co-chromatography with the respective reference standards.

Apart from isomerization (reaction 1) the main metabolic reaction in wheat was the hydrolysis of the ester group (reaction 2) of the parent compound. Furthermore, the hydroxylation (reaction 3) of the imino-methyl group and also at the meta-position of the 1,3-disubstituted trifluoromethyl ring were important. Significant portions of the hydroxylated metabolites were also detected as glycoside conjugates (reaction 4). The methyl group was partly oxidized (reaction 3) to the carboxylic acid group. A minor reaction was elimination at the methoxyimino-group, probably via a carboxylic acid intermediate, to form cyano-metabolites in hay and straw (released after microwave support).

The enzymatic starch hydrolysis of the grain matrix with diastase resulted in polar residues and polar matrix components that could not be separated either by chromatography or by partition between organic/aqueous phases. Therefore, an acid hydrolysis step and a simultaneous organic/aqueous partition were involved using hydrochloric acid and toluene. This procedure resulted in tentative identification of cleavage products containing the trifluoromethyl phenyl ring linked to an acetyl group followed by hydroxylation of the phenyl ring and glucoside conjugation.

The proposed metabolic pathway of [trifluoromethyl-phenyl-UL-^{14}C]trifloxystrobin in wheat is presented in Figure 6.2.1- 2.

Storage stability of trifloxystrobin and its metabolites in wheat

The extraction of hay, straw and grain samples of wheat was initiated within two weeks after sampling. The radio-HPLC separations for the quantitation of the isomers of parent compound and its metabolites were also performed within 1-2 weeks. The extracted solids were kept frozen until further extraction and the corresponding extracts were analysed without delay. Repeated extractions and analyses of stored wheat samples were conducted in a parallel metabolism study with glyoxyl-phenyl-radiolabelled trifloxystrobin. These analyses confirmed the storage stability the residues.

For diastase digestion the extracted grain was incubated twice for ten days at room temperature. Microbial degradation during this incubation was avoided by admixture of little sodium azide.

Conclusion

Following two foliar treatments of spring wheat with [14C-TP]trifloxystrobin using single use rates of approx. 250 g as/ha different wheat commodities were extracted with acetonitrile/water (4/1, v/v) to release radioactive residues. From wheat hay sampled three days after the second treatment 92.3% of TRR was extractable, from straw and grain sampled 35 days after the second treatment 71.9% and 66.9% of TRR was extractable.

The portion of released residues could be increased by microwave support at enhanced temperature (hay, straw) or diastase digestion of starch in grain. These procedures resulted in an increase of the extractable radioactive residues up to 97.9% of TRR from hay, 91.0% of TRR from straw and up to 88.3% of TRR from grain. In total, 80.7% of TRR (4.19 mg equ/kg) was identified in hay, 67.1% (4.11 mg equ/kg) in straw and 61.4% of TRR (0.074 mg equ/kg) in grain.

The metabolism of trifloxystrobin was very extensive as all four stereoisomers of the parent substance and at least ten free and seven conjugated metabolites could be identified. None of the isomers, except the parent substance, and metabolites exceeded 10% of TRR in each of the wheat commodities.

A significant portion of the parent *E/E*-isomer was isomerized to the *Z/E*, *E/Z* and *Z/Z* stereoisomers. Besides isomerization the main metabolic reaction revealed to be ester hydrolysis to form the corresponding carboxylic acid. Furthermore, hydroxylation occurred at the imino-methyl group and at the trifluoromethyl phenyl ring in the *m*-position. Significant portions of the hydroxylated metabolites were conjugated with glucose. The imino-methyl group was partly oxidised via a hydroxyl to a carboxylic acid group. Two very minor cyano metabolites arising from the elimination of the ester/methoxyimino-group (probably via the carboxylic acid intermediate) were only formed in hay and straw. The proposed metabolic pathway of [14C-TP]trifloxystrobin in wheat is shown in Figure 6.2.1- 2.

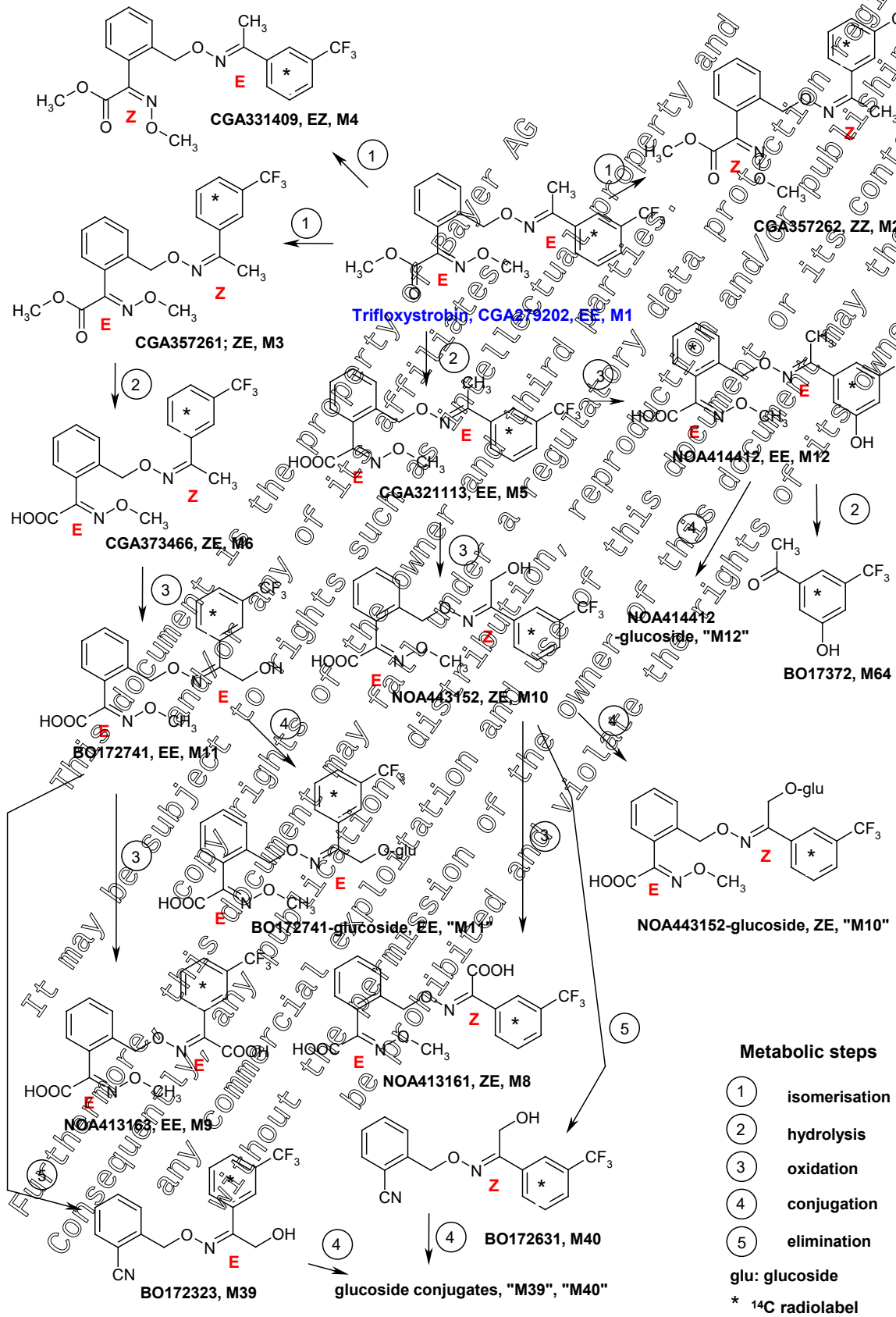
In each wheat commodity the parent substance *E/E*-trifloxystrobin proved to be the main residue component (>10% of TRR). All other residue components appeared at levels less than 10% of TRR. Therefore the parent substance may serve as analytical target for monitoring and risk assessment.

Table 6.2.1- 1: Composition of radioactive residues in wheat following two foliar treatments with [14C-TP]trifloxystrobin at a use rate of 250 g as/ha

Wheat commodity		**Hay**		**Straw**		**Grain**	
TRR [mg equ/kg]		**5.20**		**6.13**		**0.120**	
Parent/Metabolite	No.	[%TRR]	[mg/kg]*	[%TRR]	[mg/kg]*	[%TRR]	[mg/kg]*
Parent isomers		***43.5***	***2.26***	***25.5***	***1.56***	***39.7***	***0.048***
CGA279202, *E/E*	1	31.1	1.61	14.3	0.88	19.6	0.024
CGA357262, *Z/Z*	2	2.0	0.11	2.4	0.14	6.3	0.008
CGA357261, *Z/E*	3	6.7	0.35	5.3	0.32	8.0	0.010
CGA331409, *E/Z*	4	3.8	0.20	3.6	0.22	5.8	0.007
Free metabolites		***10.3***	***0.54***	***34.1***	***2.09***	***21.7***	***0.026***
CGA321113	5	1.6	0.08	4.2	0.26	2.6	0.003
CGA373466	6	0.3	0.02	1.8	0.11	1.2	0.001
NOA414412	12	2.1	0.11	7.0	0.43	5.2	0.006
NOA443152	10	1.8	0.09	6.5	0.40	4.6	0.006
BO172741	11	0.9	0.04	4.1	0.25	1.5	0.002
BO172631	40	-	-	1.0	0.06	-	-
BO172323	39	-	-	0.9	0.05	-	-
BO17372	64	-	-	0.9	0.06	-	-
NOA413163	9	3.7	0.19	5.8	0.35	2.9	0.004
NOA413161	8	n.d.	n.d.	1.8	0.11	0.3	<0.001
Conjugated metabolites		***26.8***	***1.39***	***7.6***	***0.46***	***3.4***	***0.004***
NOA414412 conj. 1	"12"	3.5	0.18	0.5	0.03	-	-
NOA414412 conj. 2	"12"	5.3	0.28	0.7	0.04	-	-
NOA443152 conj.1	"10"	8.1	0.42	2.2	0.14	3.4	0.004
NOA443152 conj.2	"10"	0.9	0.05	0.7	0.04		
BO172741 conj. 1	"11"	4.5	0.23	1.1	0.07	-	-
BO172741 conj. 2	"11"	3.7	0.19			-	-
BO172631 conj.	"40"	0.3	0.02	1.3	0.08	-	-
BO172323, conj.	"39"	0.6	0.03	1.1	0.07	-	-
Characterized fractions							
Organic fractions		4.3	0.22	4.3	0.26	10.5	0.013
Aqueous fractions		9.7	0.50	6.0	0.37	16.4	0.020
Microwave fractions		3.2	0.17	13.5	0.83	-	-
Non-extractable		2.1	0.11	9.0	0.55	11.7	0.014
Total identified		**80.7**	**4.19**	**67.1**	**4.11**	**61.4**	**0.074**
Total characterized		**17.2**	**0.89**	**23.9**	**1.46**	**26.9**	**0.032**

"n" : conjugated metabolite with number n; * [mg/kg]: mg parent equivalents/kg plant matrix

Figure 6.2.1- 2: Proposed metabolic pathway of [14C-TP]trifloxystrobin in wheat



Report:	**KCA 6.2.1/10, [redacted], [redacted], 2002** ; M-072024-01-1
Title:	Metabolism of [glyoxyl-phenyl-UL-^{14}C]Trifloxystrobin in Spring Wheat
Document No:	M-072024-01-1
Report No:	MR-028/02
Guidelines and data requirements:	US-EPA OPPTS 860.1300, Nature of Residues – Plants PMRA Ref.: DACO 6.3 – Plant Study EU Directive 91/414/EEC amended by the Commission Directive 96/68/EC
GLP	yes

Executive Summary

The metabolism of [14C-GP]trifloxystrobin was investigated in spring wheat following two spray applications at single use rates of 250 g as/ha. The wheat plants were cultivated outdoors and treated at the growth stages BBCH 33 and 69. Wheat hay was sampled three and mature straw and grain 35 days after the last application. The total radioactive residues (TRR) amounted to 5.98 mg equ/kg in hay, 6.12 mg equ/kg in straw and 0.262 mg equ/kg in grain, expressed as parent equivalents.

All samples were multiple extracted with acetonitrile/water (4/1, v/v). From wheat hay 94.1% of TRR was extractable; from straw and grain 76.3% and 66.5% of TRR were extractable. An increase of the released residues was achieved by microwave support at enhanced temperature, resulting in a total portion of extracted residues of 98.6% of TRR from hay and 93.2% of TRR from straw. Diastase digestion of grain to hydrolyse the starch increased the total portion of released residues to 93.5% of TRR. In total, 85.9% of TRR (5.13 mg equ/kg) was identified in hay, 70.4% (4.31 mg equ/kg) in straw and 48.2% of TRR (0.126 mg equ/kg) in grain.

The metabolism of trifloxystrobin in wheat was very extensive as all four stereoisomers of the parent substance and at least 11 free and 8 conjugated metabolites could be identified. None of the isomers except the parent substance and metabolites exceeded 10% of TRR in each of the wheat commodities.

A significant portion of the parent *E/E*-isomer was isomerized to the *Z/E, E/Z* and *Z/Z* stereoisomers. Besides isomerization the main metabolic reaction revealed to be ester hydrolysis to form the corresponding carboxylic acid. Furthermore, hydroxylation occurred at the imino-methyl group and at the trifluoromethyl phenyl ring in the *m*-position. Significant portions of the hydroxylated metabolites were conjugated with glucose. The imino-methyl group was partly oxidised via a hydroxyl to a carboxylic acid group. Two very minor cyano metabolites arising from the elimination of the ester/methoxyimino-group (probably via the carboxylic acid intermediate) were only formed in hay and straw. The same metabolic reactions were also observed with the alternative trifluoromethyl phenyl radiolabel.

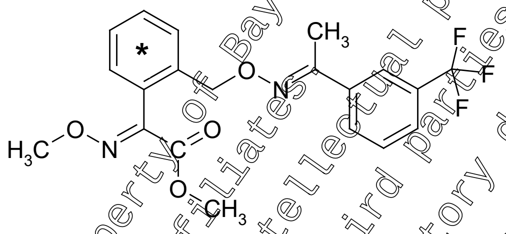
However, additional very polar metabolites were only detected when using the glyoxyl-phenyl label. These metabolites either conventionally extracted or with microwave support or after enzymatic hydrolysis of the starch in grain, were isolated from the aqueous phase following organo-aqueous partitioning. Major polar metabolites released from grain were identified as phthalic acid and its derivatives. Other metabolites of this group were characterized by a combination of phase partitioning, radio-HPLC, radio-TLC, HPLC-MS, hydrolysis, methylation and acetylation. Based on these methods the structure of a new metabolite was elucidated with very high probability, i.e. the "cyclic keto alcohol", SA04271. This metabolite was released from grain at similar portion as the parent substance

In each wheat commodity the parent substance *E/E*-trifloxystrobin proved to be the main residue component (>10% of TRR). All other residue components appeared at levels less than 10% of TRR.

The proposed metabolic pathway of [14C-GP]trifloxystrobin in wheat is shown in Figure 6.2.1- 3.

Material and Methods

Test Material

Structural formula	 * denotes the ^{14}C label
Chemical name	(*E,E*)-methoxyimino-{2-[1-(3-trifluoromethyl-phenyl)-ethylideneamino-oxymethyl]-phenyl}acetic acid methyl ester (IUPAC); (*E,E*)-α-(methoxyimino)-2-[[[[1-[3-(trifluoromethyl)phenyl]ethylidene] amino]oxy]methyl]benzene acetic acid methyl ester (CAS)
Common name	Trifloxystrobin
CAS RN	141517-21-7
Empirical formula	$C_{20}H_{19}F_3N_2O_4$
Company code	CGA 279202
Molar mass (non-labelled)	408.4 g/mole
Label	[glyoxyl-phenyl-UL-^{14}C]Trifloxystrobin, abbr. [14C-GP]
Specific radioactivity	2.48 MBq/mg = 67.0 mCi/g = 27.4 Ci/mole
Radiochemical purity	>98% (radio-HPLC, radio-TLC)
Identity	Confirmed by HPLC/MS/MS and ^{1}H-NMR

Test Plants

Test plant	Spring wheat
Variety	Thasos
Study design	Outdoor study in a 1 m^2 planting container
Growth stage at application	Two spray applications at growth stages BBCH 33 and 69, spray interval: 42 days
Harvested commodities	Hay, 3 days after the last application. Mature straw and grain at growth stage BBCH 89, 35 days after the last application

<u>Sowing of wheat grain, preparation and application of the test mixture</u>

Main experiment
Sandy loam (72.4% sand, 22.6% silt, 5.0% clay, 1.02% organic carbon, pH 6.3 [$CaCl_2$]) was filled in an outdoor planting container with a surface of 1 m^2. Spring wheat was sown and cultivated outdoors during spring and summer 2001 at [redacted] test facility (Germany) of Bayer AG. The monthly mean temperatures ranged from 8 to 20°C, the mean sunshine periods from 99 to 242 hours/month and the natural precipitation from 22.7 to 104.7 mm/month.

Supportive experiment
An additional supportive experiment was conducted in a plant container (surface of 0.5 m^2) filled with sandy loam to generate a higher amount of metabolites. The container was located in an open vegetation hall that was surrounded by a net fence and protected against rain by a flexible glass roof. Spring wheat was sown into the container at the same time as done for the main experiment. The container was watered if required. The results of this supportive experiment are not shown in the Findings' Section.

The wheat plants in both experiments were treated by two foliar spray applications approx. 7 weeks after emergence of the plants (BBCH 33, 3rd node at least 2 cm above 2nd node) and 6 weeks later (BBCH 69, end of flowering). The single application rate was approx. 250 g as/ha. The water volume rate was approx. 800 L/ha and the mean annual use rate approx. 500 g as/ha. For both experiments radiolabelled [14C-GP]trifloxystrobin was dissolved in EC 125 blank formulation and diluted with water to yield the spray solution. This spray solution was evenly sprayed over the surface of the plant containers using a flat fan nozzle that was moved manually (outdoor main experiment) or by a computer controlled track sprayer (supportive experiment).

The stability of the test substance was confirmed by radio-HPLC before and after each spraying. Both plant containers were fertilized and treated with different pesticides as required for cultivation of healthy plants.

<u>Harvest and processing</u>

Three days after the second application, wheat plants in the hay growth stage were cut shortly above the soil surface. The complete hay sample was dried at room temperature for four days, cut in pieces and homogenized with liquid nitrogen using a high-speed stirrer. At maturity, 35 days after the second application, wheat plants were cut above the soil surface. The seeds were collected by hand yielding the grain sample. The remaining ears and chaffs were combined with the straw sample. Straw was cut in pieces. Straw pieces and grain were homogenised with liquid nitrogen using a high-speed stirrer. The pulverized plant material was stored in a freezer at ≤ -20°C.

Aliquots of the homogenized plant commodities were extracted with acetonitrile/water (4/1, v/v, 4x) using a high-speed stirrer. The phases were separated by filtration. The combined extract was radioassayed, concentrated and partitioned against dichloromethane yielding an organic and an aqueous phase. The phases were separated, radioassayed, concentrated and analysed by radio-HPLC.

The remaining solids of hay and straw after the primary extraction were exhaustively extracted with acetonitrile/water (1/1, v/v) at 150°C using a microwave device. Extract and solids after extraction were radioassayed.

Enzymatic digestion of grain matrix

The remaining solids of grain after the primary extraction were hydrolysed by the starch-digesting enzyme diastase (10 days at room temperature and addition of little sodium azide to prevent microbial degradation). The hydrolysate and the filtered and air-dried solids were radioassayed. An aliquot of the diastase hydrolysate was further hydrolysed with concentrated HCl at 100°C. The hydrolysate was portioned against ethyl acetate to isolate less polar aglycons. The resulting aqueous and organic phases were radioassayed and the organic phase analysed by radio-TLC.

Derivatisation of metabolites

Methylation with diazomethane
The polar metabolites in the aqueous phase from grain extraction were purified, dried and re-dissolved in methanol and diethylether. For generation of diazomethane (CH_2N_2) N-methyl-N-nitroso-p-toluenesulfonamide dissolved in diethyl ether dropped into a solution of 10% KOH in diethylene glycol and diethyl ether in a small flow-through glass apparatus that was gently heated in a water bath. The developed diazomethane/ether vapour was directly transferred with a gentle stream of nitrogen into a small glass tube with the polar metabolites for some minutes until the solution remained constantly yellow. The resulting solution was concentrated using a stream of nitrogen. Aliquots of the metabolite before and after methylation were analysed by radio-TLC.

Acetylation with acetic acid anhydride
In a parallel experiment, the polar metabolites in the aqueous phase from grain extraction were purified, dried and re-dissolved in pyridine and acetic acid anhydride in a closed glass vial. After 5-hour incubation at room temperature the solution was concentrated to dryness using a rotary evaporator. Small amounts of ethanol were repeatedly added during this concentration procedure to destroy exceeding acetic acid anhydride and to completely co-distillate off the pyridine. The remainder was re-dissolved in little water and analysed by radio-HPLC.

Radioassaying and analysis

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). Quenching was automatically compensated using an external standard. Solid samples were firstly combusted and the formed $^{14}CO_2$ absorbed in an alkaline scintillation liquid. The detection limit was set to twice the instrument background (approx. 20 cpm).

Radio-TLC was conducted on silica gel plates (Si60 F_{254}). Following application of the residue solutions the plates were developed over a distance of 15 cm with two solvent mixtures (1) hexane : diethyl ether : tetrahydrofuran : formic acid : water (10:70:10:1:2, v:v:v:v:v) and (2) chloroform : methanol : formic acid : water (75:20:4:2, v:v:v:v). Radioactive spots were detected by a Bio-Imaging Analyzer. Co-chromatographed non-active reference standards were visualized by fluorescence extinction following excitation with mercury UV-light.

Radio-HPLC was conducted on a RP18 column (250 x 4 mm, 5 µm particle size) operated with different gradient mixtures of water and acetonitrile (both containing 1% acetic acid) at 40°C. The HPLC system was equipped with a radiomonitor with a glass scintillator (cell volume approx. 400 µL). The column recovery was examined by comparison of injected and eluted radioactivity. The eluted radioactivity amounted to 97.5 – 106.0% of the injected one (with one exemption 122.4%). The limit of quantification (LOQ) was set to a peak size of twice the detector background. The HPLC-LOQ depended on the used separation conditions and ranged between 0.001 – 0.01 mg equ/kg.

LC-MS and LC-MS/MS analyses for identification of metabolites were performed with a combination of TQS mass spectrometer connected to a HPLC system with a RP column (250 x 2 mm, particle size 5 µm) and a radiomonitor. The HPLC column was operated with gradient mixture of water and acetonitrile (both solvents containing 0.1% formic acid). Ionization was achieved by electrospray ionization. Daughter ions were generated using argon as collision gas.

Enzymatic hydrolysis of conjugated metabolites was conducted with cellulose (straw) and β-glucosidase (grain). For ^{1}H-NMR analysis a 300 MHz NMR spectrometer was used.

Findings

Total radioactive residues and their extractability

The total radioactive residues (TRR) were determined by summarizing the radioactivity in the primary extracts and the extracted plant matrix. TRR amounted to 5.98 mg equ/kg in wheat hay, 6.12 mg equ/kg in straw and 0.262 mg equ/kg for grain.

The portion of radioactive residues extractable with acetonitrile/water accounted for 94.1% of TRR in wheat hay, 76.3% of TRR in straw and for 66.5% of TRR in grain.

Partitioning of the extracted residues into methylene chloride resulted in the following distribution between organic/aqueous phases: 54.1/40.0% of TRR for hay, 50.0/26.3% of TRR for straw and 16.3/60.2% of TRR for grain.

An additional portion of radioactive residues could be extracted from hay: 4.5% of TRR by microwave support resulting in total extraction of 98.6% of TRR, from straw: 16.9% of TRR by microwave support resulting in total extraction of 93.2% of TRR, and from grain: 17.0% of TRR by diastase hydrolysis of the starch matrix resulting in total extraction of 93.5% of TRR. The non-extractable residues amounted in turn to 1.4% of TRR in hay, to 6.8% of TRR in straw and to 6.5% of TRR in grain.

The residues released from grain by diastase digestion were hydrolyzed using hydrochloric acid and partitioned between ethyl acetate and water. 14.0% of TRR portioned into the organic phase and 3.0% of TRR remained in the aqueous phase.

Residues in wheat hay, straw and grain

The composition of the radioactive residues in wheat hay mature wheat straw and grain following two foliar treatments with [14C-TP]trifloxystrobin is presented in Table 6.2.1- 2. All four cis/trans-isomers of the parent substance and at least 11 free and 8 conjugated metabolites were observed.

The parent substance *E/E*-trifloxystrobin was the main residue component in all wheat commodities amounting to 40.3% of TRR (2.41 mg/kg) in hay, 18.6% of TRR (1.44 mg/kg) in straw and to 11.1% of TRR (0.029 mg/kg) in grain.

The identified components of the extracted radioactivity shown in Table 6.2.1- 2 were grouped as isomers of the parent compound and as free and conjugated (glucoside) metabolites. In total, 85.9% of TRR (5.13 mg equ/kg) was identified in hay, 70.4% (4.31 mg equ/kg) in straw and 48.2% of TRR (0.126 mg equ/kg) in grain.

Significant amounts of the parent *E,E*-isomer were transformed into the other isomers (reaction 1 in the metabolic pathway,). The proportion of parent isomers accounted for 53.1% (3.18 mg equ/kg) of

the TRR in hay, 29.3% (1.79 mg equ/kg) in straw and 17.9% (0.047 mg equ/kg) in grain. As the individual isomers showed similar MS spectra they were differentiated by co-chromatography with the respective reference standards.

Apart from isomerization (reaction 1) the main metabolic reaction of trifloxystrobin in wheat was the hydrolysis of the ester group (reaction 2). Furthermore, the hydroxylation (reaction 3) of the imino-methyl group and also at the meta-position of the 1,3-disubstituted trifluoromethyl phenyl ring were important. Significant portions of the hydroxylated metabolites were also detected as glycoside conjugates (reaction 4). The methyl group was partly oxidized (reaction 3) to a carboxylic acid. A minor reaction was elimination at the methoxyimino-group (reaction 5), probably via a carboxylic acid intermediate, to form cyano-metabolites in hay and straw (released after microwave support). Following cleavage of the bridge between the phenyl rings, the glyoxyl-phenyl moiety was intensively degraded to different minor label-specific metabolites, such as the cyanobenzoic acid, its oxidation product the phthalic acid and, by further elimination of water, to a *"cyclic keto alcohol"* (SA04271).

The "cyclic keto alcohol" (SA04271) was isolated from the water phase following the first extraction of grain with acetonitrile/water and partition of the extract against dichloromethane. This polar metabolite was investigated by HPLC-MS and was tentatively identified as "cyclic keto alcohol". It has been demonstrated by TLC that this metabolite is different from phthalic acid and its condensation product phthalide. Following methylation with diazomethane, several methylation products were observed that obviously resulted from methylation of different products after opening of the cyclobutane ring. Following acetylation of SA04271, three acetylation products were detected. The main acetylation product was identified as mono-acetylated benzylic alcohol also formed after ring opening of the cyclobutane ring. Acidic hydrolysis of SA04271 (2N HCl, 100°C, 3 hours) proved the hydrolytic stability of this metabolite and excluded an appearance of a potential conjugate. These investigations indicated the correct identification of the structure of this metabolite with a high probability.

The enzymatic starch hydrolysis of the grain matrix with diastase resulted in polar residues and polar matrix components that could not be sufficiently separated either by chromatograph or by partition between organic/aqueous phases. Therefore, an acid hydrolysis step using hydrochloric acid was involved followed by organic/aqueous partition with ethyl acetate. Two aglycons were detected in the organic phase. The main component was phthalic acid accompanied by a small amount of its condensation product phthalide that was obviously formed due to the harsh hydrolysis conditions.

The proposed metabolic pathway of [14C-GP]trifloxystrobin in wheat is presented in Figure 6.2.1- 3.

<u>Storage stability of trifloxystrobin and its metabolites in wheat</u>

The extraction of hay, straw and grain samples of wheat was initiated within two weeks after sampling. The radio-HPLC separations for the quantitation of the isomers of parent compound and its metabolites were also performed within 1-2 weeks. The extracted solids were kept frozen until further extraction and the corresponding extracts were analysed without delay.

Repeated extraction and analysis of stored grain samples were conducted five and six months after the initial analysis. The metabolic pattern of the aqueous phase containing the major portion of the extracted residues was virtually identical after all storage periods and thus confirmed the storage stability of incurred trifloxystrobin residues in starch-containing samples for at least six months.

For diastase digestion the extracted grain was incubated twice for ten days at room temperature. Microbial degradation during this incubation was avoided by admixture of little sodium azide.

Conclusion

Following two foliar treatments of spring wheat with [14C-GP]trifloxystrobin using single use rates of approx. 250 g as/ha different wheat commodities were extracted with acetonitrile/water (4/1, v/v) to release radioactive residues. From wheat hay sampled three days after the second treatment 94.1% of TRR was extractable, from straw and grain sampled 35 days after the second treatment 76.3% and 66.5% of TRR was extractable.

The portion of released residues could be increased by microwave support at enhanced temperature (hay, straw) or diastase digestion of starch in grain. These procedures resulted in an increase of the extractable radioactive residues up to 98.6% of TRR from hay, 93.2% of TRR from straw and up to 93.5% of TRR from grain. In total, 85.9% of TRR (5.13 mg equ/kg) was identified in hay, 70.4% (4.31 mg equ/kg) in straw and 48.2% of TRR (0.126 mg equ/kg) in grain.

The metabolism of trifloxystrobin was very extensive as all four stereoisomers of the parent substance and at least 11 free and 8 conjugated metabolites could be identified. None of the isomers and metabolites except the parent substance exceeded 10% of TRR in each of the wheat commodities.

A significant portion of the parent *E/E*-isomer was isomerized to the *Z/E, E/Z* and *Z/Z* stereoisomers. Besides isomerization the main metabolic reaction revealed to be ester hydrolysis to form the corresponding carboxylic acid. Furthermore, hydroxylation occurred at the imino-methyl group and at the trifluoromethyl phenyl ring in the *m*-position. Significant portions of the hydroxylated metabolites were conjugated with glucose. The imino-methyl group was partly oxidised via a hydroxyl to a carboxylic acid group. Two very minor cyano metabolites arising from the elimination of the ester/methoxyimino-group (probably via the carboxylic acid intermediate) were only formed in hay and straw. The same metabolic reactions were also observed with the alternative trifluoromethyl phenyl radiolabel (see before).

However, additional very polar metabolites were only detected when using the glyoxyl-phenyl label. These metabolites either conventionally extracted or with microwave support or after enzymatic hydrolysis of the starch in grain, were isolated from the aqueous phase following organo-aqueous partitioning. Major polar metabolites released from grain were identified as phthalic acid and its derivatives. Other metabolites of this group were characterized by a combination of phase partitioning, radio-HPLC, radio-TLC, HPLC-MS, hydrolysis, methylation and acetylation. Based on these methods the structure of a new metabolite was elucidated with very high probability, i.e. the “cyclic keto alcohol”, SA04271. This metabolite was released from grain at similar portion as the parent substance

In each wheat commodity the parent substance *E/E*-trifloxystrobin proved to be the main residue component (>10% of TRR). All other residue components appeared at levels less than 10% of TRR. Therefore the parent substance may serve as analytical target for monitoring and risk assessment.

Table 6.2.1- 2: Composition of radioactive residues in wheat following two foliar treatments with [14C-GP]trifloxystrobin a use rate of 250 g as/ha

Wheat commodity		**Hay**		**Straw**		**Grain**	
TRR [mg equ/kg]		**5.98**		**6.12**		**0.262**	
Parent/Metabolite	No.	[%TRR]	[mg/kg]*	[%TRR]	[mg/kg]*	[%TRR]	[mg/kg]*
Parent isomers		***53.1***	***3.18***	***29.3***	***1.79***	***17.9***	***0.047***
CGA279202, *E/E*	1	40.3	2.41	18.6	1.14	11.1	0.029
CGA357262, *Z/Z*	2	2.2	0.13	2.3	0.14	1.8	0.005
CGA357261, *Z/E*	3	7.1	0.42	5.3	0.33	2.9	0.008
CGA331409, *E/Z*	4	3.6	0.21	3.1	0.19	2.1	0.005
Free metabolites		***8.9***	***0.53***	***33.5***	***2.05***	***19.2***	***0.050***
CGA321113	5	1.3	0.08	3.8	0.23	1.7	0.005
CGA373466	6	0.2	0.01	2.0	0.12	0.9	0.002
NOA414412	12	0.7	0.04	6.5	0.40	2.4	0.006
NOA443152	10	0.5	0.03	5.9	0.36	1.9	0.005
NOA413163	9	4.0	0.24	5.0	0.31	1.8	0.005
NOA413161	8	-	-	1.4	0.08	0.3	0.001
FWH0115C	54	1.4	0.09	3.0	0.18	3.6	0.009
FWH0115D	53	0.7	0.04	1.2	0.07	3.1	0.008
BO172741	11	-	-	3.5	0.21	-	-
BO172631	40	-	-	0.7	0.04	-	-
BO172323	39	-	-	0.5	0.03	-	-
Tentatively identified metabolite							
SA04271**	57	0.1	0.01	1.5	0.09	9.6	0.025
Conjugated metabolites		***23.7***	***1.42***	***6.1***	***0.37***	***4.9***	***0.013***
NOA414412 conj. 1	"12"	2.1	0.13	0.3	0.02	-	-
NOA414412 conj. 2	"12"	6.8	0.40	0.5	0.03	-	-
NOA443152 conj.1	"10"	7.2	0.43	2.0	0.12	3.4	0.009
NOA443152 conj.2	"10"	1.2	0.07	0.3	0.02		
BO172741 conj. 1	"11"	3.7	0.22	1.2	0.07	-	-
BO172741 conj. 2	"11"	2.5	0.15	-	-	-	-
BO172631 conj.	"40"	0.1	0.00	0.9	0.06	-	-
BO172323 conj.	"39"	0.2	0.01	0.9	0.05	-	-
SA04275**	56	-	-	-	-	1.5	0.004
Characterized fractions							
Organic fractions		0.5	0.03	2.7	0.16	16.4	0.043
Aqueous fractions		10.7	0.64	11.4	0.69	29.0	0.076
Microwave fractions		1.5	0.09	8.7	0.53	-	-
Non-extractable		1.4	0.08	6.8	0.42	6.5	0.017
Total identified		**85.9**	**5.13**	**70.4**	**4.31**	**48.2**	**0.126**
Total characterized		**12.7**	**0.76**	**22.8**	**1.39**	**45.3**	**0.119**

"n" : conjugated metabolite with number n * [mg/kg]: mg parent equivalents/kg plant matrix

Figure 6.2.1- 3: Proposed metabolic pathway of [14C-GP]trifloxystrobin in wheat

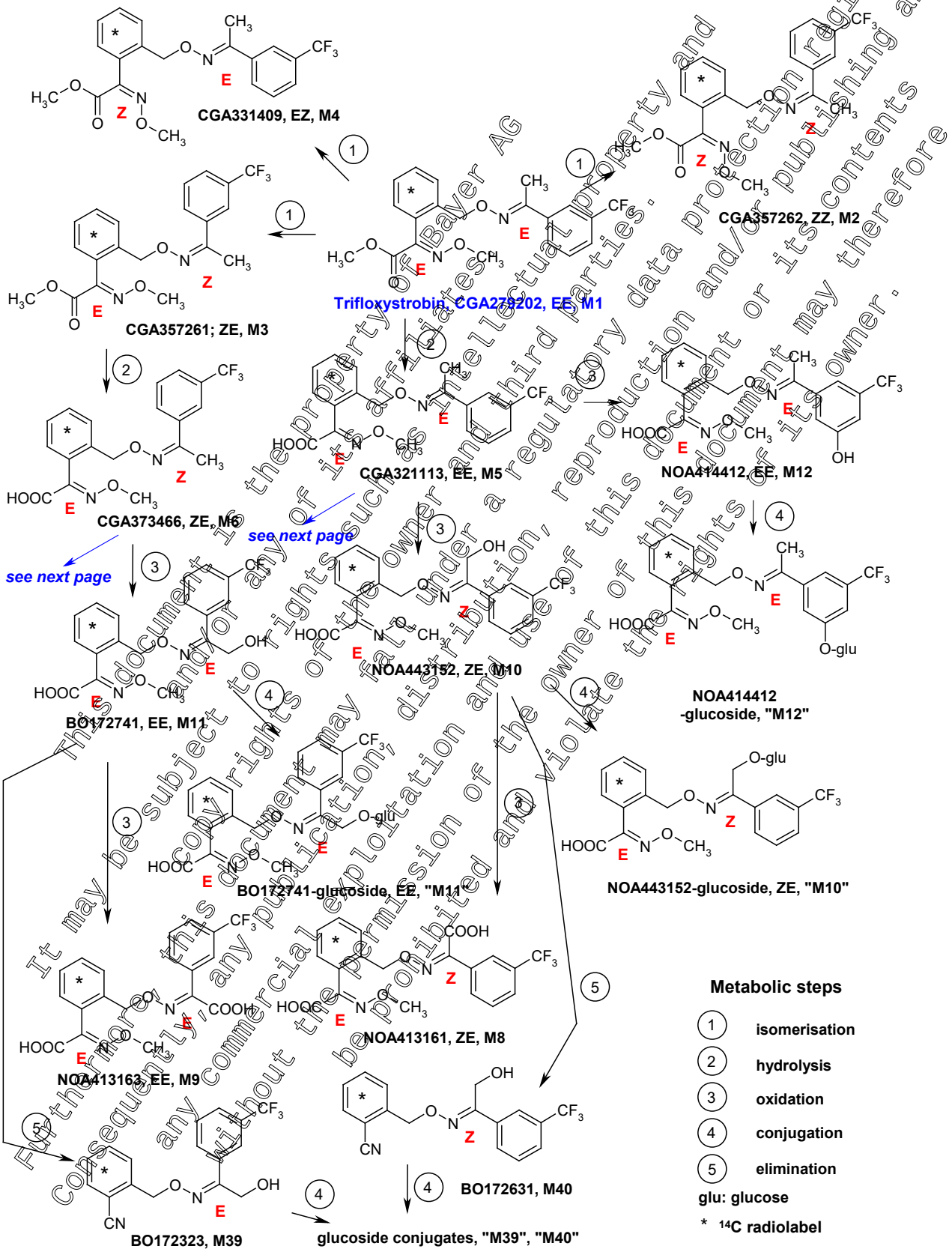
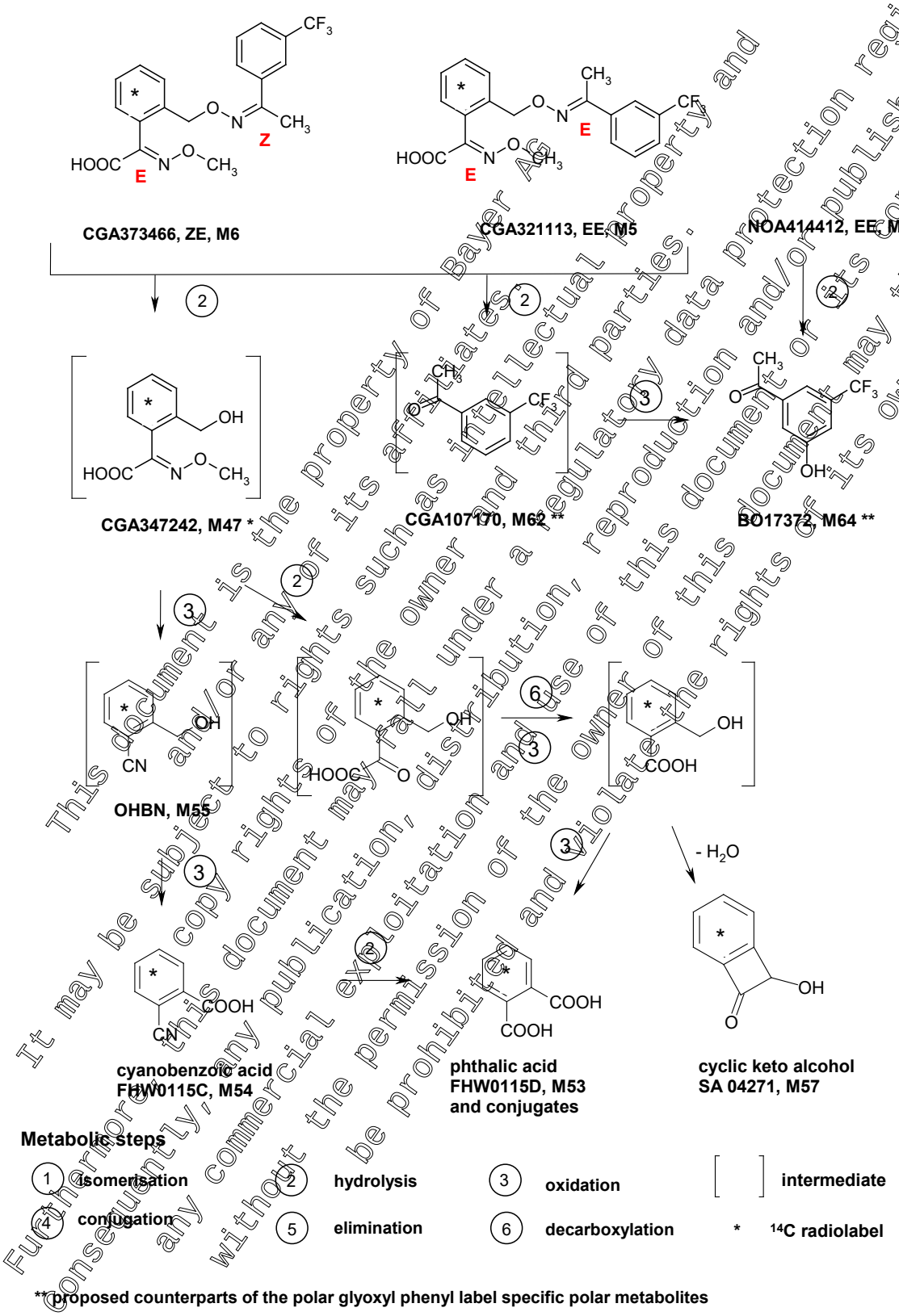


Figure 6.2.1- 3: Proposed metabolic pathway of [14C-GP]trifloxystrobin in wheat (contd.)



Sugar beet

After submission of the original submission for an Annex I inclusion of trifloxystrobin additional metabolism studies became available on sugar beets. In order to broaden the use spectrum of trifloxystrobin the metabolism was investigated in a crop of the category root and tuber crops. Two studies using the [trifluoromethyl phenyl] and the [glyoxyl phenyl]-14C label are summarised in the following abridgments.

Report:	**KCA 6.2.1/11, [redacted], 2000** ; M-069117-01-1
Title:	Behavior and Metabolism of [Trifluoromethyl-Phenyl-(U)-^{14}C] CGA-279202 in Field Grown Sugar Beets
Document No:	M-069117-01-1
Report No:	99MK09; 1266-00
Guidelines and data requirements:	US-EPA OPPTS 860.1300, Nature of Residues – Plants (1996) EU Directive 91/414/EEC amended by the Commission Directive 96/68/EC Agricultural Chemical Laws and Regulations, Japan, Metabolism Study (1985)
GLP	yes

Executive Summary

The metabolism of [14C-TP]trifloxystrobin was investigated in sugar beet following three spray applications at single use rates of approx. 130 g as/ha (1x application rate), totalling 395 g as/ha. The plants were cultivated outdoors. The first treatment was conducted at the growth stage BBCH 39 (leaves covered 90% of ground). Two subsequent treatments were performed at 3-week spray intervals. Sugar beets were sampled one day after each treatment and harvested 21 and 45 days after the last treatment. They were separated in tops (foliage) and roots (beets). An additional overdose experiment was conducted to aid in the characterization and identification of metabolites.

The total radioactive residues (TRR) in tops of the 1x application rate experiment amounted to 2.28 – 4.13 mg parent equivalents/kg matrix (mg equ/kg) 1 hour after each treatment and decreased to 0.45 mg equ/kg 45 days after the last application. The TRR levels in the beets were generally low ranging from 0.010 to 0.097 mg equ/kg throughout beet sampling.

The predominant portion of TRR could be conventionally extracted with acetonitrile/water. From the tops approx. 88 – 97% of TRR were extracted and from the beets approx. 79 – 100% of TRR. Where the extraction yield was lower a significant portion was additionally extractable with use of microwave causing evaluated temperatures. In turn, the non-extractable residues were low, i.e. approx. 1 – 5% of TRR in the tops and approx. 5 – 12% of TRR in the beets. These non-extractable residues in the beets corresponded to 0.0002 – 0.0004 mg equ/kg at the two later harvest dates (21 and 45 days after the last application).

The metabolism of trifloxystrobin in sugar beet was very intensive as approx. 20 metabolites were detected in both tops and roots. Most of these metabolites appeared at very low concentrations (<<10% of TRR). Two metabolites revealed to be major in the roots. The carboxylic acid CGA321113 (M5, EE-Isomer) accounted for 9 – 11 % of TRR and its triply hydroxylated derivative (M16, II_{19a}) accounted for 10 – 15% of TRR. Additional minor metabolites were identified as CGA373466 (M6, ZE carboxylic acid), NOA443152 (M10, hydroxylated ZE carboxylic acid) and NOA414412 (M12, phenyl hydroxylated EE carboxylic acid) and their glycoside conjugates, respectively. The metabolic pattern observed in the 1x application experiment was confirmed by the overdose experiment.

Some of the plant metabolites of trifloxystrobin were also observed in the soil. The carboxylic acid CGA311113 (M5) revealed to be the predominant residue component in soil. The parent substance was very low at the two later sampling dates.

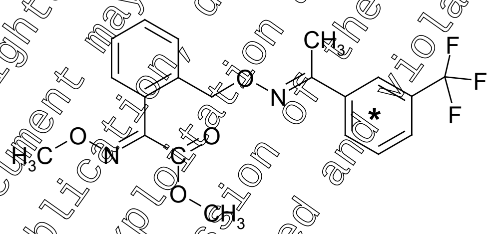
The proposed common metabolic pathway of [trifluoromethyl-phenyl-UL-14C]trifloxystrobin in plants (wheat w, cucumber c, and sugar beet sb) is shown in Figure 6.2.1- 4.

Based on the structures identified, the metabolism of trifloxystrobin in sugar beets appears to proceed via different transformations:

- Cis/trans isomerization of trifloxystrobin and/or hydrolysis of the methyl ester,
- Methyl ester cleavage to the major metabolite II_{24} (= M5, CGA321113) and cis/trans isomerization of M5 to metabolite II_{24b} (= M6, CGA373466),
- Single and double hydroxylation of the trifluoromethyl-phenyl ring of M5 resulting in metabolite II_{22} (= M12, NOA414412) and its stereoisomer II_{21a} with subsequent glycoside conjugation to form metabolites II_{10} or II_{9b},
- Hydroxylation of the methyl substituent of M5 at the 2-ethylideneaminooxymethyl group to metabolite II_{23a} (= M10, NOA443152) with subsequent glycoside conjugation to form metabolite II_{11}, and threefold hydroxylation of phenyl and methyl group to form the major metabolite fraction II_{19a} (M16) that consisted of several isomers with each isomer accounting for < 0.01 mg equ/kg (investigated in the parallel study with the 14C-GP-label, see below).
- Formation of bound residues to a low extent.
- The parent substance revealed to be the predominant residue component in sugar beet tops and roots and, therefore, is proposed as marker substance in residue analysis.

Material and Methods

Test Material

Structural formula	 * denotes the ^{14}C label
Chemical name	(*E,E*)-methoxyimino-{2-[1-(3-trifluoromethyl-phenyl)-ethylideneamino-oxymethyl]-phenyl}acetic acid methyl ester (IUPAC); (*E,E*)-α-(methoxyimino)-2-[[[[1-[3-(trifluoromethyl)phenyl]ethylidene] amino]oxy]methyl]benzene acetic acid methyl ester (CAS)
Common name	Trifloxystrobin
CAS RN	141517-21-7
Empirical formula	$C_{20}H_{19}F_3N_2O_4$
Company code	CGA 279202
Molar mass (non-labelled)	408.4 g/mole
Label	[trifluoromethyl-phenyl-UL-^{14}C]Trifloxystrobin, abbr. [14C-TP]
Specific radioactivity	1.0 MBq/mg = 27.03 mCi/g (1x dosing experiment)

	0.9 MBq/mg = 24.32 mCi/g (overdose experiment)
Radiochemical purity	95.8 – 98.7% (radio-HPLC, radio-HPLC)
Identification	LC-MS/MS with ESI ionization

Test Plants

Test plant	Sugar beet
Variety	Kassandra
Study design	Outdoor study at a 4 m^2 plot in Switzerland
Growth stage at application	Three spray applications beginning at growth stage BBCH 39 (crop cover complete, foliage cover: 90% of ground) spray interval: 3 weeks. Second and third application at growth stage BBCH 39 - 49.
Harvested commodities	Complete sugar beet plants one hour after each treatment and 21 days and 45 days after the last application. The plants were divided in tops (foliage) and roots (beets).

Sowing and cultivation of sugar beet, preparation and application of the test substance

Sugar beet seeds were sown into loam soil (49.6% sand, 30.2% silt, 20.2% clay, 1.45% organic carbon, pH 7.8 [$CaCl_2$]) of three outdoor plots located at a research station [redacted] Switzerland. The plot for the main experiment (1x application rate) had a size of 4 m^2. Two additional plots were sown for an overdose and a control experiment. The sugar beet plants were cultivated under usual agricultural conditions in spring and summer 1999. No additional pesticide treatment was performed throughout the study, apart from application of the test substance.

[^{14}C-TP]trifloxystrobin was mixed with a blank EC125 formulation. The resulting formulation was suspended in water to prepare the spray mixture. The concentration of the test substance in the formulation amounted to approx. 10.2%. Three months after sowing the first of three foliar spray treatments were performed. At the first treatment, the sugar beets reached the growth stage BBCH 39 and their leaves covered approx. 90% of the ground. Two subsequent treatments were conducted after spray intervals of 3 weeks. A small plot sprayer with four TEEJET flat-jet nozzles was used for each spray treatment. The use rates were 130 g as/ha (1st application), 137 g as/ha (2nd application) and 128 g as/ha (3rd application) resulting in a total use rate of 395 g as/ha (1x application rate). The homogeneity of the spray mixture was confirmed by repeated radioassaying before and after each spray event.

In an extra overdose experiment for generation of a higher amount of radiolabelled metabolites additional plants were sprayed at the same days as the 1x use experiment with a higher-concentrated spray mixture. The use rates of this trial were 692 g as/ha (1st application), 693 g as/ha (2nd application) and 768 g as/ha (3rd application).

Sampling and processing of sugar beet plants

Few sugar beet plants were collected one hour after each application as well as 21 and 45 days after the last application. The plants were divided into tops (leaves) and roots (beets). At each plant sampling, soil core samples (0 - 30 cm depth) were additionally taken. The soil cores were separated into three horizons: 0 - 10, 10 - 20 and 20 - 30 cm.

The plants samples (tops and roots) were chopped with plant copper in presence of liquid nitrogen. Fine homogenization was performed using a kitchen mixer. Aliquots of the homogenates were taken for radioassaying. The remaining pulverized samples were stored in frozen condition (at approx. -18°C).

The homogenized plant samples were extracted 6-times with acetonitrile/water (4/1, v/v) for at least 6 hours using a mechanical shaker. The combined extract was analysed by radio-TLC. Some extracts from the foliage (TOP extracts) were concentrated and partitioned against n-hexane. Both phases were analysed by radio-TLC and radio-HPLC. Incompletely extracted samples were subsequently extracted with 1-propanol/water (4/1, v/v) at enhanced temperatures (100 – 150°C) using a microwave oven and the extract analysed by radio-TLC.

Clean-up of crude root extracts was carried out by solid-phase extraction using C18 cartridges. The cartridges were washed with acetonitrile and 0.01N HCl before use. The primary acetonitrile/water extracts were first concentrated to remove the acetonitrile and then were applied to the cartridges. The loaded cartridges were rinsed with water followed by elution with acetonitrile/0.01N HCl. The eluted residues were analysed by radio-TLC using the solvent mixtures 2.1 and 2.2 (see below).

Enzyme cleavage of an aliquot of foliage extracts was performed with ß-glucosidase (37°C, shaking overnight) following evaporation to dryness and re-dissolution in 0.1N acetate buffer at pH 4.65. The resulting mixture was extracted with ethyl acetate and analysed by radio-TLC and radio-HPLC.

Soil core samples of the same sampling day and horizon were combined, air-dried, homogenized in a disk mill and radioassayed. They were then extracted with acetonitrile/water (4/1, v/v) in the same was manner as done with plant samples. The combined extracts were also analysed by radio-TLC.

<u>Radioassaying and analysis</u>

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). Quenching was automatically compensated using an external standard. Solid samples were firstly combusted and the formed $^{14}CO_2$ absorbed in an alkaline scintillation liquid. For quantification of the radioactivity of the spots in radio-TLC the silica gel with the spots were scrapped out, suspended in methanol and radioassayed after addition of scintillation cocktail. The LOQ for radioassaying was 0.015 – 0.018 mg equ/kg for the tops (foliage) and 0.002 – 0.005 mg equ/kg for the roots.

One and two-dimensional radio-TLC was conducted on silica gel plates (Si60 F_{254}). For two-dimensional TLC used for separation of metabolites the plates they were developed in two rectangular directions with the two solvent mixtures: (1.1) n-hexane/diethyl ether/tetrahydrofuran/formic acid/ water (10/70/10/1/2, v/v/v/v/v) and (1.2) chloroform/ethyl acetate/formic acid (60/30/10, v/v/v) as well as (2.1) chloroform/methanol/formic acid/water (75/20/4/2, v/v/v/v) and (2.2) 1-butanol/acetic acid/ water (40/10/10, v/v/v). One-dimensional TLC plates used for separation of the parent isomers was developed with toluene/ethyl acetate (9/1, v/v). Radioactive spots were detected by a Bio-Imaging Analyzer. Co-chromatographed non-labelled reference standards were visualized by fluorescence extinction following excitation by UV-light. The LOQ for radio-TLC was set to 0.001 mg equ/kg sample.

Radio-HPLC was conducted on a RP18 column (250 x 4.6 mm, 5 µm particle size) operated with a gradient mixture of aqueous phosphoric acid (0.1%) and acetonitrile. The HPLC system was equipped with a UV detector (254 nm) and a radiomonitor with a glass scintillator.

The final identification of the isomers and metabolites of trifloxystrobin was performed by a combination of LC-MS/MS (RP18 HPLC, 15 x 2 mm, particle size 5 µm, operated with an gradient mixture of water/acetonitrile both acidified with formic acid; electron spray ionization for MS) and ^{1}H-NMR (solvent D_2O/CH_3CN, ca. 1/1). In addition, non-labelled reference standards were used for chromatographic comparison.

Findings

Total radioactive residues and their extractability

The total radioactive residues (TRR) in tops (foliage) and beets, the extractable and non-extractable residues are presented in Table 6.2.1- 3. TRR in tops of the 1x application rate experiment amounted to 2.28 – 4.13 mg parent equivalents/kg matrix (mg equ/kg) 1 hour after each foliar treatment and decreased to 0.45 mg equ/kg 45 days after the last application. The TRR levels in the beets were generally low ranging from 0.010 to 0.097 mg equ/kg throughout beet sampling.

The predominant portion of TRR could be conventionally extracted with acetonitrile/water. From the tops (foliage), approx. 88 – 97% of TRR and from the beets approx. 79 – 100% of TRR were extracted. In case of a lower extraction yield, a significant portion was additionally extractable by use of microwave. In turn, the non-extractable residues were low, i.e. approx. 1 – 5% of TRR from tops and approx. 5 – 12% of TRR the beets. These non-extractable residues in the beets corresponded to 0.0002 – 0.0004 mg equ/kg at the two later harvest dates (21 and 45 days after the last application).

Residues in sugar beet tops and roots

Sugar beet roots and tops were initially extracted with acetonitrile/water at ambient temperature and subsequently with 1-propanol/water at enhanced temperature using microwave support. The extracts were partly cleaned-up by solid-phase extraction with C18 cartridges and analysed by two-dimensional radio-TLC and radio-HPLC. Co-chromatographed reference standards aided for identification. The resulting composition of the radioactive residues in sugar beet tops and roots of the last two sampling dates, 21 and 45 days after three foliar treatments with [14C-TP]trifloxystrobin is presented in Table 6.2.1- 4

The metabolism of trifloxystrobin in sugar beet was very intensive as approx. 20 metabolites were detected in both tops and roots. Most of these metabolites appeared at very low concentrations (<<10% of TRR). Two metabolites revealed to be major in the roots. The carboxylic acid CGA321113 (M5, EE-Isomer) accounted for 9 – 11 % of TRR and its triply hydroxylated derivative (M16, one hydroxyl group attached to methyl group of the bridge between the phenyl rings and two hydroxyl groups attached to the trifluoromethyl phenyl ring) accounted for 10 – 15% of TRR. Additional minor metabolites were identified as CGA373466 (M6, ZE carboxylic acid), NOA443152 (M10, hydroxylated ZE carboxylic acid) and NOA414412 (M12, phenyl hydroxylated EE carboxylic acid) and their glycoside conjugates, respectively. The metabolic pattern observed in the 1x application experiment was confirmed by the overdose experiment.

However, the parent substance trifloxystrobin (EE-Isomer, M1) accounted generally for the predominant residue components at harvest (21 – 45 days after last application) amounting to 34 – 65% of TRR in tops and to 47 – 48% of TRR in roots. Its EZ-isomer (CGA331409, M4) accounted for 1.2% of TRR in tops and for up to 3.8% of TRR in the roots. The other isomers, i.e. the ZZ-isomer (CGA357262, M2) and the ZE-isomer (CGA 357261, M3) could not be detected.

<u>Residues in soil</u>

In the top soil layer (0 – 10 cm) TRR accounted for 0.032 – 0.103 mg equ/kg one day after each application and for 0.106 and 0.162 mg equ/kg 21 and 45 days after the last application of the 1x application trial. The top layer contained ≥ 79% of the radioactivity found in the total soil core (0 – 30 cm) at all sampling intervals.

Extraction and radio-TLC of the soil extracts revealed low amounts of the parent substance at the two later sampling dates (21 and 45 days after last application to the plants). The predominant residue component in soil (top layer) was formed by ester hydrolysis of the parent substance, i.e. the carboxylic acid M5 (CGA 321113) amounting to 60 – 70% of TRR in the soil samples. Its isomeric carboxylic acid M6 (CGA373466) accounted for approx. 3 – 4% of TRR and the hydroxylated carboxylic acids M10 (NOA443152) and M12 (NOA414412) amounted to approx. 1% of TRR. The same metabolites were also detected in sugar beet roots and leaves.

<u>Storage stability</u>

All samples were stored at approx. – 18°C. Extracts were stored at 8°C, at maximum. Leaves and roots sampled 21 days after the last application were extracted and chromatographically profiled approx. two months after harvest. The extracts were re-analysed after approx. 8 months storage at maximum 8°C. After the same time period other aliquots of tops and roots of the same frozen samples were re-extracted and analysed again. Comparing the metabolite pattern, no significant change could be observed regarding the qualitative and quantitative distribution of radioactive peaks.

Conclusion

[14C-TP]trifloxystrobin metabolized very intensively in sugar beets as approximately 20 metabolites were detected in tops (foliage) and roots (beets) 21 and 45 days after the last of three foliar treatment at a total application rate of 395 g as/ha. Based on the structures identified, the metabolism of trifloxystrobin in sugar beets appears to proceed via different transformations:

- Cis/trans isomerization of trifloxystrobin and/or hydrolysis of the methyl ester,
- Methyl ester cleavage to the major metabolite II_{24} (= M5, CGA321113) and cis/trans isomerization of M5 to metabolite II_{24b} (= M6, CGA373466),
- Single and double hydroxylation of the trifluoromethyl-phenyl ring of M5 resulting in metabolite II_{22} (= M12, NOA414412) and its stereoisomer II_{21a} with subsequent glycoside conjugation to form metabolites II_{10} or II_{9b},
- Hydroxylation of the methyl substituent of M5 at the 2-ethylideneaminooxymethyl group to metabolite II_{23a} (= M10, NOA443152) with subsequent glycoside conjugation to form metabolite II_{11}, and threefold hydroxylation of phenyl and methyl group to form the major metabolite fraction II_{19a} (M16) that consisted of several isomers with each isomer accounting for < 0.01 mg equ/kg (investigated in the parallel study with the 14C-GP label).
- Formation of bound residues to a low extent.
- No cleavage of the ethylideneaminooxymethyl bridge between the phenyl rings and, therefore, formation of label-specific metabolites
- The parent substance revealed to be the predominant residue component in sugar beet tops and roots and, therefore, is proposed as marker substance in residue analysis.

The proposed common metabolic pathway of [trifluoromethyl-phenyl-UL-14C]trifloxystrobin in plants (wheat w, cucumber c, and sugar beet sb) is shown in Figure 6.2.1- 4.

Table 6.2.1- 3: Total radioactive residues (TRR) in sugar beet and extractability of the residues following three foliar treatments with [14C-TP]trifloxystrobin at a total use rate of 395 g as/ha

Sampling	Crop Part	TRR	Extraction with ACN/H_2O [% of TRR]	Microwave extraction with propanol/H_2O	Non-extractable
		[mg equ/kg]	[% of TRR]	[% of TRR]	[% of TRR]
1 h after 1st appl.	Tops	3.384	96.9	n.a.	4.0
	Roots	0.097	102.4	n.a.	10.2
1 h after 2nd appl.	Tops	2.280	98.2	n.a.	5.3
	Roots	0.010	86.0	13.2	5.2
1 h after 3rd appl.	Tops	4.133	95.1	1.1	0.9
	Roots	0.051	81.0	11.2	7.9
21 d after 3rd appl.	Tops	1.517	87.9	8.6	3.7
	Roots	0.038	79.1	17.4	11.6
45 d after 3rd appl.	Tops	0.453	97.0	5.3	2.2
	Roots	0.021	99.2	6.8	10.6

n.a.: not analysed

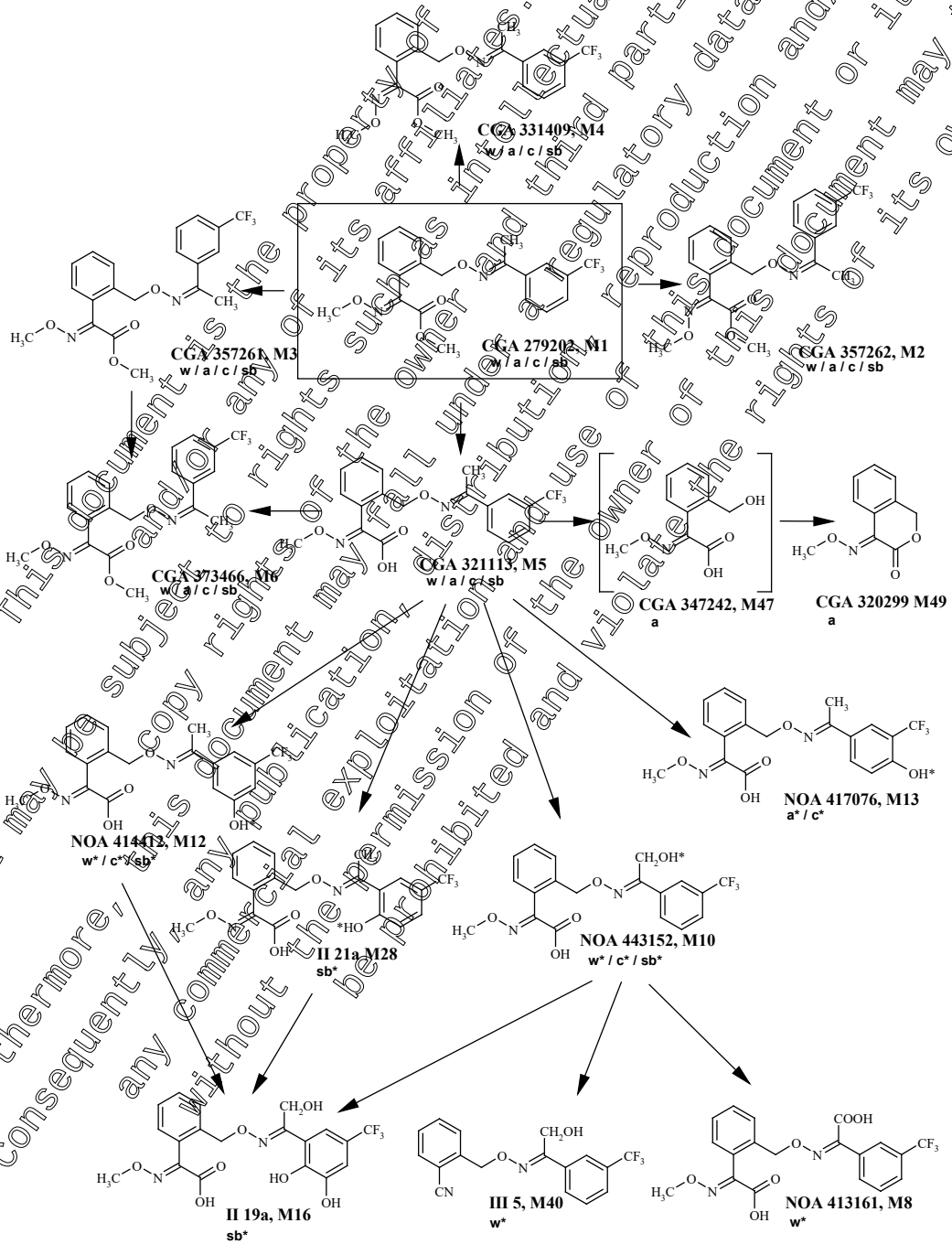
Table 6.2.1- 4: Metabolite fractions in the extracts of sugar beet tops and roots 21 and 45 days after the last of three foliar treatments with [14C-TP]trifloxystrobin at a total use rate of 395 g as/ha

Pre-harvest interval	21 Days				45 Days			
Plant part	Top		Root		Top		Root	
TRR [mg equ/kg]	1.517		0.038		0.453		0.021	
Residue composition	%TRR	ppm[1]	%TRR	ppm[1]	%TRR	ppm[1]	%TRR	ppm[1]
II_{0a}	1.6	0.024	0.7	< 0.001	2.4	0.011	0.6	< 0.001
II_{0b}	2.7	0.041	0.5	< 0.001	5.3	0.024	1.0	< 0.001
II_{1a}	n.d	n.d	0.7	< 0.001	2.2	0.010	0.6	< 0.001
II_{1b}	n.d	n.d	0.2	< 0.001	n.d.	n.d.	n.d.	n.d.
II_{2a}	2.3	0.035	0.9	< 0.001	3.6	0.016	1.5	< 0.001
$II3_a$	1.0	0.015	0.6	< 0.001	2.4	0.011	2.3	0.001
II_{3b}	n.d	n.d	n.d.	n.d.	n.d.	n.d.	0.9	0.001
II_{4a}	2.3	0.035	1.0	< 0.001	1.0	0.005	< 0.1	< 0.001
II_{5a}	0.7	0.011	1.9	< 0.001	3.2	0.014	1.3	< 0.001
II_{6a}	n.d	n.d	1.1	< 0.001	1.2	0.005	2.5	0.001
II_{7a}	n.d	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
II_{7b}	n.d	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
II_{7c}	n.d	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
II_{7d}	n.d	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
II_{7e}	n.d	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
II_{8b}	n.d	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
II_{9b} identified as M12-gly	3.0	0.046	0.7	< 0.001	4.8	0.022	1.0	< 0.001
II_{10} identified as M28-gly	0.5	0.008	0.7	< 0.001	1.9	0.009	0.6	< 0.001
II_{10c}	n.d	n.d	1.1	< 0.001	0.5	0.002	0.5	< 0.001
II_{11} identified as M10-gly	3.8	0.058	1.4	0.001	7.5	0.034	1.6	< 0.001
II_{15a}	n.d	n.d	0.4	< 0.001	n.d.	n.d.	n.d.	n.d.
II_{16a}	n.d	n.d	0.1	< 0.001	n.d.	n.d.	n.d.	n.d.
II_{17a}	0.5	0.008	0.8	< 0.001	0.4	0.002	1.0	< 0.001
II_{18a}	n.d	n.d	0.3	< 0.001	0.4	0.002	0.6	< 0.001
II_{19a} charact. as M16[2]	0.6	0.009	10.0	0.004	0.7	0.003	14.9	0.003
II_{21a} identified as M28	0.4	0.006	0.6	< 0.001	1.2	0.005	1.0	< 0.001
II_{22} = NOA414412, M12	0.9	0.014	1.1	< 0.001	3.8	0.017	2.3	0.001
II_{23a} = NOA443152, M10	0.4	0.006	0.7	< 0.001	n.d.	n.d.	1.2	< 0.001
II_{24} = CGA321113, M5	2.7	0.041	8.7	0.003	2.5	0.011	10.8	0.002
II_{24b} = CGA373466, M6	0.3	0.005	0.2	< 0.001	n.d.	n.d.	0.3	< 0.001
II_{24c}	n.d	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
II_{24d}	n.d	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unresolved	4.2	0.064	0.6	< 0.001	2.8	0.013	2.4	0.001
III_9, trifloxystrobin, M1	64.9	0.085	58.1	0.022	34.3	0.155	47.4	0.010
III_{10}, CGA357262, M2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
III_{12}, CGA331409, M4	1.2	0.018	n.d.	n.d.	1.2	0.005	3.8	0.001
E_2	8.6	0.131	7.4	0.001	5.3	0.024	4.4	<0.001
$W_3(E_2R_1)$	-	-	2.9	0.001	-	-	15.7	0.003
Non extractable	3.7	0.056	11.6	0.004	2.2	0.010	10.6	0.002
Total[3]	100.5	1.527	101.0	0.034	104.7	0.474	120.0	0.025

[1] ppm: mg parent equivalents/kg plant material
[2] the trihydroxylated metabolite fraction M16 consisted of several isomers with the same molar mass (investigated in the parallel study using the 14C-GP label)
[3] some inconsistencies in the summation as separation of the parent substance and its isomers are conducted by a separate analysis that was accompanied by some losses of radioactivity

n.d. not detected E_2 microwave-extracted (included in extracted) W_3 water phase of E_2

Figure 6.2.1- 4: Proposed common metabolic pathway of [14C-TP] and [14C-GP] trifloxystrobin in wheat w, apple a, cucumber c, and sugar beet sb



* also found as sugar conjugates

Report:	**KCA 6.2.1/12, [redacted], 2000** ; M-069125-01-1
Title:	Behavior and Metabolism of [Glyoxyl-Phenyl-(U)-^{14}C] CGA-279202 in Field Grown Sugar Beets
Document No:	M-069125-01-1
Report No:	99MK10; 1267-00
Guidelines and data requirements:	US-EPA OPPTS 860.1300, Nature of Residues – Plants (1996) EU Directive 91/414/EEC Agricultural Chemical Laws and Regulations, Japan, Metabolism Study (1985)
GLP	yes

Executive Summary

The metabolism of [14C-GP]trifloxystrobin was investigated in sugar beet following three spray applications at single use rates of approx. 130 g as/ha (1x application rate), totalling 400 g as/ha. The plants were cultivated outdoors. The first treatment was conducted at the growth stage BBCH 39 (leaves covered 90% of ground). Two subsequent treatments were performed at 3-week spray intervals. Sugar beets were sampled one day after each treatment and harvested 21 and 45 days after the last treatment. They were separated in tops (foliage) and roots (beets). An additional overdose experiment was conducted to aid in the characterization and identification of metabolites.

The total radioactive residues (TRR) in tops of the 1x application rate experiment amounted to 2.29 – 4.08 mg parent equivalents/kg matrix (mg equ/kg) 1 hour after each foliar treatment and decreased to 0.73 mg equ/kg 45 days after the last application. The TRR levels in the beets were generally low ranging from 0.025 to 0.113 mg equ/kg throughout beet sampling.

The predominant portion of TRR could be conventionally extracted with acetonitrile/water. From the tops approx. 95 – 100% of TRR were extracted and from the beets approx. 75 – 100% of TRR. In case of a lower extraction yield in roots, an additional portion up to 9% of TRR was extractable by use of microwave at elevated temperatures. In turn, the non-extractable residues were low, i.e. approx. 0.1 – 5% of TRR from tops and approx. 0.3 – 12% of TRR the beets. These non-extractable residues in the beets corresponded to 0.001 – 0.014 mg equ/kg at the two later harvest dates (21 and 45 days after the last application).

The metabolism of trifloxystrobin in sugar beet was very intensive as approx. 30 metabolites were detected in both tops and roots. Most of these metabolites appeared at very low concentrations (<<10% of TRR). Two metabolites revealed to be major in the roots. The carboxylic acid CGA321113 (M5, EE-Isomer) accounted for 7.5 – 11% of TRR and its triply hydroxylated derivative (M16, one hydroxyl group attached to methyl group of the bridge between the phenyl rings and two hydroxyl groups attached to the trifluoromethyl phenyl ring) accounted for approx. 9% of TRR. Additional minor metabolites were identified as CGA373466 (M6, ZE carboxylic acid), NOA443152 (M10, hydroxylated ZE carboxylic acid) and NOA414412 (M12, phenyl hydroxylated EE carboxylic acid) and their glycoside conjugates, respectively. These metabolites were also identified as glucose conjugates. The metabolic pattern observed in the 1x application experiment was confirmed by the overdose experiment.

Following conventional extraction of the roots further water-soluble residues could be released by extraction with boiling water. These residues proved to be incorporated into saccharose, cellulose, lignin and pectin.

Some of the plant metabolites of trifloxystrobin were also observed in the soil. The carboxylic acid CGA311113 (M5) revealed to be the predominant residue component in soil. The parent substance was very low at the two later sampling dates.

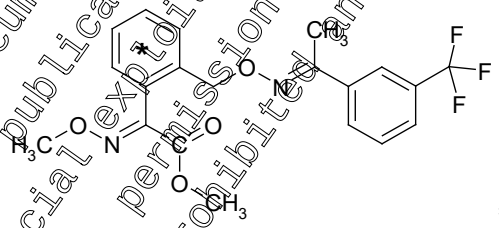
The proposed common metabolic pathway of [glyoxyl-phenyl-UL-14C]trifloxystrobin in plants (wheat w, cucumber c, and sugar beet sb) is shown in Figure 6.2.1- 4.

Based on the structures identified, the metabolism of trifloxystrobin in sugar beets appears to proceed via different transformations:

- Cis/trans isomerization of trifloxystrobin and/or hydrolysis of the methyl ester,
- Methyl ester cleavage to the major metabolite II_{24} (= M5, CGA321113) and cis/trans isomerization of M5 to metabolite II_{24b} (= M6, CGA373466),
- Hydroxylation of the trifluoromethyl-phenyl ring of M5 resulting in metabolite II_{22-1} (= M12, NOA414412) and its stereoisomer II_{21a} with subsequent glycoside conjugation to form metabolites II_{10} or II_{9b},
- Hydroxylation of the methyl substituent of M5 at the 2-ethylideneaminooxymethyl group to metabolite II_{23a} (= M10, NOA443152) with subsequent glycoside conjugation to form metabolite II_{11}, and threefold hydroxylation of phenyl and methyl group to form the major metabolite fraction II_{19a} (M16) that consisted of several isomers with each isomer accounting for < 0.01 mg equ/kg.
- Formation of bound residues to a low extent via incorporation into saccharose, cellulose, lignin and pectin.
- No cleavage of the ethylideneaminooxymethyl bridge between the phenyl rings and no formation of label-specific metabolites was found. Therefore, no significant differences were observed of the metabolites' pattern in the sugar beet metabolism studies performed with [14C-GP] and [14C-TP]trifloxystrobin.
- The parent substance revealed to be the predominant residue component in sugar beet tops and roots and, therefore, is proposed as marker substance in residue analysis.

Material and Methods

Test Material

Structural formula	 * denotes the ^{14}C label
Chemical name	(*E,E*)-methoxyimino-{2-[1-(3-trifluoromethyl-phenyl)-ethylideneamino-oxymethyl]-phenyl}acetic acid methyl ester (IUPAC); (*E,E*)-α-(methoxyimino)-2-[[[[1-[3-(trifluoromethyl)phenyl]ethylidene]amino]oxy]methyl]benzene acetic acid methyl ester (CAS)
Common name	Trifloxystrobin
CAS RN	141517-21-7
Empirical formula	$C_{20}H_{19}F_3N_2O_4$
Company code	CGA 279202

Molar mass (non-labelled)	408.4 g/mole
Label	[glyoxyl-phenyl-UL-^{14}C]Trifloxystrobin, abbr. [14C-GP]
Specific radioactivity	0.945 MBq/mg = 25.541 mCi/g
Radiochemical purity	95.5 – 98.2% (radio-HPLC, radio-HPLC)
Identification	LC-MS/MS with ESI ionization

Test Plants

Test plant	Sugar beet
Variety	Kassandra
Study design	Outdoor study at a plot in Switzerland
Growth stage at application	Three spray applications beginning at growth stage BBCH 39 (crop cover complete, foliage cover: 90% of ground) spray interval: 3 weeks. Second and third application at growth stage BBCH 39 - 49.
Harvested commodities	Complete sugar beet plants one hour after each treatment and 21 days and 45 days after the last application. The plants were divided in tops (foliage) and roots (beets).

Sowing and cultivation of sugar beet, preparation and application of the test substance

Sugar beet seeds were sown into loam soil (49.6% sand, 30.2% silt, 20.2% clay, 1.45% organic carbon, pH 7.8 [$CaCl_2$]) of three outdoor plots located at a research station [redacted] Switzerland. The plot for the main experiment (1x application rate) had a size of 4 m^2. Two additional plots were sown for an overdose and a control experiment. The sugar beet plants were cultivated under usual agricultural conditions in the summer 1999. No additional pesticide treatment was performed throughout the study, apart from application of the test substance.

[14C-GP]trifloxystrobin was mixed with a blank EC125 formulation. The resulting formulation was suspended in water to prepare the spray mixture. The concentration of the test substance in the formulation amounted to approx. 10.2%. Three months after sowing the first of three foliar spray treatments were performed. At the first treatment, the sugar beets reached the growth stage BBCH 39 and their leaves covered approx. 90% of the ground. Two subsequent treatments were conducted after spray intervals of 3 weeks. A small plot sprayer with four TEEJET flat-jet nozzles was used for each spray treatment. The use rates were 141 g as/ha (1st application), 132 g as/ha (2nd application) and 127 g as/ha (3rd application) resulting in a total use rate of 400 g as/ha (1x application rate). The homogeneity of the spray mixture was confirmed by repeated radioassaying before and after each spray event.

In an extra overdose experiment for generation of a higher amount of radiolabelled metabolites additional plants were sprayed at the same days as the 1x use experiment with a higher-concentrated spray mixture. The use rates of this trial were 830 g as/ha (1st application), 691 g as/ha (2nd application) and 683 g as/ha (3rd application).

Sampling and processing of sugar beet plants

Few sugar beet plants were collected one hour after each application as well as 21 and 45 days after the last application. The plants were divided into tops (leaves) and roots (beets). At each plant sampling, soil core samples (0 – 30 cm depth) were additionally taken. The soil cores were separated into three horizons: 0 - 10, 10 – 20 and 20 - 30 cm.

The plants samples (tops and roots) were chopped with plant copper in presence of liquid nitrogen. Fine homogenization was performed using a kitchen mixer. Aliquots of the homogenates were taken for radioassaying. The remaining pulverized samples were stored in frozen condition (at approx. -18°C).

The homogenized plant samples were extracted 6-times with acetonitrile/water (4/1, v/v) for at least 6 hours using a mechanical shaker. The combined extract (extract E_1) was analysed by radio-TLC. Some extracts from foliage (TOP extracts) and roots were concentrated and the resulting aqueous phase partitioned against n-hexane and dichloromethane. The remaining water phase was acidified with HCl and partitioned against ethyl acetate. All phases were analysed by radio-TLC and radio-HPLC. Incompletely extracted samples were subsequently extracted with 1-propanol/water (4/1, v/v) at enhanced temperatures (100 – 150°C) using a microwave oven and the resulting extract (extract E_2) analysed by radio-TLC.

Water-soluble radioactive fractions from the roots were boiled with 0.5N HCl to hydrolyse the diglycoside saccharose into glucose and fructose. Following extraction of unpolar reaction products with dichloromethane the glycosides in the remaining water phase were transferred into precipitable osazones by reaction with phenylhydrazine under acid conditions. The precipitated osazones were filtered off, washed with water, recrystallized and radioassayed via combustion.

Clean-up of microwave-supported root extracts (E_2) was carried out by solid-phase extraction (SPE) using cartridges filled with unpolar material. The cartridges were washed with acetonitrile and 0.01N HCl before use. The 1-propanol/water extracts were first concentrated to remove the organic solvent and then were applied to the cartridges. The loaded cartridges were rinsed with water followed by elution with acetonitrile/0.01N HCl. The eluted residues (solution O_3E_2) were analysed by radio-TLC using the solvent mixtures 2.1 and 2.2 (see below).

Enzyme cleavage of aliquots of foliage extracts (following evaporation of acetonitrile) was performed with ß-glucosidase (37°C, shaking overnight) following evaporation to dryness and re-dissolution in 0.1N acetate buffer at pH 4.65. The resulting mixture was extracted with ethyl acetate and analysed by radio-TLC and radio-HPLC.

A non-extractable fraction of root residues could be released by boiling with water (16 hours) and hot filtration. The filtrate containing water-soluble non-saccharides was hydrolysed with 10% aqueous NaOH (120°C), filtered while hot and the characterized as cellulose containing fraction (filtration residue) and lignin (precipitated from the filtrate after acidification with HCl).

Soil core samples of the same sampling day and horizon were combined, air-dried, homogenized in a disk mill and radioassayed. They were then extracted with acetonitrile/water (4/1, v/v) in the same was manner as done with plant samples. The combined extracts were also analysed by radio-TLC.

<u>Radioassaying and analysis</u>

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). Quenching was automatically compensated using an external standard. Solid samples were firstly combusted and the formed $^{14}CO_2$ absorbed in an alkaline scintillation liquid. The total radioactive residues (TRR) of a sample were established by the sum of extracted radioactivity and the radioactivity in the extracted sample. For quantification of the radioactivity of the spots in radio-TLC the silica gel with the spots were scrapped out, suspended in methanol and radioassayed after addition of scintillation cocktail. The LOQ for radioassaying was set to 0.017 – 0.031 mg equ/kg for foliage (tops), to 0.003 – 0.006 mg equ/kg for beets (roots) and to 0.001 mg equ/kg for soil.

One and two-dimensional radio-TLC was conducted on silica gel plates (Si60 F_{254}). For two-dimensional TLC used for separation of metabolites the plates they were developed in two rectangular directions with the two solvent mixtures: (1.1) n-hexane/diethyl ether/tetrahydrofuran/formic acid/water (10/70/10/1/2, v/v/v/v/v) and (1.2) chloroform/ethyl acetate/formic acid (60/30/10, v/v/v) as well as (2.1) chloroform/methanol/formic acid/water (75/20/4/2, v/v/v/v) and (2.2) 1-butanol/acetic acid/water (40/10/10, v/v/v). One-dimensional TLC plates used for separation of the parent isomers was developed with toluene/ethyl acetate (9/1, v/v). Radioactive spots were detected by a Bio-Imaging Analyzer. Co-chromatographed non-labelled reference standards were visualized by fluorescence extinction following excitation by UV-light. The LOQ for radio-TLC was set to 0.001 mg equ/kg sample.

Radio-HPLC was conducted on a RP18 column (250 x 4.6 mm, 5 µm particle size) operated with a gradient mixture of aqueous phosphoric acid (0.1%) and acetonitrile. The HPLC system was equipped with a UV detector (220, 254 nm) and a radiomonitor with a glass scintillator. Additional columns were used for isolation and purification of metabolites.

LC-MS/MS was performed for identification of parent substance and metabolites. Radio-HPLC separation was conducted on a reversed phase (C18) column (150 x 2.0 mm, particle size 5 µm), a water/acetonitrile gradient (both acidified with 0.5% formic acid) as liquid phase and a radiomonitor with a solid scintillator. Atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) were used for ionization. Authentic reference standard aided for identification.

Findings

Total radioactive residues and their extractability

The total radioactive residues (TRR) in tops (foliage) and beets, the extractable and non-extractable residues are presented in Table 6.2.1- 5. TRR in tops of the 1x application rate experiment amounted to 2.29 – 4.08 mg parent equivalents/kg matrix (mg equ/kg) 1 hour after each foliar treatment and decreased to 0.73 mg equ/kg 45 days after the last application. The TRR levels in the beets were generally low ranging from 0.025 to 0.113 mg equ/kg throughout beet sampling.

The predominant portion of TRR could be conventionally extracted with acetonitrile/water. From the tops (foliage), approx. 95 – 100% of TRR and from the beets approx. 75 – 100% of TRR were extracted. In case of a lower extraction yield in roots, an additional portion up to 9% of TRR was extractable by use of microwave. In turn, the non-extractable residues were low, i.e. approx. 0.1 – 5% of TRR from tops and approx. 0.3 – 12% of TRR the beets. These non-extractable residues in the beets corresponded to 0.001 – 0.014 mg equ/kg at the two later harvest dates (21 and 45 days after the last application).

Residues in sugar beet tops and roots

Sugar beet roots and tops were initially extracted with acetonitrile/water at ambient temperature and subsequently with 1-propanol/water at enhanced temperature using microwave support. The extracts were analysed by two-dimensional radio-TLC and radio-HPLC. The microwave extracts were cleaned-up by solid phase extraction prior to chromatography. Reference standards aided for identification via co-chromatography. The resulting composition of the radioactive residues in sugar beet tops and roots of the last two sampling dates, 21 and 45 days after three foliar treatments with [14C-GP]trifloxystrobin is presented in Table 6.2.1- 6.

The metabolism of trifloxystrobin in sugar beet was very intensive as approx. 30 metabolites were detected in both tops and roots. Most of these metabolites appeared at very low concentrations

(<<10% of TRR). Two metabolites revealed to be major in the roots. The carboxylic acid CGA321113 (M5, EE-Isomer) accounted for 7.5 – 11 % of TRR and its triply hydroxylated derivative (metabolite fraction M16, one hydroxyl group attached to methyl group of the bridge between the phenyl rings and two hydroxyl groups attached to the trifluoromethyl phenyl ring) accounted for approx. 9% of TRR. Further radio-RP-HPLC analyses revealed that M16 consisted of several isomers with the same molar mass and each individual isomer accounting for < 0.01 mg equ/kg. Additional minor metabolites were identified as CGA373466 (M6, ZE carboxylic acid), NOA443152 (M10, hydroxylated ZE carboxylic acid) and NOA414412 (M12, phenyl hydroxylated EE carboxylic acid) and their glycoside conjugates, respectively. These metabolites were also identified as glucose conjugates. The metabolic pattern observed in the 1x application experiment was confirmed by the overdose experiment.

The water-soluble radioactivity of sugar beet roots was analysed for the content to radioactivity incorporated into saccharose. Approximately 6% of TRR were found to be incorporated in saccharose 21 days after the last application of [14C-GP]trifloxystrobin. Analysis of the non-extractable residues in roots showed that additional portions of 0.4% of TRR were incorporated into cellulose, 2.4% of TRR into lignin and 1.5% of TRR into pectin.

However, the parent substance trifloxystrobin (EE-isomer, M1) accounted generally for the predominant residue components at harvest (21 - 45 days after last application) amounting to 21 - 43% of TRR in tops and to approx. 23% of TRR in roots. Its isomers CGA 357262 (ZZ-isomer, M2) and CGA 331409 (EZ-isomer, M4) were detected at very low levels <0.1 – 0.8% of TRR (M2) and 0.9 – 3.2% of TRR (M4). The ZE-isomer (CGA 357161 (M3) was not observed in any commodity.

<u>Residues in soil</u>

In the top soil layer (0 – 10 cm) TRR accounted for 0.005 – 0.262 mg equ/kg one day after each application and for 0.098 and 0.127 mg equ/kg 21 and 45 days after the last application of the 1x application trial.

Extraction and radio-TLC of the soil extracts revealed low amounts of the parent substance at the two later sampling dates (21 and 45 days after last application to the plants). The predominant residue component in soil (top layer) was formed by ester hydrolysis of the parent substance, i.e. the carboxylic acid M5 (CGA 321113) amounting to 57 - 65% of TRR in the soil samples. Its isomeric carboxylic acid M6 (CGA373466) accounted for approx. 2 - 3% of TRR and the hydroxylated carboxylic acids M10 (NOA443152) and M12 (NOA414412) amounted to less than 1% of TRR. The same metabolites were also detected in sugar beet roots and leaves.

<u>Storage stability</u>

All samples were stored at approx. – 18°C. Extracts were stored at 8°C, at maximum. Leaves and roots sampled 21 days after the last application were extracted and chromatographically profiled approx. two months after harvest. The extracts were re-analysed after approx. 8 - 9 months storage at maximum 8°C. Further aliquots of tops and roots of the same frozen samples were re-extracted approx. 4 – 6 months after the initial extraction and analysed again. Comparing the metabolite pattern, no significant change could be observed regarding the qualitative and quantitative distribution of radioactive peaks.

Conclusion

[14C-GP]trifloxystrobin metabolized very intensively in sugar beets as approximately 30 metabolites were detected in tops (foliage) and roots (beets) 21 and 45 days after the last of three foliar treatment at a total application rate of 400 g as/ha. Most of these metabolites appeared at a very low level (< 1% of TRR in the roots and < 5% of TRR in the leaves at harvest). Based on the structures identified, the metabolism of trifloxystrobin in sugar beets appears to proceed via different transformations:

- Cis/trans isomerization of trifloxystrobin and/or hydrolysis of the methyl ester,
- Methyl ester cleavage to the major metabolite II_{24} (= M5, CGA321113) and cis/trans isomerization of M5 to metabolite II_{24b} (= M6, CGA373466),
- Hydroxylation of the trifluoromethyl-phenyl ring of M5 resulting in metabolite II_{22} (= M12, NOA414412) and its stereoisomer II_{21a} with subsequent glycoside conjugation to form metabolites II_{10} or II_{9b},
- Hydroxylation of the methyl substituent of M5 at the 2-ethylideneaminooxymethyl group to metabolite II_{23a} (= M10, NOA443152) with subsequent glycoside conjugation to form metabolite II_{11}, and threefold hydroxylation of phenyl and methyl group to form the major metabolite fraction II_{19a} (M16) that consisted of several isomers with each isomer accounting for < 0.01 mg equ/kg.
- Formation of bound residues to a low extent via incorporation into saccharose, cellulose, lignin and pectin.
- No cleavage of the ethylideneaminooxymethyl bridge between the phenyl rings and no formation of label-specific metabolites was found. Therefore, no significant differences were observed of the metabolites' pattern in the sugar beet metabolism studies performed with [14C-GP] and [14C-TP]trifloxystrobin.
- The parent substance revealed to be the predominant residue component in sugar beet tops and roots and, therefore, is proposed as marker substance in residue analysis.

The proposed common metabolic pathway of [14C-GP]trifloxystrobin in plants (wheat w, cucumber c, and sugar beet sb) is shown Figure 6.2.1- 4.

Table 6.2.1- 5: Total radioactive residues (TRR) in sugar beet and extractability of the residues following three foliar treatments with [14C-GP]trifloxystrobin at a total use rate of 400 g as/ha

Sampling	Crop Part	TRR	Extraction with ACN/H_2O [% of TRR]	Microwave extraction with 1-propanol/H_2O	Non-extractable
		[mg equ/kg]	[% of TRR]	[% of TRR]	[% of TRR]
1 h after 1st appl.	Tops	3.204	96.5	0.1	0.1
	Roots	0.055	106.7	0.7	0.3
1 h after 2nd appl.	Tops	2.286	95.2	n.a.	1.1
	Roots	0.033	88.2	5.2	3.0
1 h after 3rd appl.	Tops	4.077	99.8	0.3	0.5
	Roots	0.063	87.0	6.3	6.9
21 d after 3rd appl.	Tops	1.396	98.9	n.a.	3.9
	Roots	0.113	75.6	7.5	12.2
45 d after 3rd appl.	Tops	0.727	96.9	n.a.	4.7
	Roots	0.025	84.5	9.2	3.5

n.a.: not analysed

Table 6.2.1- 6: Metabolite fractions in the extracts of sugar beet tops and roots 21 and 45 days after the last of three foliar treatments with [14C-GP]trifloxystrobin at a total use rate of 400 g as/ha

Pre-harvest interval	21 Days				45 Days			
Plant part	Top		Root		Top		Root	
TRR [mg equ/kg]	1.369		0.113		0.727		0.025	
Residue composition	%TRR	ppm[1]	%TRR	ppm[1]	%TRR	ppm[1]	%TRR	ppm[1]
II_{0a}	2.1	0.029	2.2	0.002	2.6	0.019	0.2	< 0.001
II_{0b}	2.1	0.029	1.2	0.001	1.6	0.012	0.6	< 0.001
II_{1a}	1.2	0.017	2.4	0.002	2.6	0.019	0.5	< 0.001
II_{1b}	n.d.	n.d.	1.0	0.001	0.2	0.001	0.5	< 0.001
II_{2a}	3.2	0.045	1.5	0.002	3.7	0.027	1.5	< 0.001
$II3_a$	1.1	0.015	0.6	0.001	0.8	0.006	1.1	< 0.001
II_{3b}	n.d.	n.d.	n.d.	n.d.	0.5	0.004	0.5	0.001
II_{4a}	0.7	0.010	1.0	0.001	5.3	0.039	0.9	< 0.001
II_{5a}	1.8	0.025	1.7	0.002	2.1	0.015	1.2	< 0.001
II_{6a}	0.5	0.007	0.6	0.001	0.5	0.004	0.5	< 0.001
II_{7a}	n.d.	n.d.	1.7	0.002	2.3	0.017	0.7	< 0.001
II_{7b}	n.d.	n.d.	1.0	0.001	1.4	0.010	0.3	< 0.001
II_{7c}	n.d.	n.d.	1.1	0.001	1.5	0.011	0.3	< 0.001
II_{7d}	n.d.	n.d.	0.3	< 0.001	1.5	0.011	n.d.	n.d.
II_{7e}	n.d.	n.d.	0.6	0.001	2.2	0.016	0.4	< 0.001
II_{8b}	0.7	0.010	2.5	0.003	1.6	0.012	1.1	< 0.001
II_{9b} identified as M12-gly	3.4	0.047	1.7	0.002	6.2	0.045	0.7	< 0.001
II_{10} identified as M28-gly	1.5	0.021	0.5	0.001	1.8	0.013	0.3	< 0.001
II_{10c}	n.d.	n.d.	0.9	0.001	1.6	0.012	0.8	< 0.001
II_{11} identified as M10-gly	5.0	0.070	0.9	0.001	8.2	0.060	0.6	< 0.001
II_{15a}	1.0	0.014	0.6	0.001	0.7	0.005	0.9	< 0.001
II_{16a}	0.6	0.008	0.6	0.001	0.5	0.004	0.6	< 0.001
II_{17a}	0.7	0.010	0.9	0.001	1.2	0.009	1.5	< 0.001
II_{18a}	0.6	0.008	0.6	0.001	0.2	0.001	0.9	< 0.001
II_{19a} charact. as M16 [2]	1.4	0.020	9.0	0.010	1.6	0.012	9.2	0.002
II_{21a} identified as M28	0.6	0.008	0.8	0.001	0.9	0.007	1.1	< 0.001
II_{22} = NOA414412, M12	1.7	0.024	1.6	0.002	1.7	0.012	1.3	< 0.001
II_{23a} = NOA443152, M10	0.4	0.006	0.8	0.001	0.4	0.003	1.0	< 0.001
II_{24} = CA32G1113, M5	5.2	0.073	10.8	0.012	2.8	0.020	7.5	0.002
II_{24b} = CGA373466, M6	1.1	0.015	1.1	0.001	0.9	0.007	0.4	< 0.001
II_{24c}	n.d.	n.d.	0.2	< 0.001	n.d.	n.d.	n.d.	n.d.
II_{24d}	n.d.	n.d.	0.3	< 0.001	n.d.	n.d.	0.4	< 0.001
Unresolved	5.6	0.078	7.3	0.008	10.7	0.078	6.3	0.002
III_9, Trifloxystrobin, M1	43.2	0.603	22.9	0.026	21.3	0.155	23.3	0.006
III_{10}, CGA357262, M2	0.8	0.011	0.5	0.001	< 0.1	<0.001	n.d	n.d.
III_{12}, CGA331409, M4	1.3	0.018	1.9	0.002	0.9	0.007	3.2	0.001
E_2	-	-	5.5	0.008	-	-	9.2	0.002
$W_3(E_2R_1)$	-	-	1.7	-	-	-	3.6	0.001
Non extractable	3.9	0.054	12.2	0.014	4.7	0.034	3.5	0.001
Total[3]	103.1	1.439	95.8	0.109	102.0	0.745	108.3	0.028

[1] ppm: mg parent equivalents/kg plant material

[2] the trihydroxylated metabolite fraction M16 consisted of several isomers with the same molar mass

[3] some inconsistencies in the summation as separation of the parent substance and its isomers are conducted by a separate analysis that was accompanied by some losses of radioactivity

n.d. not detected E_2 microwave-extracted (included in extracted) W_3 water phase of E_3

Peanuts

In order to show common metabolism of trifloxystrobin in different crop categories an additional metabolism study on peanuts is reported by the following summary. This study was originally intended for submission of this use to the US EPA. It is reported in two reports describing the biological and the analytical part.

Report:	**KCA 6.2.1/13, [redacted], 1997**; M-137152-01-1
Title:	Uptake and metabolism of CGA-279202 in field grown peanuts after spray treatment with phenyl(A)-14C- CGA-279202 and phenyl(B)- CGA-279202
Document No:	M-137152-01-1
Report No:	ABR-97084
Guidelines and data requirements:	US-EPA: 40 CFR 158.240 Pesticide Assessment Guidelines, Subdivision O, Residue Chemistry Series 171-4(a), Nature of the Residues in Plants
GLP	yes

Report:	**KCA 6.2.1/14, [redacted], 1997**; M-038413-01-1
Title:	Biological phase report uptake and metabolism of CGA-279202 in field grown peanuts after spray treatment with phenyl(A)-14C- CGA-279202 and phenyl(B)-CGA-279202
Document No:	M-038413-01-1
Report No:	BIOL-96024
Guidelines and data requirements:	US-EPA: 40 CFR 158.240 Pesticide Assessment Guidelines, Subdivision O, Residue Chemistry Series 171-4(a), Nature of the Residues in Plants
GLP	yes

Executive Summary

The metabolism of [glyoxyl-phenyl-UL-^{14}C] and [trifluoromethyl-phenyl-UL-^{14}C]trifloxystrobin ([14C-GP] and [14C-TP]trifloxystrobin) was investigated in peanuts following four spray applications at single use rates of approx. 0.5 lb ai/A (0.56 kg as/ha), totalling 2.02 kg as/ha for the [14C-GP] and 2.13 kg as/ha for the [14C-TP] label. The plants were cultivated outdoors. They reached the following growth stages at the respective treatments: bloom, bloom/pegging, nut formation, and nut maturity. Plant samples were taken directly after the first treatment, 14 days after the first treatment (immature vines) and 14 days after the last treatment (mature hay and shelled nuts/nutmeat).

The total radioactive residues (TRR) amounted to 20.7 and 24.9 mg equ/kg in the vines directly after the first application based on the [14C-GP] and the [14C-TP] label. TRR values decreased to 7.7 and 9.1 mg equ/kg in vines during the following 14 days and increased again in mature hay to 26.3 and

27.9 mg equ/kg for the two labels 14 days after the last application. Mature nutmeat showed significantly lower residue levels, amounting to 0.305 and 0.184 mg equ/kg.

The extractability with acetonitrile/water was high in vines and hay amounting to 86 – 91% of TRR in vines and 68 – 74% of TRR in hay. From nutmeat, only 21 – 27% of TRR was extractable with acetonitrile/water. Partitioning against dichloromethane resulted in organosoluble residues amounting to 56.5 – 66.9% of TRR in immature vines, 42.4 – 55.4% of TRR in mature hay, but to less than 10% of TRR in nutmeat. However, a significant portion could be extracted from nutmeat in a prior extraction with hexane accounting to 25.6 – 30.9% of TRR based on the two radiolabels.

The radio-HPLC and radio-TLC profiles of the organosoluble metabolites extracted from immature vines and mature hay were similar in both radiolabels. The major metabolites for both labels were identified as the parent substance trifloxystrobin (CGA-279202) and its isomers CGA-357261 (M3), CGA-357262 (M2) and CGA-331409 (M4), as well as the hydrolysed carboxylic acids CGA-321113 (M5) and its isomer CGA-373466 (M6). Parent substance and the carboxylic acid isomers CGA-321113 (M5) and CGA-373466 (M6) were also identified in the dichloromethane fraction of nutmeat. The hexane fraction from mature nutmeat was shown to be composed of the parent substance, its hydrolysed carboxylic acid and the isomers as well as of radiolabeled triglycerides.

The parent substance trifloxystrobin (EE isomer) proved to be the predominant residue component in peanut hay using both radiolabels. In nutmeat, the sum of parent isomers was too low (0.009 – 0.011 mg/kg) to achieve a separation into the individual isomers.

However, the respective radio-profiles of the aqueous soluble (polar) metabolites in vines and hay resulting from the two labels were different, indicating the cleavage of the N-O bridge between the phenyl rings and the formation of label-specific metabolites. All metabolites in mature hay and nutmeat exceeding 1% of TRR could be identified.

The metabolism study on ^{14}C-trifloxystrobin in field grown peanuts revealed the following transformations resulting in at least 12 – 13 metabolites:

- Cis/trans isomerization of parent substance trifloxystrobin (CGA-279202) to form CGA-357261 (M3), CGA-357262 (M2) and CGA-331409 (M4)

- Methylester cleavage to the carboxylic acids CGA-321113 (M5) and its cis/trans isomer CGA-373466 (M6). The subsequent conjugation with malonyl glucose leads to different malonyl glucoside metabolites (A-9a11, A-9b1, A-9c1, and A9c2).

- Hydroxylation of the aminooxymethyl group on the bridge between the phenyl rings, or hydroxylation of the CF_3 bearing phenyl ring at the meta position, followed by glucose conjugation, leads to metabolites NOA-443152 (M10)- and NOA-414412 (M12)-glucosides (A-7a, A-7a2, A-7c); upon cleavage of the N-O bridge and subsequent glucose conjugation the metabolites CGA-107170 (M62)- and BO-17372 (M64)-glucosides (A-7a, A-7a2, A-7b) are formed.

- The cleavage of N-O bridge in trifloxystrobin or its carboxylic acid CGA-321113 (M5), followed by oxidation and/or conjugation to form metabolites CGA-328365 (M61, A-4b), CGA 300624 (M59, A-5b); CGA-347242 (M47, F2b2-3a&b (A1))-glucoside, methyl ester of CGA-373463 (M46, F2b2-3a&b (A2)); CGA-367619 (M53, phthalic acid, A2b2 (top)); WFX-IX-86 (M51, peak 3,4-1 or 8-1);

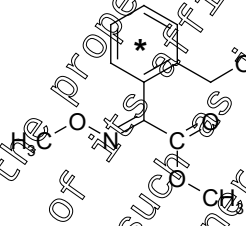
- Complete oxidation of the CF_3 bearing phenyl ring resulted in low formation of trifluoroacetate.

- The parent substance trifloxystrobin (EE-isomer) proved to be the main residue component in immature vines and mature hay of peanuts and, therefore, can be used as analytical target in the residue analysis. In nutmeat, no single residue component exceeded 10% of TRR, also applying to the parent substance. The major portion of radioactivity represented ^{14}C-triglycerides obviously formed by mineralization of the ^{14}C-trifloxystrobin in soil and uptake of the formed $^{14}CO_2$ via photosynthesis.

The proposed metabolic pathway of trifloxystrobin in peanuts is presented in Figure 6.2.1- 5.

Material and Methods

Test Material

Structural formula	 * denotes ^{14}C label A (14C-GP) # denotes ^{14}C label B (14C-TP)
Chemical name	(*E,E*)-methoxyimino-[2-[1-(3-trifluoromethyl-phenyl)-ethylideneamino-oxymethyl]-phenyl]acetic acid methyl ester (IUPAC); (*E,E*)-α-(methoxyimino)-2-[[[1-[3-(trifluoromethyl)phenyl]ethylidene] amino]oxy]methyl]benzene acetic acid methyl ester (CAS)
Common name	Trifloxystrobin
CAS RN	141517-21-7
Empirical formula	$C_{20}H_{19}F_3N_2O_4$
Company code	CGA 279202
Molar mass (non-labelled)	408.4 g/mole
Label	[glyoxyl-phenyl-UL-^{14}C]Trifloxystrobin, abbr. [14C-GP] Label A in the original report [trifluoromethyl-phenyl-UL-14C]Trifloxystrobin, abbr. [14C-TP] Label B in the original report
Specific radioactivity	[14C-GP], label A: 23.8 mCi/g (880.6 MBq/g) [14C-TP], label B: 26.2 mCi/g (964.4 MBq/g)
Radiochemical purity	[14C-GP], label A: 97.9% (> 94.7% in the formulation) [14C-TP], label B: 98.5% (>93.8% in the formulation)
Chemical purity	[14C-GP], label A: 99.8% [14C-TP], label B: 99.6%

Test Plants

Test plant	Peanuts
Variety	Florunner
Cultivator	Montgomery Seed and Supply Comp. Inc., Montgomery, Alabama, USA
Study design	Outdoor study at two plots in Greenville, Mississippi, USA
Growth stage at application	Four spray applications at the following growth stages 1. Bloom, 57 days after peanut planting (height: 25 - 30 cm) 2. Bloom/Pegging, 85 days after planting (height: 30 – 45 cm) 3. Nut formation, 120 days after planting (height: 45 – 60 cm) 4. Nut maturity, 148 days after planting (height: 45 - 60 cm)
Harvested commodities	Complete peanut plants immediately after the 1st treatment and 14 days after the 2nd treatment. Final harvest at maturity, i.e. 162 days after planting and 14 days after the last treatment: mature plants, separated into hay and peanuts (not shelled). All samples were transferred from the field site to the analytical laboratory where the peanuts were shelled to get the nutmeat.

<u>Sowing and cultivation of sugar beet, preparation and application of the test substance</u>

Peanuts were sown into silt loam soil (27.0% sand, 56.0% silt, 17.0% clay, 1.3% organic carbon, pH 7.3 [H_2O]) of two outdoor plots located at the [redacted], USA. The plots, one for the [14C-GP] and one for the [14C-TP] label, had a size of 6 ft x 12 ft (1.8 m x 3.6 m). The peanuts were planted in three rows to each plot and cultivated under usual agricultural conditions during the summer 1995.

Batches of [14C-GP; label A] and [14C-TP; label B] trifloxystrobin were mixed separately with a EC 250 blank formulation. The final EC 250 formulations consisted of 25% active substance and 75% inert ingredients. These formulations were transferred into water to prepare the spray mixtures and stirred for homogenization. The homogeneity of the spray mixture and the stability of the test substance were examined by radioassaying and radio-TLC analysis of several small aliquots.

Four spray treatments were conducted using a hand sprayer with four nozzles that were operated with compressed CO_2. At spraying, the peanut plants had the following growth stages (1) bloom; (2) bloom/pegging, (3) nut formation, and (4) nut maturity. The nominal application rate per treatment was 0.5 lb ai/A (0.56 kg as/ha). The actual total use rate amounted to 1.8 lb ai/A (2.02 kg as/ha) for the [14C-GP; A] label and 1.9 lb ai/A (2.13 kg as/ha) for the [14C-TP; B] label. The rate of the spray mixture was approx. 300 - 350 L/ha.

<u>Sampling and processing of the peanuts and soil samples</u>

Whole immature peanuts plants (vines) were sampled 0 days after the first and 14 days after the 2nd application. At the final harvest, 162 days after planting and 14 days after the last application, mature pods (shelled peanuts) were dug and separated from hay. All samples were stored in plastic bags in a freezer at approx. -20°C until transfer deep-frozen to the analytical laboratories of Novartis Vero Beach, Florida, and Novartis Greensboro, North Carolina, USA.

The peanut pods were shelled manually. Vines, hay and shelled peanuts (nutmeat) were homogenized separately with dry ice in mills fitted with a 2 mm diameter screen. After homogenization, the dry ice

was allowed to sublime in a freezer. Aliquots of the resulting plant and soil samples were radioassayed by combustion and liquid scintillation counting.

In addition, soil cores (depth: 0 – 3 inch; 0 – 7.6 cm, 2 cm diameter) were sampled before application, 4 hours after the first application of the test substance and at harvest of mature peanuts. The soil samples were stored in a freezer until shipment to the analytical laboratory.

<u>Extraction of peanut plant, clean-up, hydrolysis and derivatisation of metabolites</u>

The homogenized vines and hay samples were extracted with acetonitrile/water (4/1, v/v, 2x) using a high-speed stirrer. The extracts were radioassayed and cleaned-up by flash chromatography (solid phase extraction) using a flash C18 column. The non-extractable residues were radioassayed and stored frozen until further investigation of the non-extractable residues by hydrolysis steps.

Clean-up of the primary plant extracts from vines and hay was performed using C18 flash chromatography (solid phase extraction). Following application of the crude extract to the top of the C18 column the polar by-products (e.g. chlorophyll) were sucked through while the less polar metabolites were retarded. The loaded resin was rinsed with acetonitrile/water and pure acetonitrile. The acetonitrile eluates were concentrated and partitioned against dichloromethane. The primary acetonitrile/water extracts were also partitioned against dichloromethane and the organosoluble residues analysed by radio-HPLC and radio-TLC. Residues in the aqueous phase were characterized by different derivatisation reactions (see below).

Homogenized nutmeat samples were first extracted with hexane using a high-speed stirrer. The hexane extract was partitioned against acetonitrile and methanol. In a second step, the nutmeat sample were additionally extracted with acetonitrile/water (4/1, v/v, 2x). The combined aqueous extract was partitioned against dichloromethane. Subsamples of the hexane and the dichloromethane extract were analysed by normal and reversed phase radio-HPLC and two-dimensional radio-TLC. The post extraction-solids of hay and nutmeat with the non-extractable extractable residues were radioassayed and subjected to sequential hydrolysis procedures with increasing rigorousness (see below).

A subsample of the hexane fraction from nutmeat was also incubated with the lipase enzyme for at least 12 hours (37°C, pH 7.5) to hydrolyse the lipid components (glyceride esters). The hydrolysis was completed by agitation with KOH. Subsequent partitioning with ethyl acetate at acid pH resulted in isolation of the fatty acid fraction. This fraction was analysed by radio-HPLC. Other subsamples of the hexane extract were cleaned-up using flash silica gel columns that were eluted with acetonitrile, methanol and hexane. Fractions were analysed by radio-HPLC.

Subsamples of the aqueous fractions of vines and hay samples were hydrolysed with cellulase as well as with 3N and 6N hydrochloric acid (HCl). The resulting mixture was concentrated to dryness and reconstituted in methanol before analysis. The reaction products were analysed by two-dimensional radio-TLC and/or radio-HPLC.

Subsamples of the aqueous fractions following C18 flash chromatography and partitioning against dichloromethane were concentrated to dryness and then characterized by different derivatising reactions, e.g. acetylated with acetanhydride/pyridine, butylated with 3N HCl in butanol, or methylated with diazomethane. The resulting mixtures were brought to dryness using a stream of nitrogen, redissolved in methanol and analysed by radio-TLC.

Characterization of the radioactivity in the post-extraction solids

Following extraction with acetonitrile/water the remaining solids were subjected to various sequential analytical conditions: (1) reflux with acetonitrile/water (4/1), (2) reflux with 1% aqueous sodium chloride, (3) cellulase enzyme hydrolysis (37°C, pH 4.6, 24 hours), (4) protease enzyme hydrolysis (37°C, pH 7, 24 hours), (5) mild acid hydrolysis at room temperature using 1N HCl, (6) mild base hydrolysis at room temperature using 0.8N NaOH, strong acid hydrolysis with 6N HCl under reflux and strong base hydrolysis with 6N NaOH under reflux.

All fractions were radioassayed.

Extraction of soil samples

Soil samples taken at maturity of the peanuts were extracted with acetonitrile/water (4/1, v/v; 4x) by mechanic shaking for 15 minutes. The extracts and extracted soil were radioassayed. The extracts were combined, concentrated and analysed by radio-HPLC and two-dimensional radio-TLC.

Radioassaying and analysis

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). Quenching was automatically compensated using an external standard. Solid samples were firstly combusted and the formed $^{14}CO_2$ absorbed in an alkaline scintillation liquid. The combustion values were corrected for the combustion efficiency. The total radioactive residues (TRR) of a sample were established by the sum of extracted radioactivity and the no-extractable radioactivity in the extracted sample. The limit of quantification (LOQ) depended on the typical aliquot size, the background and the specific radioactivity of the test substance. The following LOQs were used in the study:

[14C-GP], label A: plant 0.005 mg equ/kg; nutmeat 0.020 mg equ/kg; soil 0.0005 mg equ/kg
[14C-TP], label B: plant 0.004 mg equ/kg; nutmeat 0.018 mg equ/kg; soil 0.0004 mg equ/kg.

One- and two-dimensional analytical and one-dimensional preparative radio-TLC was conducted on silica gel plates (SiO_2 F_{254}) of different thickness of the separation layer. The plates were developed with six different solvent systems, four of them contained formic or acetic acid. Radioactive spots were detected by a radioanalytic imaging system. Co-chromatographed non-radiolabelled reference standards were visualized by fluorescence extinction following excitation with UV light.

Radio-HPLC was conducted on a semi-preparative RP18 column (250 x 9.4 mm) operated with different gradient mixtures of aqueous phosphoric acid (0.1%) and acetonitrile. For profiling of hexane soluble fractions of the nutmeat the same column was also operated with different gradient mixtures of acetone and acetonitrile. In addition, organosoluble fractions of the nutmeat were also chromatographed on a semi-preparative straight–phase silica column (250 x 9.4 mm) that was operated with a gradient mixture of n-heptane and methanol. The HPLC systems were equipped with a UV detector (251 nm) and a radiomonitor. The flow rate was generally 2 mL/min.

Preparative column chromatography was also conducted with a C18 bulk packing (50 µm size). The sample was applied to the column and eluted with mixture of acetonitrile and water. For examination of acidic metabolites anion exchange chromatography was used with a diethylaminoethyl packing operated with a gradient mixture of water and 1N potassium bromide.

LC-MS/MS was performed for identification of parent substance and metabolites. Radio-HPLC separation was conducted on a reversed phase (C18) column (150 x 2.0 mm, particle size 5 µm), a

water/methanol gradient (both acidified with 0.1% formic acid) as liquid phase and a radiomonitor with a solid scintillator. Atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) were used for ionization. Authentic reference standards aided for identification.

GC-MS was performed using DB-625 capillary column that was coupled to a mass spectrometer. The GC separation was achieved using a temperature gradient between 70 and 250°C.

^{1}H-NMR analysis was performed using a 400 MHz NMR spectrometer. Samples were dissolved in deuterated d_3-acetonitrile or d_4-methanol. Chemical shifts were given in ppm relative to the partially deuterated solvent, which in turn referenced to TMS (tetramethylsilane).

Findings

Total radioactive residues and their extractability

The total radioactive residues (TRR) in immature vines of peanut plants, mature hay and shelled nuts (nutmeat) are shown in Table 6.2.1- 7. Initial TRR directly after the first application amounted to 20.7 and 24.9 mg parent equivalents/kg matrix (mg equ/kg) in vines for the [14C-GP] and [14C-TP] label. The TRR values decreased to 7.7 and 9.1 mg equ/kg in vines during the following 14 days. Due to three additional applications and withering TRR values increased again to 26.3 and 27.9 mg equ/kg for the two labels in mature hay 14 days after the last application. Mature hulls had radioactivity levels of approx. 1.1 mg equ/kg. Mature nutmeat had the lowest residue levels, amounting to 0.305 and 0.184 mg equ/kg for the [14C-GP] and the [14C-TP] label 14 days after the last application.

The extractability of radioactive residues from vines, hay and nutmeat is presented in Table 6.2.1- 8. From immature vines and mature hay the main portion of radioactive residues was extractable with acetonitrile and water amounting to approx. 86 – 91% of TRR in vines and to approx. 68 – 74% of TRR in hay with both radiolabels. The primary extract was concentrated and partitioned against dichloromethane and water. 56 – 67% of TRR in immature vines and 42 – 55% of TRR in mature hay were organosoluble. The non-extractable residues (post-extraction solids) accounted for approx. 10% of TRR in vines and for 24 – 30% of TRR in hay.

Nutmeat, sampled 14 days after the last treatment, was first extracted with hexane resulting in an organosoluble portion of approx. 26 - 31% of TRR for both radiolabels. Subsequent extraction with acetonitrile/water released 21 – 27% of TRR with the [14C-TP] and the [14C-GP] label. Partitioning of the aqueous extract against dichloromethane resulted in another organosoluble fraction of 12.5% of TRR for the [14C-TP] and 4.7% of TRR for the [14C-GP] label. Approx. 53 – 55% of TRR remained non-extractable by conventional extraction (post-extraction solids, PES).

The PES radioactivity remaining non-extractable following acetonitrile/water extraction were released by sequential treatments including mild, enzymatic and more drastic hydrolysis steps. The terminal residues remaining after these steps were <10% of TRR for all samples and both radiolabels. The radioactivity released by these hydrolysis steps were represented by the same metabolites as identified in the extracted organosoluble fraction of vines and hay (see Table 6.2.1- 9). From nutmeat, significant portions of the PES (53 – 57% of TRR) were released by refluxing with acetonitrile/water (20 – 26% of TRR) and aqueous 1% sodium chloride solution (11% of TRR) as well as by enzymatic hydrolysis with cellulase (9 – 11% of TRR) as shown in Table 6.2.1- 11.

Residues in peanut vines, hay and nutmeat

The organosoluble metabolites were characterized by co-chromatography with authentic reference standards using reversed phase radio-HPLC and/or normal phase two-dimensional radio-TLC. The organosoluble radioactivity separated into 5 regions by HPLC (designated as O-l through O-5) while the aqueous soluble radioactivity separated into 9 or 10 regions (A-l to A-10), depending upon the label. The metabolites in each HPLC region of the organic and aqueous fractions were isolated and identified by co-chromatography with the reference standard and/or MS and NMR analysis.

The radio-HPLC and radio-TLC profiles of the organosoluble fractions from both labels of immature vines and mature hay were similar. The main residue components using both labels were identified as parent substance trifloxystrobin (CGA-279202). Its isomers CGA-357261 (M3), CGA-357262 (M2) and CGA-331409 (M4) as well as the hydrolysed carboxylic acids CGA-321113 (M5) and its isomer CGA-373466 (M6) proved to be minor metabolites. Greater than 98% of the organosoluble radioactivity in mature hay was identified. The metabolites in immature vines and mature hay of peanut plants are compiled in Table 6.2.1- 9.

In nutmeat, the parent substance CGA-279202 and its carboxylic acids CGA-321113 (M5) and CGA-373466 (M6) were also identified in the dichloromethane fraction. The primary hexane fraction from mature nutmeat was shown to be composed of the parent substance, its hydrolysed carboxylic acid and their isomers as well as of radiolabeled triglycerides. Due to the low radioactivity level in nutmeat a satisfying separation of the radioactive residues into individual components was not possible. The composition of partly separated residues in nutmeat is presented in Table 6.2.1- 10.

The radio-HPLC and 2D-radio-TLC profiles of the aqueous fractions from [14C-GP] (label A) and [14C-TP] (label B) were different, indicating the cleavage of the N-O bond between the phenyl rings and formation of the label-specific metabolites. For each label, the HPLC profiles of the aqueous fractions from mature nutmeat, immature vines and mature hay were similar. The major aqueous soluble metabolites, which occurred primarily as malonyl glucosides and glucoside conjugates, were isolated and identified. No single unidentified metabolite accounted for more than 1% of TRR in either label of mature hay or nutmeat.

The post-extraction solids (PES) with the non-extractable residues following acetonitrile/water extraction were solubilized by following the sequential treatments with increasing hydrolytic reactivity: acetonitrile/water reflux, aqueous sodium chloride reflux, cellulase and protease incubation, HCl and NaOH hydrolyses. The terminal residues remaining after these hydrolysis steps were <10% of TRR for all samples. The radioactive residues released by these steps were characterized by radio-HPLC and 2D-radio-TLC. Similar to the organosoluble extractable metabolites, the parent substance trifloxystrobin (CGA-279202) and its isomers CGA-357261 (M3), CGA-357262 (M2) and CGA-331409 (M4) as well as its carboxylic acids CGA-321113 (M5) and CGA-373466 (M6) were identified in non-extractable radioactivity from both labels.

Residues in soil

In the top soil layer (0 – 7.6 cm) TRR remained at an approximately constant level ranging from 0.2 to 0.4 mg equ/kg for both labels throughout the study period. 60.5 – 70.7% of TRR (0.22 – 0.24 mg equ/kg) were extractable from the soil samples taken a maturity of the peanuts using both radiolabels. The main residue component revealed to by the carboxylic acid CGA-321113 (M5) amounting to 36.8% of TRR (0.136 mg equ/kg) or 52.0% of TRR (0.176 mg equ/kg) using the [14C-GP; A] or the [14C-TP; B] label. Other residue components identified in soil were CGA-373466 (M6), CGA-357276 (M2) and the parent substance trifloxystrobin.

Storage stability

All samples were stored at approx. – 20°C. Extracts were stored at 4°C, at maximum. Initial extractions and analysis of the organic and aqueous fractions from all samples occurred within 6 months of harvest.

The stability residues in the homogenized samples was shown by comparing the initial HPLC profile for mature hay with that generated after 15 months [14C-GP] or 14 months [14C-TP] storage. The stability of residues in the extracts from hay, nutmeat and immature vines was shown by comparing the initial HPLC profiles to those after 14 – 25 months of frozen storage. The profiles did not change significantly.

Conclusion

[14C-GP] and [14C-TP] trifloxystrobin metabolized intensively in peanuts as approximately 12 - 13 metabolites were detected in immature vines 14 days after the first foliar treatment and in mature hay 14 days after the last of four foliar treatments at an application rate of 0.5 lb ai/A (0.56 kg as/ha) each. The majority of radioactive residues were extractable from immature vines and mature hay using acetonitrile/water. The percentage of extractable radioactivity ranged from 86.25% - 90.89% of TRR in immature vines to 20.66% - 26.99% of TRR in mature nutmeat. The organosoluble metabolites represented 56.45% - 66.89% of TRR in immature vines and 42.42% - 55.35% of TRR in mature hay, but less than 10% of TRR in the nutmeat. However, a significant portion could be extracted from nutmeat in a prior extraction with hexane accounting to 25.6 – 30.9% of TRR based on the two radiolabels.

The radio-HPLC and radio-TLC profiles of the organosoluble metabolites extracted from immature vines and mature hay were similar in both radiolabels. The main residue component using both labels was identified as the parent substance trifloxystrobin (CGA-279202). Its isomers CGA-357261 (M3), CGA-357262 (M2) and CGA-331409 (M4), as well as the hydrolysed carboxylic acids CGA-321113 (M5) and its isomer CGA-373466 (M6) proved to be minor metabolites. Parent substance and the carboxylic acid isomers CGA-321113 (M5) and CGA-373466 (M6) were also identified in the dichloromethane fraction of nutmeat. The hexane fraction from mature nutmeat was shown to be composed of radiolabeled triglycerides. Due to the very low residue levels in nutmeat the separation of the radioactivity into individual residue components was only partly feasible.

However, the respective radio-profiles of the aqueous soluble (polar) metabolites in vines and hay resulting from the two labels were different, indicating the cleavage of the N-O bridge between the phenyl rings and the formation of label-specific metabolites. All metabolites in mature hay and nutmeat exceeding 1% of TRR could be identified.

The metabolism study on ^{14}C-trifloxystrobin in field grown peanuts revealed the following transformations:

- Cis/trans isomerization of parent substance trifloxystrobin (CGA-279202) to form CGA-357261 (M3), CGA-357262 (M2) and CGA-331409 (M4)

- Methylester cleavage to the carboxylic acids CGA-321113 (M5) and its cis/trans isomer CGA-373466 (M6). The subsequent conjugation with malonyl glucose leads to different malonyl glucosides metabolites (A-9a11, A-9b1, A-9c1, A9c2).

- Hydroxylation of the aminooxymethyl group on the bridge between the phenyl rings, or hydroxylation of the CF_3 bearing phenyl ring at the meta position, followed by glucose conjugation, leads to metabolites NOA-443152 (M10)- and NOA-414412 (M12)-glucosides (A-7a, A-7a2, A-7c); upon cleavage of the N-O bridge and subsequent glucose conjugation the metabolites CGA-107170 (M62)- and BO-17372 (M64)-glucosides (A-7a, A-7a2, A-7b) are formed .

- The cleavage of N-O bridge in trifloxystrobin or its carboxylic acid CGA-321113 (M5), followed by oxidation and/or conjugation to form metabolites CGA-328365 (M61, A-4b), CGA 300624 (M59, A-5b); CGA-347242 (M47, F2b2-3a&b (A1))-glucoside, methyl ester of CGA-373463 (M46, F2b2-3a&b (A2)); CGA-367619 (M53, phthalic acid, A2b2 (top)); WFX-IX-86 (M51, peak 3,4-1 or 8-1).

- Complete oxidation of the CF_3 bearing phenyl ring resulted in low formation of trifluoroacetate.

- The parent substance trifloxystrobin (EE isomer) proved to be the main residue component in immature vines and mature hay of peanuts and, therefore, can be used as analytical target in the residue analysis. In nutmeat, no single residue component exceeded 10% of TRR, also applying to the parent substance. The major portion of radioactivity represented ^{14}C-triglycerides obviously formed by mineralization of the ^{14}C-trifloxystrobin in soil and uptake of the formed $^{14}CO_2$ via photosynthesis.

The proposed metabolic pathway of trifloxystrobin in peanuts is presented in Figure 6.2.1- 5.

Table 6.2.1- 7: Total radioactive residues (TRR) in peanuts treated with [14C-GP; A] and [14C-TP; B] labelled trifloxystrobin using four foliar applications at nominal use rates of 0.5 lb ai/A (0.56 kg as/ha) each

Crop part	Growth stage	Day *)	[14C-GP], label A	[14C-TP], label B
			[mg equ/kg]	[mg equ/kg]
Vines	(initial residues)	0 DAFA	20.742	24.899
Vines	immature	14 DAFA	7.734	9.114
Hay	mature	14 DALA	26.340	27.922
Hull (shell)	mature	14 DALA	1.148	1.081
Nutmeat	mature	14 DALA	0.305	0.184

*) DAFA: days after the first application

DALA: days after the last application

Table 6.2.1- 8: Extractability of radioactive residues from peanuts treated with [14C-GP; A] and [14C-TP; B]-labelled trifloxystrobin using four foliar applications at nominal use rates of 0.5 lb ai/A (0.56 kg as/ha) each

Crop part	Hexane	ACN/H_2O	Partitioning of ACN/H_20 extract		Non-extractable	Recovery
			CH_2Cl_2	H_20		
	[% of TRR]					
[14C-GP], label A						
Vines, immature	-	90.89	- 56.45	- 26.05	10.65	101.54
Hay, mature	-	67.68	- 42.42	- 21.01	30.40	98.08
Nutmeat	25.63	26.99	- 4.70	- 22.35	55.36	107.98
[14C-TP], label B						
Vines, immature	-	86.25	- 66.89	- 17.89	9.91	96.16
Hay, mature	-	74.09	- 55.35	- 16.84	24.32	98.41
Nutmeat	30.89	20.66	- 12.53	- 7.46	53.17	104.72

Table 6.2.1- 9: Metabolite fractions in immature vines (14 days after the 1st application) and mature hay (14 days after the last of four foliar applications) with [14C-GP] and [14C-TP]trifloxystrobin at nominal use rates of 0.5 lb ai/A (0.56 kg as/ha) each

Label position	[14C-GP], label A				[14C-TP], label B			
Plant part	**Immature Vines**		**Mature Hay**		**Immature Vines**		**Mature Hay**	
TRR [mg equ/kg]	**7.734**		**26.340**		**9.114**		**27.922**	
	%TRR	**ppm[1]**	**%TRR**	**ppm[1]**	**%TRR**	**ppm[1]**	**%TRR**	**ppm[1]**
Extractable	**90.89**	**7.030**	**67.68**	**17.827**	**86.25**	**7.860**	**74.09**	**20.686**
CGA-373466 (M6)	0.00	0.000	1.65	0.436	0.33	0.030	0.55	0.155
CGA-321113 (M5)	1.35	0.105	3.14	0.827	2.04	0.185	3.87	1.082
CGA-357261 (M3)	2.94	0.227	2.21	0.581	2.94	0.268	2.66	0.742
CGA-279202 (parent)	32.40	2.506	29.02	7.643	38.60	3.518	43.51	12.148
CGA-357262 (M2)	2.48	0.192	1.32	0.346	2.41	0.219	1.77	0.495
CGA-331409 (M4)	3.22	0.249	2.63	0.693	3.34	0.305	3.54	0.989
OH-CGA-321113	0.00	0.000	0.47	0.123	1.49	0.135	1.82	0.510
CGA-328365-mal-glu[2]	-	-	-	-	1.25	0.114	2.48	0.692
CGA-300624-mal-glu	-	-	-	-	0.40	0.036	0.00	0.000
CGA-321113-mal-glu	11.63	0.899	4.86	1.279	15.19	1.384	1.62	0.453
CGA-321113-glu[3]	1.52	0.117	0.07	0.019	0.15	0.014	0.00	0.000
Phthalic acid (M53)	1.42	0.110	1.17	0.307	-	-	-	-
F2b2-3a&b (A2), (M46)	1.14	0.088	1.20	0.315	-	-	-	-
F2b2-3a&b (A1), (M47)	6.36	0.492	3.72	0.980	-	-	-	-
WFX-IX-86 (M51)	1.54	0.119	2.48	0.653	-	-	-	-
Trifluoro acetate (M66)	-	-	-	-	0.28	0.025	0.78	0.217
A-7a/A-7a2 (glucoside)	-	-	-	-	0.95	0.087	1.87	0.522
A-7b (glucoside)	-	-	-	-	1.47	0.133	3.00	0.838
Post-Extraction-Solids	**10.65**	**0.823**	**30.40**	**8.007**	**9.91**	**0.903**	**24.32**	**6.790**
CGA-373466 (M6)	0.24	0.019	0.00	0.000	0.17	0.015	0.20	0.057
CGA-321113 (M5)	0.42	0.033	0.09	0.024	0.41	0.037	1.22	0.341
CGA-373465 (M7)	0.07	0.005	0.00	0.000	0.06	0.005	0.00	0.000
CGA-357261 (M3)	0.24	0.018	0.00	0.000	0.21	0.020	0.50	0.139
CGA-279202 (parent)	0.81	0.062	0.30	0.079	0.90	0.082	3.00	0.837
CGA-357262 (M2)	0.22	0.017	0.00	0.000	0.25	0.022	0.41	0.115
CGA-331409 (M4)	0.27	0.021	0.00	0.000	0.27	0.024	0.54	0.151
Phthalic acid (M53)	0.14	0.011	1.51	0.398	-	-	-	-
F2b2-3a&b (A2), (M46)	0.14	0.011	0.00	0.000	-	-	-	-
F2b2-3a&b (A1), (M47)	0.40	0.031	4.21	1.110	-	-	-	-
Terminal residue[4]	6.61	0.511	3.92	0.357	0.40	0.104	0.47	0.131
Total identified	**68.94**	**5.332**	**60.06**	**15.813**	**73.11**	**6.660**	**73.34**	**20.483**

[1] ppm: mg parent equivalents/kg plant material "-" not detectable due to missing label

[2] mal-glu: malonyl glucose conjugate [3] glu: glucose conjugate

[4] terminal residues after different hydrolysis steps of the post-extraction solids determined by combustion

Conjugated aglycons: CGA-321113 (M5); CGA-328365 (M60); CGA-300624 (M59)

Table 6.2.1- 10: Residue fractions in mature nutmeat of peanuts 14 days after the last of four foliar applications with [14C-GP] and [14C-TP]trifloxystrobin at nominal use rates of 0.5 lb ai/A (0.56 kg as/ha) each

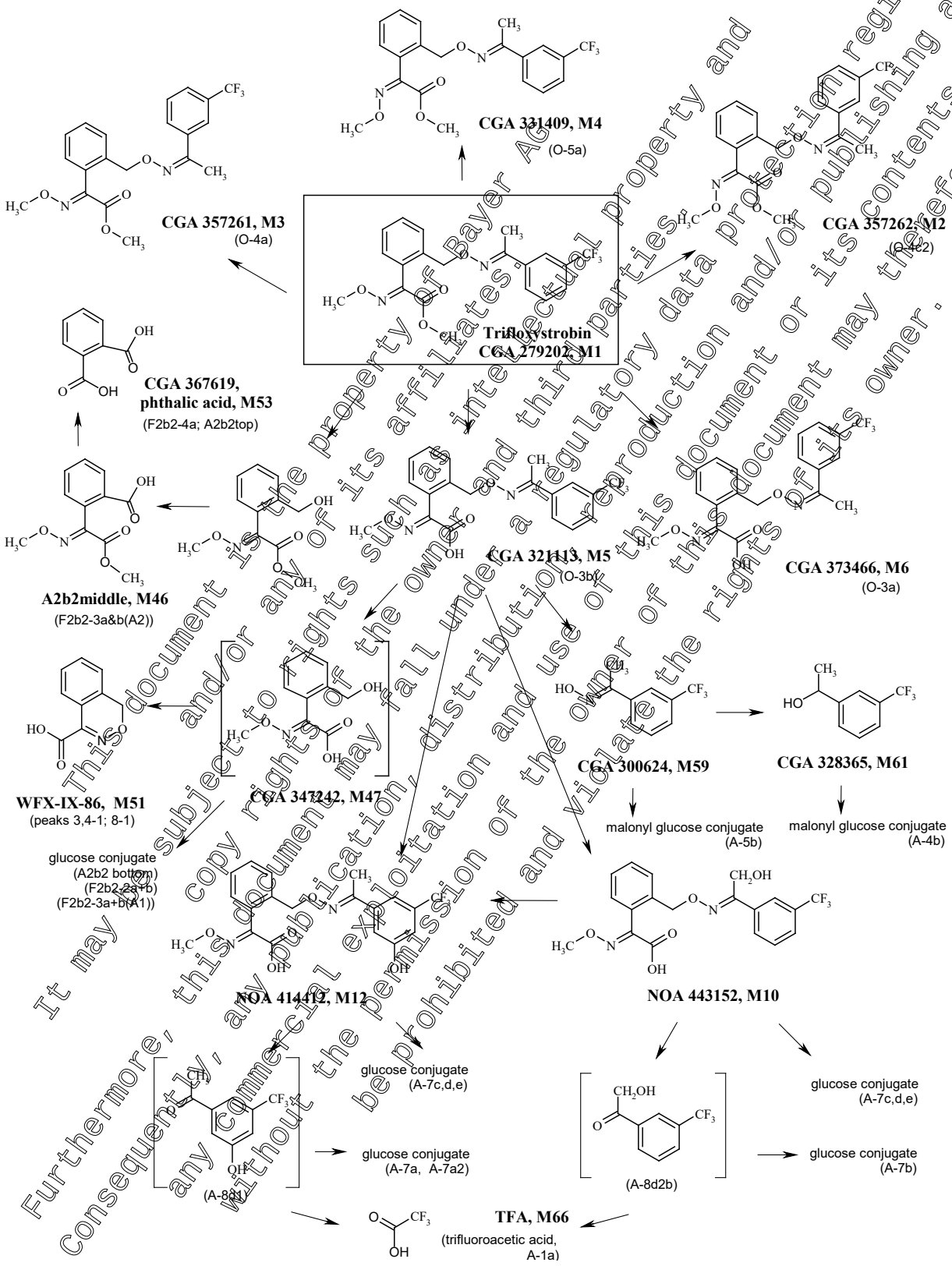
Label position	**[14C-GP], label A**		**[14C-TP], label B**	
TRR [mg equ/kg] of extracted nutmeat sample	0.274		0.165	
	%TRR	mg equ/kg	%TRR	mg equ/kg
Extractable	**52.63**	**0.144**	**51.55**	**0.85**
Hexane Fraction	**25.63**	**0.070**	**30.89**	**0.051**
CGA-321113 (M5)				
CGA-279202 (parent) and its isomers	1.98	0.005	1.61	0.003
^{14}C-triglycerides	23.65	0.065	29.28	0.048
Dichloromethane Fraction	**4.70**	**0.013**	**12.53**	**0.021**
CGA-373466 (M6)				
CGA-321113 (M5)	1.11	0.003	2.04	0.003
CGA-279202 (parent)			2.34	0.004
CGA-357261 (M2)	-	-		
Aqueous Fraction	**22.35**	**0.061**	**7.45**	**0.012**
Phthalic acid (M53)	5.97	0.016		
F2b2-3a&b (A2), (M46)	3.35	0.009		
F2b2-3a&b (A1), (M47)	8.80	0.024		
Total characterized	**52.68**	**0.144**	**50.87**	**0.084**
Total identified	**44.87**	**0.123**	**35.24**	**0.058**

Table 6.2.1- 11: Characterization of the non-extractable residues (post-extraction solids) in mature nutmeat of peanuts 14 days after the last of four foliar applications with [14C-GP] and [14C-TP] trifloxystrobin at nominal use rates of 0.5 lb ai/A (0.56 kg as/ha) each

Label position	**[14C-GP], label A**		**[14C-TP], label B**	
TRR [mg equ/kg] of extracted nutmeat sample	0.274		0.165	
	%TRR	mg equ/kg	%TRR	mg equ/kg
Post extraction solids (PES)	**55.36**	**0.152**	**53.17**	**0.088**
Sequential releasing steps				
Acetonitrile/water (4/1) reflux	25.59	0.070	19.87	0.033
1% aqueous NaCl reflux	11.38	0.031	10.56	0.017
Cellulase hydrolysis	9.40	0.026	11.45	0.019
Protease hydrolysis	2.37	0.007	4.08	0.007
Mild acid (1N HCl) treatment	0.32	0.001	0.74	0.001
Mild base (0.8N NaOH) treatment	1.14	0.003	0.95	0.002
Terminal residue[1]	1.50	0.004	1.89	0.003
Total recovered	**51.70**	**0.142**	**49.54**	**0.082**

[1] Terminal residue values after different hydrolysis steps of PES were determined by combustion.

Figure 6.2.1- 5: Metabolic pathway of trifloxystrobin in field grown peanuts



Summary of the metabolism of trifloxystrobin in plants including the new metabolism studies

Metabolism of trifloxystrobin was investigated in wheat, apple, cucumber, sugar beet and peanuts by foliar application of the active substance in radiolabelled form. According to OECD guideline 501 'Metabolism in crops', four different crop categories were covered: cereal/grass crops, fruits and fruiting vegetables, root crops and pulses and oilseeds. The ^{14}C-radiolabel was located in both phenyl rings, the labels are designated as [14C-GP] and [14C-TP] trifloxystrobin. The application pattern used was approximately:

Wheat:	2 x 0.25 kg as/ha (two studies)
Apple:	4 x 0.10 kg as/ha
Cucumber:	3 x 0.31 kg as/ha
Sugar beet:	3 x 0.13 kg as/ha
Peanuts:	4 x 0.56 kg as/ha

At harvest, the total radioactive residues (TRR) were measured in mature commodities.

Wheat grain:	GP: 0.099 mg equ/kg (first study) and 0.262 mg equ/kg (second study)
	TP: 0.056 mg equ/kg (first study) and 0.120 mg equ/kg (second study)
Wheat straw	GP: 7.48 mg equ/kg (first study) and 6.12 mg equ/kg (second study)
	TP: 3.85 mg equ/kg (first study) and 6.13 mg equ/kg (second study)
Apple fruit	GP: 1.276 mg equ/kg
	TP: 0.883 mg equ/kg
Cucumber fruit	GP: 2.289 mg equ/kg (small fruits < 20 cm, larger fruits had lower TRR)
	TP: 0.586 mg equ/kg (small fruits < 20 cm, larger fruits had lower TRR)
Sugar beet root	GP: 0.025 mg equ/kg
	TP: 0.021 mg equ/kg
Sugar beet tops	GP: 0.73 mg equ/kg
	TP: 0.45 mg equ/kg
Peanut nutmeat	GP: 0.305 mg equ/kg
	TP: 0.184 mg equ/kg
Peanut hay	GP: 26.34 mg equ/kg
	TP: 27.92 mg equ/kg

From the pattern of metabolites in the different crops as compiled in Table 6.2.1-12 (given as % of TRR) and in Table 6.2.1-13 (given as mg equ/kg) similar metabolic reactions can be deduced. These reactions comprise:

- Cis/trans isomerization at the two C=N double bonds to form the isomers CGA 357261 (M3), CGA 331409 (M4) and CGA 357262 (M2). The amount of the single isomers does not exceed 10% of the TRR, with exemption of the parent substance trifloxystrobin (EE isomer).

- Methyl ester hydrolysis to form the carboxylic acid CGA 321113 (M5) amounting to a maximum portion of 10.8% of TRR (corresponding to 0.002 mg equ/kg) in sugar beet roots and, to a lower portion, its isomer CGA 373466 (M6)

- Hydroxylation of the trifluoromethyl-phenyl ring to form the hydroxylated carboxylic acid NOA 414412 (M12) with subsequent glucoside conjugation (mainly detected in wheat straw).

- Hydroxylation of the aminooxymethyl substituent in the bridge between the phenyl rings to form the metabolite NOA 443152 (M10) with subsequent glucoside conjugation or further oxidation to dicarboxylic acid NOA 413161 (M8) and its isomer NOA 413163 (M9), also found in wheat straw.

- In sugar beet, a carboxylic acid was hydroxylated three-fold, once at the methyl group in the bridge and twice at the trifluoromethyl phenyl ring reaching a maximum amount of 14.9% of TRR (metabolite fraction M16). Further analysis revealed that M16 consisted of several isomers, each of them amounting to less than 0.01 mg equ/kg.

- In wheat and peanuts, some label-specific metabolites were formed after cleavage of the ethylideneaminooxy bridge. Most prominent was the “cyclic keto-alcohol” (M57) amounting up to 9.6% of TRR (0.025 mg equ/kg) in wheat grain, obviously formed from the glyoxyl phenyl moiety via hydrolysis, oxidation and four-ring formation by elimination of water. In peanuts, a glucoside conjugate of hydroxylated cleavage product CGA 347242 (M47) was found up to an amount of 8.8% of TRR (0.024 mg equ/kg). Trifluoroacetate, TFA (M66) formed by complete degradation of the trifluoromethyl phenyl ring was detected in non-edible peanut hay as a very minor metabolite (0.8% of TRR, 0.217 mg equ/kg)

- Almost all of the metabolites occurred as minor metabolites (< 10% of TRR). Only in sugar beet roots the carboxylic acid CGA 321113 (M5) and the fraction of “trihydroxy metabolites” (M16) consisting of several isomers slightly exceeded the 10% of TRR, but only using one of the two radiolabels. Given in absolute values, both metabolites accounted for < 0.01 mg equ/kg

- In all crops, the parent substance trifloxystrobin, in principle, proved to be the main residue component and, therefore, can be used as marker substance in the residue analysis of trifloxystrobin residues in food of plant origin.

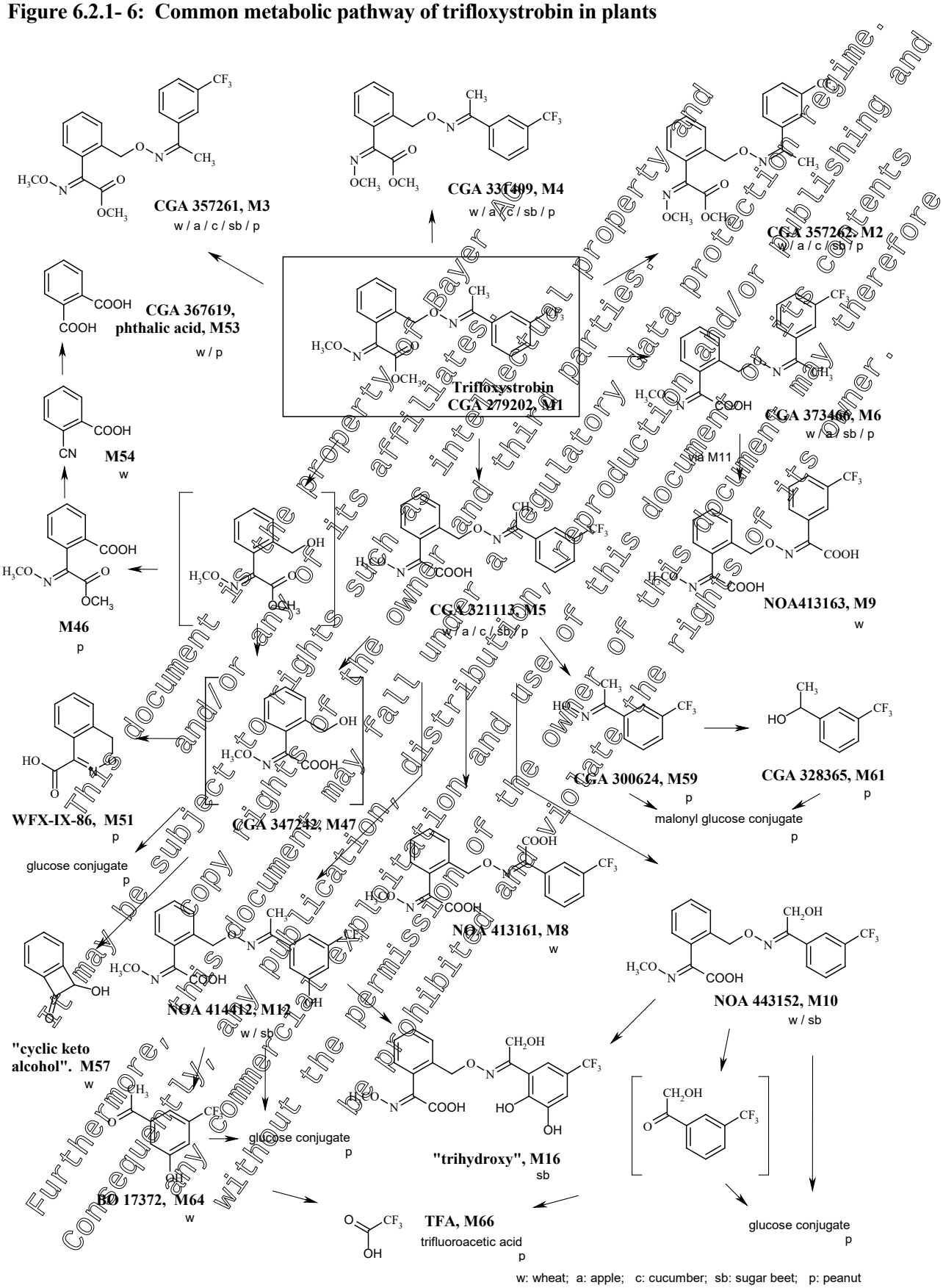
Table 6.2.1- 12: Metabolites of trifloxystrobin in plant metabolism studies at harvest following application of [14C-GP] and [14C-TP] label in % of TRR

Crop, Commodity	Wheat Grain		Wheat Straw		Apple Fruit		Cucumber Fruit		Wheat Grain		Wheat Straw		Sugar beet Root		Sugar beet Top		Peanut Nutmeat		Peanut Hay	
Appl. Rate [kg as/ha]	2 x 0.25				4 x 0.10		3 x 0.31		2 x 0.25		2 x 0.25		3 x 0.13				4 x 0.56			
Label	GP	TP	GP	TP	GP	TP	GP	TP	GP	TP	GP	TP	GP	TP	GP	TP	GP	TP	GP	TP
TRR [mg equ/kg]	0.099	0.056	5.48	3.85	1.276	0.883	2.289*	0.586*	0.262	0.120	6.12	6.13	0.025*	0.021*	0.73*	0.45*	0.306	0.184	26.34	27.92
Parent Substance and Metabolites (% of TRR)																				
Parent substance and its isomers																				
A.S., M1 (parent)	1.3	3.2	2.2	0.9	83.0	80.7	87.0	86.6	11.1	19.6	18.6	14.3	23.3	47.6	21.3	34.3	3.1	6.0	29.3	46.5
M2, CGA 357262	-		0.4	0.4	1.4	2.2	<0.1	2.1	1.8	6.3	2.3	2.4	-	-	<0.1	-			1.3	2.2
M3, CGA 357261	1.2		1.0	0.7	3.3	5.2	0.9	0.5	2.9	8.0	5.3	5.3	-	-	-	-			2.2	3.2
M4, CGA 331409	-		0.7	0.6	2.2	3.4	2.0	1.2	2.1	5.8	3.1	3.6	3.2	3.8	0.9	1.2			2.6	4.1
Metabolites with intact basic molecular structure including both phenyl rings																				
M5, CGA 321113	-	1.1	1.3	0.8	0.4	0.6	2.3	2.9	1.7	2.6	3.8	4.2	7.5	10.8	2.8	2.5			3.2	5.1
M6, CGA 373466									0.9	1.2	2.0	1.8	0.4	0.3	2.8				1.7	0.8
M8, NOA 413161	10.1	1.5	0.9	1.4					0.3	0.3	1.4	1.8								
M9, NOA 413163		2.6	3.3	4.2					1.8	2.9	5.0	5.8								
M12, NOA 414412	≤2.1	4.6	4.7	7.0					2.4	5.2	6.5	7.0	1.3	2.3	1.7	3.8				
M10, NOA 443152									1.9	4.6	5.9	6.5	1.2	1.0	0.4	-				
M16, "trihydroxy"													9.2	14.9	1.6	0.7				
M11, BO 172741										1.5	3.5	4.1								
Label specific metabolites formed by cleavage of the ethylideneaminooxy bridge between the phenyl rings																				
M54, cyanobenzoic acid									3.6		3.0									
M53, phthalic acid									3.1		1.2						6.0		2.7	
M57, "cyclic keto alcohol"									9.6		2.5									
M47, CGA 347242																	8.8		7.9	
M5-, CGA 321113-gluc, malonyl gluc.																			4.9	1.6
M66, TFA																				0.8
References	[redacted], 1997 (GP), M-034368-01-1; [redacted], 1997 (TP), M-034053-01-1				[redacted], 1997 (GP), M-034389-04-1; [redacted], 1998 (TP), M-034423-01-1		[redacted], 1997 (GP), M-034445-01-1; [redacted], 1997 TP, M-034442-01-1, small fruit		[redacted], [redacted], 2002 (GP), M-072024-01-1; [redacted], [redacted], 2002 (TP), M-070885-01-1				[redacted], 2000 (GP), M-069125-01-1; [redacted], 2000 (TP), M-069117-01-1, * PHI = 45 days				[redacted], 1997, M-137152-01-1			

Table 6.2.1- 13: Metabolites of trifloxystrobin in plant metabolism studies at harvest following application of [14C-GP] and [14C-TP] label in mg equ/kg

Crop, Commodity	Wheat Grain		Wheat Straw		Apple Fruit		Cucumber Fruit		Wheat Grain		Wheat Straw		Sugar beet Root		Sugar beet Top		Peanut Nutmeat		Peanut Hay	
Appl. [kg as/ha]	2 x 0.25				4 x 0.10		3 x 0.31		2 x 0.25		2 x 0.25		3 x 0.13				4 x 0.56			
Label	GP	TP	GP	TP	GP	TP	GP	TP	GP	TP	GP	TP	GP	TP	GP	TP	GP	TP	GP	TP
TRR [mg equ/kg]	0.099	0.056	5.48	3.85	1.276	0.883	2.289*	0.586*	0.262	0.120	6.12	6.13	0.025*	0.021*	0.73*	0.45*	0.305	0.184	26.34	27.92
Parent Substance and Metabolites (mg equ/kg)																				
Parent substance and its isomers																				
A.S., M1 (parent)	0.001	0.002	0.123	0.033	1.059	0.672	1.991	0.507	0.029	0.024	0.14	0.88	0.006	0.010	0.155	0.155	0.009	0.011	7.722	12.99
M2, CGA 357262	-		0.023	0.017	0.018	0.018	<0.002	0.012	0.005	0.008	0.14	0.14		-	0.000				0.346	0.610
M3, CGA 357261	0.001		0.057	0.028	0.042	0.043	0.021	0.008	0.008	0.010	0.33	0.32	-		-	-			0.581	0.881
M4, CGA 331409	-		0.037	0.024	0.028	0.028	0.046	0.007	0.005	0.007	0.19	0.22	0.001	0.001	0.007	0.005			0.693	1.140
Metabolites with intact basic molecular structure including both phenyl rings																				
M5, CGA 321113	-	0.000	0.071	0.031		-	0.053	0.017	0.005	0.003	0.23	0.26	0.002	0.002	0.020	0.011			0.851	1.423
M6, CGA 373466	-	-	-	-	0.005	0.005	-	-	0.002	0.001	0.12	0.11	0.000	0.000	0.007	-			0.436	0.212
M8, NOA 413161	0.010	0.001	0.049	0.024	-	-	-	-	0.001	0.000	0.08	0.11	-	-	-	-				
M9, NOA 413163		0.001	0.181	0.162	-	-	-	-	0.005	0.004	0.31	0.35	-	-	-	-				
M12, NOA 414412	≤ 0.002	0.003	0.258	0.270	-		-	-	0.006	0.006	0.40	0.43	0.000	0.000	0.012	0.017				
M10, NOA 443152	-								0.005	0.006	0.36	0.40	0.000	0.000	0.003	-				
M16, "trihydroxy"	-								-	-	-	-	0.002	0.003	0.012	0.003				
M11, BO 172741	-								-	0.002	0.21	0.25								
Label specific metabolites formed by cleavage of the ethylideneaminooxy bridge between the phenyl rings																				
M54, cyanobenzoic acid									0.009		0.18									
M53, phthalic acid									0.008		0.07						0.016		0.705	-
M57, "cyclic keto alcohol"									0.025		0.09						-		-	-
M47, CGA 347242																	0.024		2.090	-
M5-, CGA 321113-gluc, malonyl gluc,																	-		1.279	0.453
M66. TFA																			-	0.217
References	[redacted]. 1997 (GP). M-034306-01-1; [redacted] 1997 (TP) M-034053-01-1		[redacted].1997(GP) M-034389-04-1 [redacted] 1998(TP) M-034423-01-1		[redacted] 1997GP M-034445-01-1; [redacted] .1997TP M-034442-01-1 *small fruit		[redacted]. [redacted]. 2002 (GP) M-072024-01-1: [redacted]. [redacted]. 2002 (TP) M-070885-01-1		[redacted]. 2000 (GP) M-069125-01-1; [redacted]. 2000 (TP) M-069117-01-1 * PHI = 45 days		[redacted] 1997. M-137152-01-1					

Figure 6.2.1- 6: Common metabolic pathway of trifloxystrobin in plants



CA 6.2.2 Poultry

Data / information on poultry metabolism studies were reviewed during the peer review under Directive 91/414/EEC. For further information please refer to the Annex II dossier.

CA 6.2.3 Lactating ruminants

Data / information on ruminant metabolism studies were reviewed during the peer review under Directive 91/414/EEC. For further information please refer to the Annex II dossier.

CA 6.2.4 Pigs

A 'Summary of metabolism in domestic animals" was presented in Section B.7.2.4 of the Draft Assessment Report (2000) on trifloxystrobin (p. 288). From this summary the following quotations are repeated here to show that the metabolism in goat (ruminant), hen (poultry) and rat (lab animal used in toxicity studies) are essentially the same and. Therefore, a pig metabolism study is not required.

"Goats were given daily doses of [14C-TP] Trifloxystrobin at 103.8 mg/kg diet (40N) and [14C-GP] Trifloxystrobin at 100.4 mg/kg diet (39N) for four days. Goats were sacrificed 6 hours after the last dose. … Highest residue levels in milk were 0.121 and 0.153 mg/kg and a plateau was reached after 48 hours. CGA 279202 was the dominant residue. … . Identified metabolites comprised up to 93.5% TRR (milk), 84.5% (muscle), 93% (fat), 90% (kidney) and 60% (liver). There was some evidence of cleavage of the molecule between to two phenyl rings with the formation of metabolites 11U and 12U (CGA 354870). **The goat metabolites were all identified in the rat metabolism studies**."

"Hens were given daily doses of [14C-TP] Trifloxystrobin at 100.7 mg/kg diet (1060N) and [14C-GP] Trifloxystrobin at 98.9 mg/kg diet (1041N) for four days and were sacrificed 6 hours following the last dose. … Parent compound predominated in muscle and fat + skin, accounting for up to 27.8 and 55.3% TRR respectively. Major metabolites were CGA 321113, 1U and 6U (egg white), 2F (NOA 405637) (fat + skin : muscle), 12U (CGA 354870) (muscle) and L13b & L14 (liver). Other identified metabolites did not individually exceed 8% TRR. Unextracted residues were up to 79% and 35% TRR in liver and egg yolk respectively, however residues in poultry products are highly unlikely to be determinable at 1N rate exposure.

The hen metabolites were all identified in the rat metabolism studies, except for the following: Met EW1b, Met L13b, Met EW11, Met L24, EGR10a-c, EGR8, EGR1, EX5. These metabolites are not considered to be of toxicological concern at the levels found."

As a conclusion it can be stated, that apart from some metabolites in the hen being of no toxicological concern, the metabolism in goat and hen is principally the same as in the rat. Therefore, a metabolism study in pigs is not required.

CA 6.2.5 Fish

Since no guideline on a metabolism study in food fish and its typical feed is currently available a bioconcentration study with bluegill sunfish is summarised in the following. The main objective of this study was the determination of a potential bioaccumulation of a test substance in fish during long-term exposure in water. However, the nature of residues of radiolabelled trifloxystrobin in fillet and viscera of the fish was also disclosed in this study following a long-term uptake of continuously added trifloxystrobin with the inflowing water in 28-day flow-through study. As this study yields the same information as a metabolism study in fish it can be used as surrogate study according to Section 6.2.5 of the official data requirements (EU) No. 283/2013 of 1-March-2013 in accordance with Regulation (EC) No. 1107/2009. This study was submitted with the original Annex II dossier in section IIA 8.2.3 and evaluated in the DAR under point B.9.2.2 d) 'Bioaccumulation' of the ecotoxicology section. p 450 ff. (April 2000). It is here summarised in more detail with special focus on the metabolism of trifloxystrobin in fish.

Report:	**KCA 6.2.5/01, [redacted]; 1997** ; M-032004-01-1
Title:	[Phenyl(A)-U-^{14}C]-CGA-279202-Flow-Through Bioconcentration and Metabolism Study with Bluegill Sunfish (*Lepomis macrochirus*)
Document No:	M-032004-01-1
Report No:	96-8-6608 of Springborn Lab.. Health and Environmental Sciences. Wareham. MA. USA. now Bayer CropScience
Guidelines and data requirement:	US EPA Guidelines for Pesticide Registration: 40 CFR 158. Subdivision N. Section 165-4 'Accumulation in Fish'. which is comparable to OECD No. 305E guideline (May 1989)
GLP	yes

Executive summary

Groups of approximately 250 bluegill sunfish (Lepomis macrochirus) were exposed for 28 days under flow-through conditions to nominal concentrations of 0.16 µg/L and 1.6 µg/L radiolabelled [14C-GP]trifloxystrobin (^{14}C-CGA 279202, label A) for determination of the bioconcentration factors (BCF) and the metabolism in fish (only high dose). The test material was dispensed via syringe pump into dilution water flowing to each test vessel at the rate of approximately 8 volume turnovers per day (initial bioloading ca. 0.6 g/L/day). Some fish and water samples were collected at different exposure intervals. Following a 28-day exposure, the remaining fish were held in untreated control water for 14 days to provide data on the rate of elimination of trifloxystrobin and its residues from fish tissues (depuration period). A concurrent 28-day metabolism study was also conducted using a nominal concentration of 1.6 µg/L [14C-GP]trifloxystrobin.

At different intervals of the exposure (0 – 28 days in water with the test substance) and the depuration period (0 – 14 days in pure water) each five fish and water samples were collected and radioassayed. Five fish were also collected from the metabolism aquarium on days 21 and 28. These fish were dissected into three portions, edible, viscera and carcass. On day 28 of exposure, the remaining 238 fish from the metabolism aquarium were harvested for metabolite identification.
Results of water analyses indicate that mean measured concentrations represented 97.4 and 82.0% of the nominal [14C-GP]trifloxystrobin concentration (0.16 and 1.6 µg/L trifloxystrobin, respectively). The mean BCFs measured as ratio of the radioactivity levels in fish and water at steady state in fish amounted to 90 - 131 (edible fillet). 530 – 835 (non-edible viscera), and 280 – 431 (whole body) for

the high and low exposure level. The steady state of uptake and elimination was reached within 3 days at the low exposure level and within 14 days at the high level.

Within 24 h of being placed in clean water, ^{14}C-levels in fish had fallen to 69 and 73.4% of final 28 day exposure levels for the high and low exposure concentration, respectively. At the end of the 14-day depuration period, greater than 98% of the accumulated radioactive residue was eliminated from the fish tissue. The respective times for 50 and 90% depuration were given as 0.5 to 2.4 days and 1.5 to 7.8 days.

Following 28-day exposure of 1.6 µg/L [14C-GP]trifloxystrobin the parent substance proved to be the predominant residue component in edible tissue of fish (total radioactive residues, TRR = 0.107 mg equ/kg) amounting to 0.082 mg/kg (76.6% of TRR). The hydrolysed carboxylic acid CGA 321113 (M5) was detected at a level of 0.012 mg equ/kg (11.2% of TRR). Other identified (CGA 331409, M4, Metabolite B) and unknown metabolites (Metabolites A) were below 0.01 mg equ/kg. Approximately 96% of TRR in the edible tissue was identified.

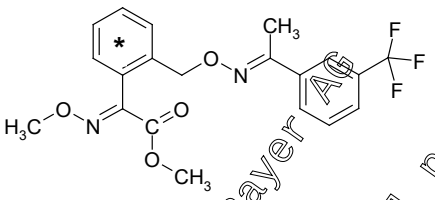
In non-edible viscera (TRR = 1.72 mg equ/kg), the parent substance also revealed to be the main residue component amounting to 0.537 mg/kg (31.2% of TRR). The carboxylic acid CGA 321113 (M5) could be detected at a level of 0.179 mg equ/kg (10.4 % of TRR). In addition, a cysteine conjugate of the demethylated carboxylic acid (Metabolite B) was detected at a level of 0.503 mg equ/kg (29.2% of TRR). A glucuronide conjugate of the parent substance (Metabolite C, glucuronic acid linked to the demethylated aminooxi substituent of the glyoxyl-phenyl ring; NOA 405637 (M27)-glucuronide) was found at a level of 0.190 mg equ/kg (11.0% of TRR). Minor metabolites in viscera were the *EZ*-isomer of the parent substance, i.e. CGA 331409 (M4. 2.7% of TRR), a *ZE* isomer of the carboxylic acid CGA 373466 (M6. 0.8% of TRR), a cyano metabolite CGA 357276 (M42. 3.4% of TRR), and a very minor cyclised derivative CGA 320299 (M49). Approximately 89% of TRR in non-edible viscera tissue was identified. The same metabolites were also detected in the aquarium water.

All main metabolites in fish and water contained the intact basic molecular structure with both phenyl rings. As a consequence, fish metabolism using the second [14C-TP] label would result in the same metabolites and, therefore, deemed not to be needed.

A proposal of the metabolic pathway of trifloxystrobin in fish is shown in Figure 6.2.5- 1. The metabolic pattern in fish provided the parent substance trifloxystrobin as the dominant residue component in edible and main residue component in non-edible fish tissue. These results suggest using the parent substance as marker substance in potential residue analysis of trifloxystrobin residues in fish.

Materials and Methods

Test Material

Structural formula	 * denotes ^{14}C label A. [14C-GP]
Chemical name	(*E.E*)-methoxyimino-{2-[1-(3-trifluoromethyl-phenyl)-ethylideneamino-oxymethyl]-phenyl}acetic acid methyl ester (IUPAC); (*E.E*)-α-(methoxyimino)-2-[[[[1-[3-(trifluoromethyl)phenyl]ethylidene]amino]oxy]methyl]benzene acetic acid methyl ester (CAS)
Common name	Trifloxystrobin
CAS RN	141517-21-7
Empirical formula	$C_{20}H_{19}F_3N_2O_4$
Company code	CGA 279202
Molar mass (non-labelled)	408.4 g/mole
Label	[glyoxyl-phenyl-UL-^{14}C]Trifloxystrobin. abbr. [14C-GP] Label A in the original report
Specific radioactivity	82 mCi/g (3034 MBq/g)
Radiochemical purity	98.4% (primary stock solution); 98.0 and 97.5% (diluter stock solution)
Chemical purity	

Test Organism and Test Conditions

Test species	Bluegill sunfish (*Lepomis macrochirus*)
Breeder	[redacted]. USA
Acclimatization	14 days in a 1000 L steel tank using 16 h light/8 h dark photoperiods
Mean body weight	1.5 g at test initiation
Mean body length	44 mm at test initiation
Test aquaria	Glass aquaria: 75 cm x 39 cm x 30 cm (length. width. height). filled with 73 L water
Number of fish	250 fishes per aquarium at initiation of the exposure
Total biomass	380 g / aquarium

	corresponding to 0.628 g/L of the 24-hour flow-through volume
Feeding	Commercial dry pellet food. approx. 2% of the total biomass; uneaten food and fecal matter were removed (by siphon) 30 min after feeding
Exposure concentration	1.6 and 0.16 µg as/L plus solvent control for BCF determination 1.6 µg/L for metabolism investigation
Exposure period	28 days
Depuration period	14 days
pH of aquarium water	6.8 – 7.1
Temperature	16 - 19°C
Dissolved oxygen	> 60% saturation
Hardness	32 - 36 mg/L
Alkalinity ($CaCO_3$)	22 – 24 mg/L
Photoperiods	16h light / 8h dark cycles

Set-up of the flow-through system

[14C-GP]trifloxystrobin was dissolved in acetone (primary stock solution) at a concentration of 1.15 mg/mL. This solution was diluted with acetone to prepare the diluter stock solutions, i.e. 30.5 mg/L for the 0.16 µg/L exposure and 305 mg/L for the 1.6 µg/L exposure. The exact concentrations were measured via radioassaying (LSC). The diluter stock delivery rates were 0.0022 mL/min for the diluter stock solutions and 420 mL/min for the inflowing water. This delivery rate provided a turnover rate equivalent to 8 aquarium volumes per 24 hours. The diluter stock solutions were delivered by syringe pumps equipped with 10 mL gas-tight glass syringes. This system produced an acetone concentration in the aquarium of 5 µL/L water for the low exposure level and of 0.1 mL/L water for the high exposure level. Due to the rapid metabolism of trifloxystrobin in fish and excretion of metabolites into the water the stock solution and water inflow were increased by approx. 50% on Day 10 of exposure resulting in a turnover rate of 12 aquarium volumes per 24 hours. The exposure concentration in the water was measured by radioassaying (LSC).

Sampling of fish and aquarium water as well as radioassaying

During the study, 5 fish of the BCF experiment were removed from each group for radioassaying of the edible and viscera tissues at days 1, 3, 7, 10, 14, 16, 21 and 28 of exposure, and at 1, 3, 7, 10 and 14 days after the depuration phase was initiated. Five fish were also collected from the metabolism aquarium on days 21 and 28. These fish were dissected into three portions, edible, viscera and carcass. On day 28, the remaining 238 fish from the metabolism aquarium were harvested for metabolite identification.

Together with fish sampling triplicate water samples were also collected (50 mL low exposure or 5 mL high exposure). The radioactivity of 5-mL water samples were directly measured by LSC after mixing with scintillation cocktail (radioassaying). The 50-ml water samples were acidified to pH 2 with phosphoric acid and extracted with ethyl acetate. The organic phase was separated, concentrated to dryness re-dissolved in 5 mL acetonitrile and radioassayed and analysed by radio-HPLC. Fish were dissected into edible (muscle. fillet) and non-edible portion (viscera and carcass). These matrices were weighed (wet weight) and radioassayed after air-drying overnight, combustion and radioactivity determination of the formed $^{14}CO_2$.

Radioassaying was conducted by liquid scintillation counting (LSC) using counters with automatic quench correction. The LOQ of LSC depended on the specific radioactivity the test substance, counting efficiency, sample size and acceptable minimum net counts per minute (cpm). For water samples the LOQ was set to 0.00476 µg/L. for tissue samples LOQ was set to 0.685µg/kg.

Determination of the lipid content

Eight total fish were weighed and homogenized in chloroform and methanol using a biohomogenizer. The mixture was filtered and the filtrate partitioned against aqueous sodium chloride. The chloroform phase was separated, dried with anhydrous sodium sulfate and concentrated to dryness. The remaining lipid sample was weighed again and compared to the original wet weight of the fish.

Extraction and clean-up of the residues from tissues

Edible and non-edible tissue samples were separately extracted (2x) with acetonitrile using a biohomogenizer. The extracts were combined, concentrated, centrifuged, radioassayed and analysed by radio-TLC and radio-HPLC. The remaining solids were further extracted with acetonitrile/water (1/1). The extract was concentrated and cleaned-up by solid phase extraction (SPE) using C18 columns. The residues absorbed to the C18 phase were eluted with acetonitrile. All fractions were radioassayed. The extracts from viscera were analysed by radio-LSC, radio-HPLC and LC/MS.

Chromatographic analysis

Radio-HPLC was performed using a RP18 column (250 x 4.0 mm. particle size 5 µm) that was operated with a gradient mixture of 0.1% aqueous formic acid and acetonitrile. The system was equipped with an UV detector (250 nm) and a fraction collector for collection HPLC eluent in 1-min intervals. The fractions were radioassayed for construction of radiohistograms of the HPLC eluent. Non-labelled reference standards were used for identification.

One- and two-dimensional radio-TLC was performed normal phase silica gel and RP18 F_{254} coated silica gel plates. One-dimensional normal phase plates were developed with pure acetonitrile, one-dimensional reversed phase plates with a mixture of acetonitrile/water/acetic acid (50/50/0.5. v/v/v). Two dimensional silica gel plates were developed in perpendicular directions with the two solvent mixtures (1) chloroform/methanol/formic acid/water (83/13/3/1. v/v/v/v) and (2) toluene/ethyl acetate/acetic acid (90/10/4. v/v/v). Radiolabelled spots were visualized by a radioanalytic imaging system, non-labelled reference standards by extinction of fluorescence caused be UV excitation (254 nm) of the fluorescence dye added to the silica gel separation layer.

LC/MS was performed using a HPLC system with a RP18 column (250 x 4 mm. particle size 5 µm) that was operated with a gradient mixture of acetic acid and acetonitrile and coupled with triple quadruple mass spectrometer. Ionization was achieved by an atmospheric pressure ionization (API) interface fitted with an electrospray ionization (ESI) head.

Findings

Quality control

Throughout the study, no undissolved material was observed in the dilution system or the test aquaria. On Days 13 and 26, one mortality was observed in the 0.16 µg/L and 1.6 µg/L aquaria, respectively.

The mean measured concentrations of [14C-GP]trifloxystrobin in water of the BCF trials represented 97.4% (0.156 ± 0.028 µg/L) and 82.0% (1.31 ± 0.27 µg/L) of the low and high nominal concentrations (measured as total radioactive residues, TRR). In the metabolism trial with a nominal water concentration of 1.6 µg/L, the mean measured concentration amounted to 84.9% (1.36 ± 0.20 µg/L) over the 28-day exposure.

Total radioactive residues (TRR) in fish tissue and derived bioconcentration factors (BCF)

For the bluegill exposed to the low concentration of radiolabelled trifloxystrobin, the radioactive residues (TRR) accumulated rapidly and plateaued within one day for the edible tissue, and within 3 days for non-edible and whole body tissues. For the bluegill exposed to the high concentration, the radioactive residues plateaued within 14 days for edible, non-edible and whole body tissues, respectively. The mean BCFs measured as ratio of the radioactivity levels in fish and water at steady state in fish amounted to 90 - 131 (edible fillet). 530 – 835 (non-edible viscera), and 280 – 431 (whole body) for the high and low exposure level. The mean TRR in water and fish tissue during the plateau period and the derived bioconcentration factor (BCF) are presented in Table 6.2.5- 1.

After the exposure the remaining fish were transferred to pure water for examination of the depuration of radioactive residues. 69% (low exposure level) or 73% (high exposure level) of TRR in the tissue of the last day of exposure had depurated within 24 hours in clean water. The half-life of elimination was thus derived as $DT50_{elim}$ = 0.6 days for the low dose and $DT50_{elim}$ = 0.7 days for the high exposure level.

Extractability of residues from fish and identification/characterization of extracted residues

A separate trial for investigation of the metabolism of trifloxystrobin in fish was conducted using the high exposure level (nominal1.6 µg/L). Following a 28-day exposure fish were collected, radioassayed and extracted with acetonitrile and acetonitrile/water. Extractability of radiolabelled residues from fish tissue was very high amounting to 99.6% of TRR in the edible tissue (TRR: 0.107 mg equ/kg) and 91.1% of TRR in the viscera tissue (TRR: 1.72 mg equ/kg).

The residues extracted from viscera were analysed by radio-HPLC. Some of the radiolabelled peaks were eluted with the same retention times as the non-labelled reference standards of parent trifloxystrobin, i.e. CGA 373466 (M6), CGA 321113 (M5), CGA 357276 (M42), and CGA 331409 (M4). The identity of these radioactive compounds was finally confirmed by LC/MS. Thus, the ester hydrolysis metabolite CGA 321113 (M5) accounted for 10.4% of TRR (0.179 mg equ/kg), the isomer of the parent substance CGA 331409 (M4) for 2.7% of TRR and an isomer of the hydroxylated carboxylic acid CGA 373466 (M6) for 0.8% of TRR. A cyano derivative CGA 357276 (M42) amounted to 3.4% of TRR. A very minor cyclized derivative CGA 320299 (M49) was only found in trace levels.

The residue level of Metabolite A was below 0.05 mg equ/kg; therefore it was not further identified or characterized. Metabolites B and C were isolated from viscera extracts by radio-HPLC with fraction collection and purified by radio-TLC. LC/MS of the purified metabolite B resulted in protonated molecule ion at m/z of 502 amu. Natural isotopic abundance of the involved elements and LC/MS/MS investigation (including distinct fragmentation) suggested an elemental composition of $C_{21}H_{22}N_3O_6F_3S$. This formula could be interpreted by a cysteine conjugate of the carboxylic acid NOA 412443 (M29). LC/MS of Metabolite C resulted in a protonated molecule ion at m/z 571 amu. It could be interpreted as glucuronide conjugate of the parent substance following demethylation of methoxyamino group. Incubation with glucuronidase released the aglycon that was identified as NOA 405637 (M27) by LC/MS.

The concentration of the residue components extracted from edible tissues (fillet) was significantly lower than those in viscera. Therefore, spectroscopic identification was not possible. However, they could be assigned by comparative chromatographic profiling with the viscera metabolites. The most prominent residue component was the parent substance trifloxystrobin amounting to 76.6% of TRR (equivalent of 0.082 mg/kg). The composition of parent and metabolites in fillet and viscera is shown in Table 6.2.5- 2.

Conclusion

Bluegill sunfish were exposed to [14C-GP]trifloxystrobin via a flow-through study design at two exposure levels (nominal 0.16 and 1.6 µg/L) for a total exposure period of 28 days. The main objective of this study was the determination of a bioconcentration factor (BCF). Nevertheless, in an additional trial also the metabolism of trifloxystrobin in fish was investigated following a 28-day exposure of 1.6 µg ^{14}C-trifloxystrobin/L water.

The steady state between uptake and elimination of radiolabelled residues was reached after 3 or 14 days of exposure for the low or high exposure level. The BCF values at steady state accounted for 90 - 131 (edible fillet). 530 – 835 (non-edible viscera), and 280 – 431 (whole body) for the high and low level. Following transfer of remaining fish into pure water without test substance the half-life of elimination was less than one day for low and high exposure level.

The metabolites in non-edible viscera (TRR = 1.72 mg equ/kg) were identified by co-chromatography with reference standards and by LC-MS/MS analysis. The metabolites in edible fillet tissue (TRR = 0.107 mg equ/kg) could by identified by comparative profiling with the metabolites identified in viscera. Major metabolites were formed by hydrolytic ester cleavage of trifloxystrobin resulting in the carboxylic acid CGA 321113 (M5) and by cysteine conjugation of the aminooxy-demethylated carboxylic acid resulting in M29-cysteine (Metabolite B). Glucuronic acid conjugation of the aminooxy-demethylated parent substance resulted in the NOA 405637 (M27) glucuronide (Metabolite C). Minor metabolites were identified as an isomer of the parent substance (CGA 331409, M4), an isomer of the primary carboxylic acid CGA 373466 (M6) as well as a cyano derivative CGA 357276 (M42) and cyclized metabolite CGA 320299 (M49). The same metabolites were also detected in the aquarium water. All main metabolites in fish and water contained the intact basic molecular structure with both phenyl rings. As a consequence, fish metabolism using the second [14C-TP] label would result in the same metabolites and, therefore, deemed not to be needed.

A proposal of the metabolic pathway of trifloxystrobin in fish is shown in Figure 6.2.5- 1. The metabolic pattern in fish provided the parent substance trifloxystrobin as the dominant residue component in edible and main residue component in non-edible fish tissue. These results suggest using the parent substance as marker substance in residue analysis of trifloxystrobin residues in fish.

A conclusion on a potentially required fish feeding study cannot be drawn as no official feeding table for fish feed is currently available. Therefore, it is not possible to estimate whether the expected residue level in edible fish tissue may exceed the trigger level of 0.01 mg/kg.

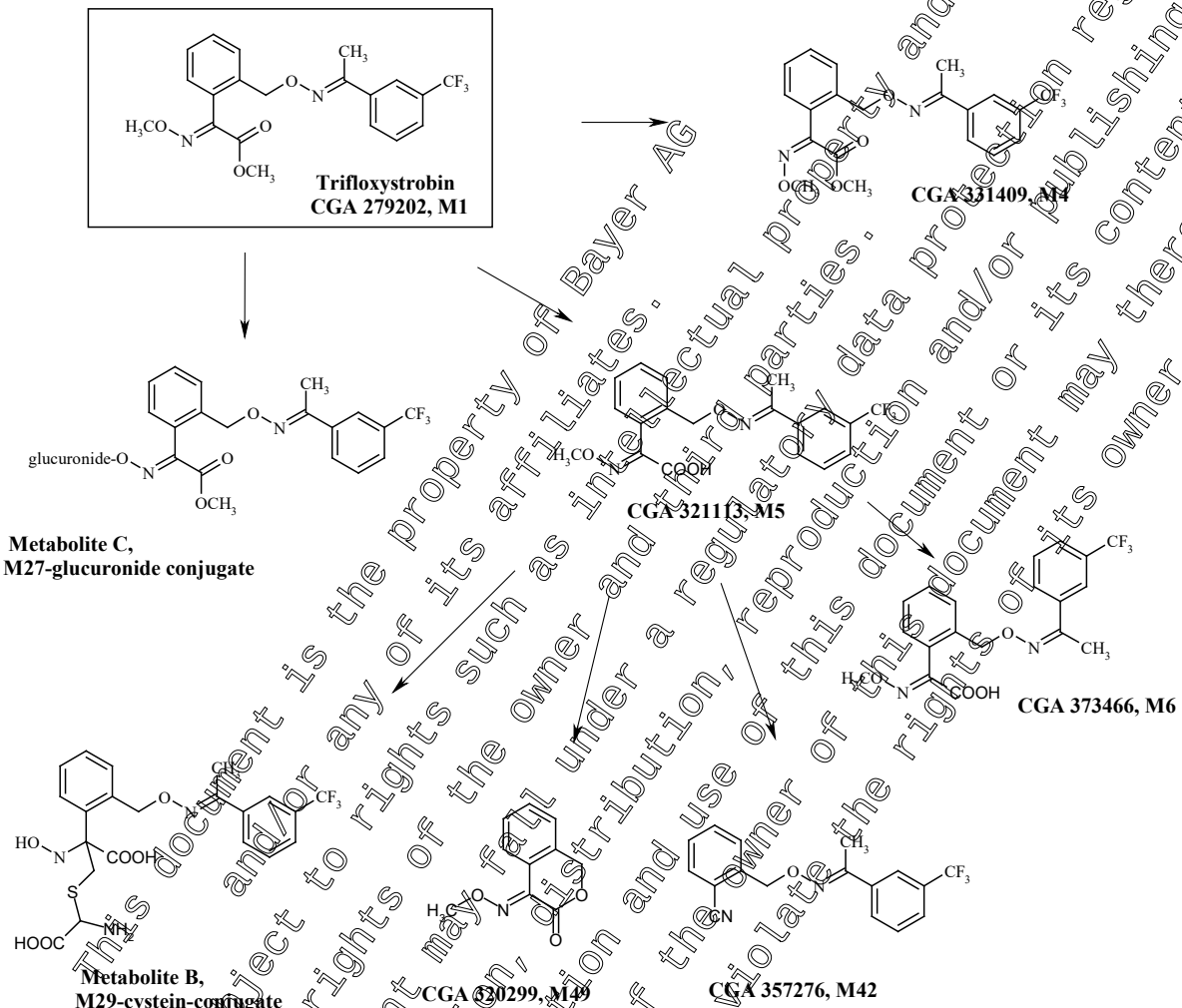
Table 6.2.5- 1: Total radioactive residues (TRR) in water and fish and bioconcentration factors (BCF)

Exposure level	Mean TRR in water [µg equ/L]	Mean TRR in fish [µg equ/kg]			Bioconcentration factor (BCF)		
		Edible	Non-edible	Whole body	Edible	Non-edible	Whole body
Low Exposure		during the plateau period 1; 4 - 28 days					
	0.156	20.5	13	67.2	131	835	431
High Exposure		during the plateau period 14 – 28 days					
	1.31	118	695	367	90	530	280

Table 6.2.5- 2: Residues in fillet and viscera tissue of fish exposed to [14C-GP]trifloxystrobin at a nominal concentration of 1.6 µg/L for 28 days (mean of two extractions)

Residue component	Edible fillet		Non-edible viscera	
TRR [mg equ/kg]	0.170		1.72	
	[% of TRR]	[mg equ/kg]	[% of TRR]	[mg equ/kg]
Parent trifloxystrobin (CGA 279202)	**76.6**	**0.082**	**31.2**	**0.537**
Metabolite A (unknown)	3.74	0.004	1.45	0.025
Metabolite B (M29-cystein conjugate)	3.74	0.004	29.2	0.503
Metabolite C (glucuronide of parent hydroxyl amine)	-	-	11.0	0.190
M5, CGA 321113	11.2	0.012	10.4	0.179
M4, CGA 331409	4.67	0.005	2.73	0.047
M6, CGA 373466	-	-	0.81	0.014
M42, CGA 357276	-	-	3.43	0.059
Sum	**99.95**	**0.170**	**90.22**	**1.554**

Figure 6.2.5- 1: Proposed metabolic pathway of trifloxystrobin in Bluegill sunfish



CA 6.3 Magnitude of residue trials in plants

Residue data on pomefruit, grape and strawberry is summarised below. Further data (cereals and other crops) may be found in chapter 6.10.

The detailed tables (Tier 1) of the supplementary trials and the processing trials are submitted in a separate document as additional information for the evaluator:

Report:	KCA 6.3 /02; [redacted];2014;M-467298-02; Amended: 2014-11-26
Title:	Tier 1 Summary of the residues data and processing studies for trifloxystrobin
Report No: Document No: Guidelines:	M-467298-02-1 M-467298-02-1 EU Regulation 1107/2009 & EU Regulation 283/2013
GLP/GEP:	no

CA 6.3.1 Pome fruit

Former Annex II dossier

In the Annex II dossier, the critical GAP for trifloxystrobin supported at the European level (northern and southern Europe) consisted of up to 10 foliar spray applications at rates of 75 g a.s./ha trifloxystrobin and a PHI of 14 days.

Annex I renewal process: New studies

The critical Good Agricultural Practice (cGAP) supported at the European level in the Annex I renewal (AIR) process consists of 3 foliar spray applications at 75 g a.s./ha trifloxystrobin in northern Europe and 112.5 g a.s./ha trifloxystrobin in southern Europe, with a minimum spray interval of 10 days and a PHI of 14 days.

Table 6.3.1-1: Summary of the critical GAP for the proposed uses of Trifloxystrobin WG 50

Crop	Region*	F, G or I**	Maximum Number of Applications	Minimum Application Interval (days)	Maximum Rate (g a.s./ha)	Minimum PHI (days)
Pome fruit	EU-N	F	3	10	75	14
Pome fruit	EU-S	F	3	10	112.5	14

* EU-N northern Europe, EU-S southern Europe ** F Field; G Greenhouse; I Indoor

Trials available to support the European GAP relevant for Annex I renewal are summarised in Table 6.3.1-2 and Table 6.3.1-3

Table 6.3.1- 2: Residue trials conducted per geographical region and formulation

Region	Crop	Formulation	Number of Trials					Total	Report-No.	Document N°	Dossier-Ref.
			Vegetation period								
			1999	2003	2004	2006	2011				
Supplementary data											
N-EU	Apple Pear	WG 50	1	-	-	-	-	13	2007/99	M-024932-01-1	KCA 6.3.1/31
			1	-	-	-	-		2109/99	M-030455-01-1	KCA 6.3.1/32
			1	-	-	-	-		2124/99	M-030187-01-1	KCA 6.3.1/33
			1	-	-	-	-		2125/99	M-136411-01-1	KCA 6.3.1/34
			-	-	-	-	4		11-2117	M-457963-01-1	KCA 6.3.1/39
		WG 75	-	-	-	1	-		RA-2006/06	M-292645-01-1	KCA 6.3.1/35
		WG 64	-	1	-	-	-		RA-2170/03	M-061865-01-1	KCA 6.3.1/36
		WG 68.8	-	-	1	-	-		RA-2044/04	M-256712-01-1	KCA 6.3.1/37
			-	-	2	-	-		RA-2046/04	M-257107-01-1	KCA 6.3.1/38
S-EU	Apple Pear	WG 50	-	-	-	-	4	9	11-2116	M-457957-01-1	KCA 6.3.1/43
		WG 64	-	1	-	-	-		RA-2171/03	M-061855-01-1	KCA 6.3.1/40
		WG 68.8	-	-	2	-	-		RA-2045/04	M-255883-01-1	KCA 6.3.1/41
			-	-	2	-	-		RA-2047/04	M-257391-01-1	KCA 6.3.1/42

N-EU northern Europe S-EU southern Europe
WG 50: wettable granule formulation containing 50% trifloxystrobin
WG 75: wettable granule formulation containing 50% tebuconazole and 25% trifloxystrobin
WG 64: wettable granule formulation containing 60% captan and 4% trifloxystrobin
WG 68.8: wettable granule formulation containing 62.5% tolylfluanid and 6.3% trifloxystrobin

Table 6.3.1- 3: Overall summary of residue data on pome fruit covering the critical GAP for Annex I renewal

Application Rate	Region	Formulation	Crop	Sample material	n	Residue level (mg/kg) trifloxystrobin		
						Min.	Max.	STMR
3 applications at about 75 g/ha	N-EU	WG	Apple / Pear	Fruit	13	<0.02	0.11	0.05
3 applications at about 112.5 g/ha	S-EU	WG	Apple / Pear	Fruit	9	0.02	0.17	0.10

N-EU northern Europe S-EU southern Europe n: number of trials

Field trials – northern Europe:

Report:	**KCA 6.3.1/31, [redacted]; 2000** ; M-024932-01-1
Title:	Residue study with CGA 279202 in or on apples in France (north)
Document No & Report No:	M-024932-01-1 2007/99
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.3.1/32, [redacted]; 2000;** M-030455-01-1
Title:	Residue study with CGA 279202 in or on apples in Netherlands

Document No & Report No:	M-030455-01-1 2109/99
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.3.1/33, [redacted]; 2000**; M-030187-01-1
Title:	Residue study with CGA 279202 in or on apples in Switzerland
Document No & Report No:	M-030187-01-1 2124/99
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.3.1/34, [redacted]; 2000** ; M-136411-01-1
Title:	Residue study with CGA 279202 in or on apples in Switzerland
Document No & Report No:	M-136411-01-1 2125/99
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.3.1/35, [redacted]; 2007** ; M-292645-01-1
Title:	Determination of the residues of trifloxystrobin and tebuconazole in/on apple after spraying of CGA 279202 & HWG 1608 (75 WG) in the field in Germany
Document No & Report No:	M-292645-01-1 RA-2006/06
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.3.1/36, [redacted], [redacted]; 2004** ; M-061865-01-1
Title:	Determination of residues of trifloxystrobin and captan in/on apple after spraying and low-volume spraying of trifloxystrobin & Captan (64 WG) in Germany, Belgium and Great Britain
Document No & Report No:	M-061865-01-1 RA-2170/03
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.3.1/37, [redacted]; 2005** ; M-256712-01-1
Title:	Determination of the residues of trifloxystrobin and tolylfluanid in/on apple after spraying of CGA279202 & KUE 13183B (68.8 WG) in the field in Belgium and the Netherlands

Document No & Report No:	M-256712-01-1 RA-2044/04
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.3.1/38, [redacted]; 2005**; M-257107-01-1
Title:	Determination of the residues of trifloxystrobin and tolylfluanid in/on pear after spraying of CGA279202 & FUE13183B (68.8 WG) in the field in United Kingdom and Germany
Document No & Report No:	M-257107-01-1 RA-2046/04
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Test system

In 1999 to 2006 nine trials were performed in northern Europe on apple or pear trees with trifloxystrobin WG formulations according to the use pattern supported within this dossier. The products were applied three times at application rates of about 0.075 (0.062 – 0.079) kg trifloxystrobin/ha. The treatments were performed with intervals of about 10 (7 to 12) days.
Fruits samples were taken on day 14 (13) after the last application in all trials. Additional samples of fruit were taken at earlier or later time points.

Residues of trifloxystrobin and CGA 321113 were determined according to methods AG-659, 00839, 00839/E001 or 00742/M002. The analytical methods were validated by recovery experiments prior to and during the analysis of the samples by spiking control samples. The limit of quantitation was 0.01 or 0.02 mg/kg for both analytes.

Findings

- Method performance: Overall mean recoveries at fortification levels between 0.01 and 1.0 mg/kg were within the acceptable range of 70-110 %, RSD <20% as shown in Table 6.3.1-4.

Table 6.3.1-4: Recoveries for trifloxystrobin and CGA321113 in/on apple and pear

Report No.	Analyte	Sample Material	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
2007/99	Trifloxystrobin	apple fruit	0.02	103			0.02
			0.2	104			
2007/99	CGA 321113	apple fruit	0.02	91			0.02
			0.2	101			
2009/99	Trifloxystrobin	apple fruit	0.02	81			0.02
			0.2	90			
	CGA 321113	apple fruit	0.02	70			0.02
			0.2	94			

Report No.	Analyte	Sample Material	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
2124/99	Trifloxystrobin	apple fruit	0.02	90			0.02
			0.2	104			
	CGA 321113	apple fruit	0.02	87			0.02
			0.2	113			
2125/99	Trifloxystrobin	apple fruit	0.01	97; 92; 130	106	19.4	0.01
			0.1	94; 94; 102	97	4.8	
			Overall Recovery (n =6)		102	14.2	
	CGA 321113	apple fruit	0.01	76; 75	75	-	0.01
			0.1	89; 92	91	-	
			Overall Recovery (n =4)		83	10.9	
RA-2170/03	Trifloxystrobin	apple fruit	0.02	93; 98; 95	95	2.6	0.02
			0.2	97; 95; 94; 97; 96; 94; 93; 96	95	1.8	
			Overall Recovery (n =11)		95	1.9	
	CGA 321113	apple fruit	0.02	71; 87; 86	81	11.0	0.02
			0.2	94; 92; 92; 96; 100; 102; 92; 96	96	4.0	
			Overall Recovery (n =11)		92	9.1	
RA-2044/04*	Trifloxystrobin	apple fruit	0.02	100; 100	100	-	0.02
			0.2	97; 101; 101; 101	100	2.0	
			Overall Recovery (n =6)		100	1.5	
	CGA 321113	apple fruit	0.02	98; 113	106	-	0.02
			0.2	109; 109; 104; 103	106	3.0	
			Overall Recovery (n =6)		106	5.1	
RA-2046/04*	Trifloxystrobin	pear fruit	0.02	97; 94	96	-	0.02
			0.2	92; 91; 87; 91	90	2.5	
			Overall Recovery (n =6)		92	3.6	
	CGA 321113	pear fruit	0.02	89; 94	92	-	0.02
			0.2	94; 89; 90; 97	93	4.0	
			Overall Recovery (n =6)		92	3.6	
RA-2006/06	Trifloxystrobin	apple fruit	0.01	92; 92; 100	95	4.9	0.01
			1.0	86; 88; 89	88	1.7	
			Overall Recovery (n =6)		91	5.4	
	CGA 321113	apple fruit	0.01	86; 103, 92	94	9.2	0.01
			1.0	86; 91; 89	89	2.8	
			Overall Recovery (n =6)		91	6.9	

RSD = relative standard deviation n = number of tests
* Single recoveries are not available in the report, but given in the BCS residue data base and the raw data

- Storage stability: The maximum storage period of deep-frozen samples was up to 272 days for trifloxystrobin and CGA 321113 and is covered by the storage stability studies.

- Residue results: In the northern European field trials the residues in apple and pear fruit at a PHI of 14 (13) days ranged from <0.02 to 0.09 mg/kg for trifloxystrobin and were <0.01 mg/kg or <0.02 mg/kg for CGA 321113.

- No residues above the LOQ of 0.01 mg/kg could be detected in any of the corresponding control samples.

Table 6.3.1-5: Application data and residues of trifloxystrobin and CGA 321113 in/ on apple and pear treated with Trifloxystrobin WG formulations in the field in northern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Trifloxystrobin (mg/kg)	CGA 321113 (mg/kg)
2007/99 FRA-2007-99 GLP: yes 1999	Apple Golden Smothee	France [redacted] Europe, North	50 WG	3	0.075	0.015	85	fruit	0 7 **14** **14**	0.10 0.03 0.03 0.02	<0.02 <0.02 <0.02 <0.02
2109/99 NIE-2109-99 GLP: yes 1999	Apple Jonagored	Nether-lands [redacted] Europe, North	50 WG	3	0.0643 0.0763	0.0299-0.0300	81	fruit	0 7 **14** **14**	0.08 0.04 0.03 0.04	<0.02 <0.02 <0.02 <0.02
2124/99 SWZ-2124-99 GLP: yes 1999	Apple Prime-rouge	Switzer-land [redacted] Europe, North	50 WG	3	0.075	0.0075	84 85	fruit	0 7 **14** **14**	0.19 0.08 0.03 0.04	<0.02 <0.02 <0.02 <0.02
2125/99 SWZ-2125-99-A GLP: yes 1999	Apple Golden Smoothy	Switzer-land [redacted] Europe, North	50 WG	3	0.075	0.0075	89	fruit	0 7 **14** **14**	0.08 0.06 0.05 0.03	<0.01 <0.01 <0.01 <0.01
RA-2006/06 R 2006 0124/7 0124-06 GLP: yes 2006	Apple Golden Delicius	Germany D-[redacted] Europe, North	75 WG	3	0.065	0.0050	87	fruit	0* 0 3 7 **14**	0.10 0.05 0.08 0.06 0.05	<0.01 <0.01 <0.01 <0.01 <0.01
RA-2170/03 R 2003 0079/4 0079-03 GLP: yes 2003	Apple Jonagold	Belgium B-[redacted] Europe, North	64 WG	3	0.0625	0.0250	85	fruit	0 **14**	0.05 <0.02	<0.02 <0.02
RA-2044/04 R 2004 0182/5 0182-04 GLP: yes 2004	Apple Jonagold	Nether-lands NL-[redacted] Europe, North	68.8 WG	3	0.079	0.0063	78	fruit	0* 0 3 7 **14** 21	<0.02 0.06 0.04 0.04 0.03 <0.02	<0.02 <0.02 <0.02 <0.02 <0.02 <0.02

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Trifloxystrobin (mg/kg)	CGA 321113 (mg/kg)
RA-2046/04 R 2004 0183/3 0183-04 GLP: yes 2004	Pear Confe-rence	United Kingdom GB-CB4 5HG Cambrid-ge Europe, North	68.8 WG	3	0.063	0.0063	81	fruit	0* 0 3 6 **13** 20	0.05 0.18 0.10 0.09 0.05 0.05	<0.02 <0.02 <0.02 <0.02 <0.02 <0.02
RA-2046/04 R 2004 0184/1 0184-04 GLP: yes 2004	Pear Gellerts Butter-birne	Germany D-65366 Geisen-heim Europe, North	68.8 WG	3	0.063	0.0063	85	fruit	0* 0 3 7 **14** 21	0.06 0.13 0.12 0.10 0.09 0.05	<0.02 <0.02 <0.02 <0.02 <0.02 <0.02

FL: Formulation No: number of applications
GS = growth stage (BBCH code) at last application DALT = days after last treatment * prior to last treatment
RA-2006/06, 0124-06: It can be assumed that sample 0 and 0* were be mixed up and that the higher values belong to the 0 sample

Report:	**KCA 6.3.1/39, [redacted] 2013**; M-457963-01-1
Title:	Determination of the residues of trifloxystrobin in/on apple and pear after spray application of trifloxystrobin WG 50 in the field in Germany, northern France and United Kingdom
Document No & Report No:	M-457963-01-1 11-2117
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Test system

In 2011 four trials were performed in northern Europe on apple or pear trees with trifloxystrobin WG 50 according to the use pattern supported within this dossier. The product was applied three times at application rates of 0.075 kg trifloxystrobin/ha. The treatments were performed with intervals of 9-10 days.
Fruits samples were taken on day 14 (15) after the last application in all trials. Additional samples of fruit were taken at earlier or later time points in two trials.

Residues of trifloxystrobin (CGA 279202), its isomers CGA 331409, CGA 357261, CGA 357262, as well as the metabolite CGA 321113 and its isomer CGA 373466 were determined according to method 01313. The analytical method was validated by recovery experiments prior to and during the analysis of the samples by spiking control samples. The limit of quantitation was 0.01 mg/kg for all analytes.

Findings

- Method performance: Overall mean recoveries at fortification levels between 0.01 and 0.5 mg/kg per analyte were within the acceptable range of 70-110 %, RSD <20% as shown in Table 6.3.1-6.

Table 6.3.1-6: Recoveries for trifloxystrobin, CGA321113, CGA 357261, CGA 357262, CGA 331409, CGA 373466 in/on apple and pear

Report No.	Analyte	Sample Material	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
11-2117	Trifloxystrobin	apple fruit	0.01	87	87	-	0.01
			0.1	79	79	-	
			0.5	86	86	-	
			Overall Recovery (n =3)		84	5.2	
	Trifloxystrobin	pear fruit	0.01	86; 89	88	-	0.01
			0.1	104	104	-	
			0.5	96	96	-	
			Overall Recovery (n =4)		94	8.5	
	CGA 321113	apple fruit	0.01	83	83	-	0.01
			0.1	74	74	-	
			0.5	81	81	-	
			Overall Recovery (n =3)		79	6.0	
	CGA 321113	pear fruit	0.01	83; 86	85	-	0.01
			0.1	94	94	-	
			0.5	92	92	-	
			Overall Recovery (n =4)		89	5.8	
	CGA 357261	apple fruit	0.01	84	84	-	0.01
			0.1	78	78	-	
			0.5	84	84	-	
			Overall Recovery (n =3)		82	4.2	
	CGA 357261	pear fruit	0.01	91; 93	92	-	0.01
			0.1	103	103	-	
			0.5	96	96	-	
			Overall Recovery (n =4)		96	5.5	
	CGA 357262	apple fruit	0.01	92	92	-	0.01
			0.1	81	81	-	
			0.5	86	86	-	
			Overall Recovery (n =3)		86	6.4	
	CGA 357262	pear fruit	0.01	90; 91	91	-	0.01
			0.1	100	100	-	
			0.5	102	102	-	
			Overall Recovery (n =4)		96	6.4	
	CGA 331409	apple fruit	0.01	88	88	-	0.01
			0.1	84	84	-	
			0.5	90	90	-	
			Overall Recovery (n =3)		87	3.5	

Report No.	Analyte	Sample Material	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
11-2117	CGA 331409	pear fruit	0.01	88; 94	91	-	0.01
			0.1	100	100	-	
			0.5	103	103	-	
			Overall Recovery (n =4)		96	6.9	
	CGA 373466	apple fruit	0.01	73	73	-	0.01
			0.1	74	74	-	
			0.5	81	81	-	
			Overall Recovery (n =7)		76	5.7	
	CGA 373466	pear fruit	0.01	74; 79	77	-	0.01
			0.1	104	104	-	
			0.5	96	96	-	
			Overall Recovery (n =7)		88	16.0	

RSD = relative standard deviation n = number of tests

- Storage stability: The maximum storage period of deep-frozen samples was up to 368 days for trifloxystrobin and its metabolite / isomers and is covered by the storage stability studies.

- Residue results: In the northern European field trials conducted in 2011 the residues at a PHI of 14 (15) days ranged from 0.044 to 0.11 mg/kg for trifloxystrobin and were <0.01 mg/kg for CGA 321113 and CGA 373466 in apple or pear fruit. Residues of CGA 357261 were between <0.01 and 0.035, residues of CGA 357262 were <0.01 or 0.0.012 mg/kg, and residues of CGA 331409 were <0.01 or 0.017 mg/kg at the PHI of 14 days.

- No residues above the LOQ of 0.01 mg/kg could be detected in any of the corresponding control samples.

Table 6.3.1-7: Application data and residues of trifloxystrobin and CGA 321113 in/ on apple and pear treated with Trifloxystrobin WG 50 in the field in northern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Trifloxy- strobin (mg/kg)	CGA 321113 (mg/kg)
11-2117 11-2117-01 11-2117-01-T GLP: yes 2011	Apple Jonagold	Germany [redacted] Europe, North	50 WG	3	0.075	0.015	79	fruit	0* 0 7 **15** 22 29	0.048 0.15 0.072 0.058 0.051 0.050	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
11-2117 11-2117-02 11-2117-02-T GLP: yes 2011	Pear Beurré Hardy	France [redacted] Europe, North	50 WG	3	0.075	0.0075	81	fruit	0 **14**	0.15 0.01	<0.01 <0.01
11-2117 11-2117-03 11-2117-03-T GLP: yes 2011	Pear Williams Christ	Germany [redacted] Europe, North	50 WG	3	0.075	0.0075	78	fruit	0* 0 7 **14** 21 28	0.061 0.17 0.084 0.057 0.034 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
11-2117 11-2117-04 11-2117-04-T GLP: yes 2011	Apple Cox's Early	United Kingdom [redacted] Europe, North	50 WG		0.075	0.0050	81	fruit	0 **14**	0.075 0.044	<0.01 <0.01

FL: Formulation No: number of applications
GS = growth stage (BBCH code) at last application DALT = days after last treatment * prior to last treatment

Table 6.3.1-8: Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in/ on apple and pear treated with Trifloxystrobin WG 50 in the field in northern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Residues Portion analysed	DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
11-2117 11-2117-01 11-2117-01-T GLP: yes 2011	Apple Jonagold	Germany [redacted] Europe, North	fruit	0* 0 7 **15** 22 29	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
11-2117 11-2117-02 11-2117-02-T GLP: yes 2011	Pear Beurre Hardy	France [redacted] Europe, North	fruit	0 **14**	0.022 0.035	<0.01 0.012	<0.01 0.017	<0.01 <0.01

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Residues Portion analysed	DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
11-2117 11-2117-03 11-2117-03-T GLP: yes 2011	Pear Williams Christ	Germany [redacted] Europe, North	fruit	0* 0 7 **14** 21 28	<0.01 <0.01 0.013 0.011 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
11-2117 11-2117-04 11-2117-04-T GLP: yes 2011	Apple Cox's Early	United Kingdom [redacted] Europe, North	fruit	0 **14**	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01

DALT = days after last treatment * prior to last treatment

Field trials – southern Europe:

Report:	**KCA 6.3.1/40, [redacted], [redacted]; 2004** ; M-061855-01-1
Title:	Determination of the residues of trifloxystrobin and captan in/on apple following spray application and low-volume spray application of Trifloxystrobin & Captan (64 WG) in Spain, Southern France, Portugal and Italy
Document No & Report No:	M-061855-01-1 RA-2171/03
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.3.1/41, [redacted]; 2005** ; M-255883-01-1
Title:	Determination of the residues of trifloxystrobin and tolylfluanid in/on pear after spraying of CGA279202 & KUE13183B (68.8 WG) in the field in Italy and Spain
Document No & Report No:	M-255883-01-1 RA-2045/04
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.3.1/42, [redacted]; 2005** ; M-257391-01-1
Title:	Determination of the residues of trifloxystrobin and tolylfluanid in/on pear after spraying of CGA279202 & KUE13183B (68.8 WG) in the field in Italy and Spain
Document No & Report No:	M-257391-01-1 RA-2047/04
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Test system

In 2003 and 2004 five trials were performed in southern Europe on apple or pear trees with trifloxystrobin WG formulations according to the use pattern supported within this dossier. The products were applied three times at application rates of 0.093 to 0.104 kg trifloxystrobin/ha. The treatments were performed with intervals of about 10 (7-11) days.
Fruits samples were taken on day 13/14 after the last application in all trials. Additional samples of fruit were taken at earlier or later time points in some trials.

Residues of trifloxystrobin, and CGA 321113 were determined according to method 00742/M002 or 00839/E001. The analytical methods were validated by recovery experiments prior to and during the analysis of the samples by spiking control samples. The limit of quantitation was 0.02 mg/kg for trifloxystrobin and CGA 321113.

Findings

- Method performance: Overall mean recoveries at fortification levels between 0.02 and 0.2 mg/kg per analyte were within the acceptable range of 70-110 %, RSD <20% as shown in Table 6.3.1-9.

Table 6.3.1-9: Recoveries for trifloxystrobin and CGA321113 in/on apple and pear

Report No.	Analyte	Sample Material	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
RA-2171/03	Trifloxystrobin	apple fruit	0.02	93; 98; 95	95	2.6	0.02
			0.2	97; 95; 94; 97; 96; 94; 92; 96	95	1.8	
			Overall Recovery (n =11)		95	1.9	
	CGA 321113	apple fruit	0.02	71; 87; 86	81	11.0	0.02
			0.2	94; 92; 92; 96; 100; 102; 92; 96	96	4.0	
			Overall Recovery (n =11)		92	9.1	
RA-2045/04*	Trifloxystrobin	apple fruit	0.02	100; 100	100	-	0.02
			0.2	97; 101; 101; 101	100	2.0	
			Overall Recovery (n =6)		100	1.5	
	CGA 321113	apple fruit	0.02	98; 113	106	-	0.02
			0.2	109; 109, 104; 103	106	3.0	
			Overall Recovery (n =6)		106	5.1	
RA-2047/04*	Trifloxystrobin	pear fruit	0.02	97; 94	96	-	0.02
			0.2	92; 91; 87; 91	90	2.5	
			Overall Recovery (n =6)		92	3.6	
	CGA 321113	pear fruit	0.02	89; 94	92	-	0.02
			0.2	94; 89; 90; 97	93	4.0	
			Overall Recovery (n =6)		92	3.6	

RSD = relative standard deviation n = number of tests
* Single recoveries are not available in the report, but given in the BCS residue data base and the raw data

- Storage stability: The maximum storage period of deep-frozen samples was up to 238 days for trifloxystrobin and CGA 321113 and is covered by the storage stability studies.

- Residue results: In the southern European field trials the residues at a PHI of 13/14 days ranged from 0.02 to 0.12 mg/kg for trifloxystrobin and were <0.02 mg/kg for CGA 321113.

- No residues above the LOQ of 0.02 mg/kg could be detected in any of the corresponding control samples.

Table 6.3.1-10: Application data and residues of trifloxystrobin and CGA 321113 in/on apple and pear treated with Trifloxystrobin WG formulations in the field in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
RA-2171/03 R 2003 0083/2 0083-03 GLP: yes 2003	Apple Jona-gold	Portugal P-[redacted] Europe, South	64 WG	3	0.093-0.104	0.0250	81	fruit	0 **14**	0.32 0.12	<0.02 <0.02
RA-2045/04 R 2004 0186/8 0186-04 GLP: yes 2004	Apple Florina	Italy I-[redacted] Europe, South	68.8 WG	3	0.095	0.0063	81	fruit	0* 0 3 7 **14** 21	0.02 0.09 0.06 0.03 0.02 0.02	<0.02 <0.02 <0.02 <0.02 <0.02 <0.02
RA-2045/04 R 2004 0187/6 0187-04 GLP: yes 2004	Apple Golden	Spain E-[redacted] Europe, South	68.8 WG	3	0.095-0.102	0.0063	85	fruit	0* 0 3 6 **14** 20	0.07 0.12 0.09 0.10 0.05 0.05	<0.02 <0.02 <0.02 <0.02 <0.02 <0.02
RA-2047/04 R 2004 0188/4 0188-04 GLP: yes 2004	Pear Abate Fetel	Italy I-[redacted] Europe, South	68.8 WG	3	0.0945	0.0063	81	fruit	0* 0 3 7 **14** 21	0.06 0.15 0.10 0.09 0.07 0.06	<0.02 <0.02 <0.02 <0.02 <0.02 <0.02
RA-2047/04 R 2004 0189/2 0189-04 GLP: yes 2004	Pear Confe-rence	Spain E-[redacted] Europe, South	68.8 WG	3	0.0945	0.0063	81	fruit	6 **13** 21	0.11 0.10 0.07	<0.02 <0.02 <0.02

FL: Formulation No: number of applications
GS = growth stage (BBCH code) at last application DALT = days after last treatment * prior to last treatment.

Report:	**KCA 6.3.1/43, [redacted]; 2013** ; M-457957-01-1
Title:	Determination of the residues of trifloxystrobin in/on apple and pear after spray application of trifloxystrobin WG 50 in the field in southern France, Portugal, Italy and Spain
Document No & Report No:	M-457957-01-1 11-2116
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Test system

In 2011 four trials were performed in southern Europe on apple or pear trees with trifloxystrobin WG 50 according to the use pattern supported within this dossier. The product was applied three times at application rates of 0.1125 kg trifloxystrobin/ha (in one trial up to 0.12 kg trifloxystrobin/ha). The treatments were performed with intervals of about 10 (9-11) days.
Fruit samples were taken on day 14 after the last application in all trials. Additional samples of fruit were taken at earlier or later time points in two trials.

Residues of trifloxystrobin (CGA 279202), its isomers CGA 331409, CGA 357261, CGA 357262, as well as the metabolite CGA 321113 and its isomer CGA 373466 were determined according to method 01313. The analytical method was validated by recovery experiments prior to and during the analysis of the samples by spiking control samples. The limit of quantitation was 0.01 mg/kg for all analytes.

Findings

- Method performance: Overall mean recoveries at fortification levels between 0.01 and 0.4 mg/kg were within the acceptable range of 70-110 %, RSD <20% as shown in Table 6.3.1-11, except for pear and CGA 331409 with an overall mean recovery of 115%, which was accepted since the RSD was in line and the values for apple were below 110%.

Table 6.3.1-11: Recoveries for trifloxystrobin, CGA321113, CGA 357261, CGA 357262, CGA 331409, CGA 373466 in/on apple and pear

Report No.	Analyte	Sample Material	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
11-2116	Trifloxystrobin	apple fruit	0.01	88; 92; 93	91	2.9	0.01
			0.1	102; 103; 104; 105	104	1.2	
			0.4	103	103	-	
			Overall Recovery (n =8)		99	6.7	
	Trifloxystrobin	pear fruit	0.01	103; 105; 106	105	1.5	0.01
			0.1	92; 96; 98	95	3.2	
			0.4	101	101	-	
			Overall Recovery (n =7)		100	5.1	

Report No.	Analyte	Sample Material	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
11-2116	CGA 321113	apple fruit	0.01	77; 78; 81	79	2.6	0.01
			0.1	82; 86; 88; 94	88	5.7	
			Overall Recovery (n =7)		84	7.2	
	CGA 321113	pear fruit	0.01	95; 99; 113	102	9.2	0.01
			0.1	92; 94; 97	94	2.7	
			0.4	97	97	-	
			Overall Recovery (n =7)		98	7.1	
	CGA 357261	apple fruit	0.01	88; 88; 93	90	3.2	0.01
			0.1	95; 96; 102; 102	99	3.8	
			Overall Recovery (n =7)		95	6.1	
	CGA 357261	pear fruit	0.01	98; 99; 104	100	3.2	0.01
			0.1	95; 95; 98	96	1.8	
			0.4	101	101	-	
			Overall Recovery (n =7)		99	3.3	
	CGA 357262	apple fruit	0.01	93; 93; 96	94	1.8	0.01
			01	98; 100; 102; 111	103	5.6	
			Overall Recovery (n =7)		99	6.3	
	CGA 357262	pear fruit	0.01	107; 111; 114	111	3.2	0.01
			0.1	96; 99; 99	98	1.8	
			0.4	106	106	-	
			Overall Recovery (n =7)		105	6.5	
	CGA 331409	apple fruit	0.01	101; 102; 102	102	0.6	0.01
			0.1	93; 97; 98; 114	101	9.2	
			Overall Recovery (n =7)		101	6.5	
	CGA 331409	pear fruit	0.01	121; 134; 137	131	6.5	0.01
			0.1	99; 101; 102	101	1.5	
			0.4	113	113	-	
			Overall Recovery (n =7)		115	13.7	
	CGA 373466	apple fruit	0.01	72; 80; 84	79	7.8	0.01
			0.1	84; 86; 86; 95	88	5.6	
			Overall Recovery (n =7)		84	8.3	
	CGA 373466	pear fruit	0.01	94; 96; 115	102	11.4	0.01
			0.1	90; 95; 96	94	3.4	
			0.4	92	92	-	
			Overall Recovery (n =7)		97	8.6	

RSD = relative standard deviation n = number of tests

- Storage stability: The maximum storage period of deep-frozen samples was up to 364 days for trifloxystrobin and CGA 321113 and is covered by the storage stability studies.

- Residue results: In the southern European field trials conducted in 2011 the residues at a PHI of 14 days ranged from 0.055 to 0.17 mg/kg for trifloxystrobin and were <0.01 mg/kg for CGA 321113 and CGA 373466 in apple or pear fruit. Residues of CGA 357261 were between 0.010 and 0.068, residues of CGA 357262 were between <0.01 and 0.031 mg/kg, and residues of CGA 331409 were between <0.01 and 0.036 mg/kg at the PHI of 14 days.

- No residues above the LOQ of 0.01 mg/kg could be detected in any of the corresponding control samples.

Table 6.3.1-12: Application data and residues of trifloxystrobin and CGA 321113 in/on apple and pear treated with Trifloxystrobin WG 50 in the field in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Trifloxystrobin (mg/kg)	CGA 321113 (mg/kg)
11-2116 11-2116-01 11-2116-01-T GLP: yes 2011	Apple Granny smith	France [redacted] Europe, South	50 WG	3	0.1125	0.0075	85	fruit	0* 0 7 **14** 21 28	0.065 0.14 0.067 0.055 0.028 0.024	<0.01 0.01 0.01 0.01 0.01 0.01
11-2116 11-2116-02 11-2116-02-T GLP: yes 2011	Apple Royal Gala	Portugal [redacted] Europe, South	50 WG	3	0.1125	0.0075	76	fruit	0 **14**	0.26 0.15	<0.01 <0.01
11-2116 11-2116-03 11-2116-03-T GLP: yes 2011	Pear Conference	Italy [redacted] Europe, South	50 WG	3	0.1125	0.0075	77	fruit	0* 0 7 **14** 22 29	0.12 0.25 0.18 0.12 0.11 0.076	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
11-2116 11-2116-04 11-2116-04-T GLP: yes 2011	Pear Conference	Spain [redacted] Europe, South	50 WG	3	0.1125 -0.120	0.0075- 0.00757	85	fruit	0 **14**	0.28 0.17	<0.01 <0.01

FL: Formulation No: number of applications
GS = growth stage (BBCH code) at last application DALT = days after last treatment
* prior to last treatment.

Table 6.3.1-13: Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in/ on apple and pear treated with Trifloxystrobin WG 50 in the field in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Portion analysed	DALT (days)	Residues CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
11-2116 11-2116-01 11-2116-01-T GLP: yes 2011	Apple Granny smith	France [redacted] Europe, South	fruit	0* 0 7 **14** 21 28	<0.01 <0.01 0.010 0.010 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
11-2116 11-2116-02 11-2116-02-T GLP: yes 2011	Apple Royal Gala	Portugal [redacted] Europe, South	fruit	0 **14**	0.031 0.032	0.013 0.014	0.017 0.017	<0.01 <0.01
11-2116 11-2116-03 11-2116-03-T GLP: yes 2011	Pear Confe-rence	Italy [redacted] Europe, South	fruit	0* 0 7 **14** 22 29	0.028 0.034 0.036 0.036 0.042 0.035	0.014 0.014 0.016 0.017 0.020 0.018	0.017 0.018 0.022 0.021 0.025 0.020	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
11-2116 11-2116-04 11-2116-04-T GLP: yes 2011	Pear Confe-rence	Spain [redacted] Europe, South	fruit	0 **14**	0.049 0.068	0.019 0.031	0.028 0.036	<0.01 <0.01

DALT = days after last treatment * prior to last treatment

CA 6.3.5 Grape

Former Annex II dossier

In the Annex II dossier, the critical GAP for trifloxystrobin supported at the European level (northern and southern Europe) consisted of up to 6 foliar spray applications at rates of 187.5 g a.s./ha trifloxystrobin and a PHI of 35 days.

Annex I renewal process/ New studies

The critical Good Agricultural Practice (cGAP) supported at the European level in the Annex I renewal (AIR) process consists of 3 foliar spray applications at 125 g a.s./ha trifloxystrobin in northern Europe and southern Europe, with a minimum spray interval of 10 days and a PHI of 14 days.

Table 6.3.5-1: Summary of the critical GAP for the proposed uses of Trifloxystrobin WG 50

Crop	Region*	F, G or I**	Maximum Number of Applications	Minimum Application Interval (days)	Maximum Rate (g a.s./ha)	Minimum PHI (days)
Grape	EU-N	F	3	10	125	14
Grape	EU-S	F	3	10	125	14

* EU-N northern Europe EU-S southern Europe ** F Field; G Greenhouse; I Indoor

Trials available to support the European GAPs relevant for Annex I renewal are summarised in Table 6.3.5-2 and Table 6.3.5-3.

Table 6.3.5- 2: Residue trials conducted per geographical region and formulation

Region	Crop	Formulation	Number of Trials			Report No.	Document N°	Dossier Ref.
			Vegetation period		Total			
			2011	2012				
Supplementary data								
N-EU	Grape	WG 50	4	4	8	11-2115	M-456337-01-1	KCA 6.3.5/32
			-			12-2010	M-453336-02-1	KCA 6.3.5/33
S-EU	Grape	WG 50	4	5	9	11-2114	M-454927-01-1	KCA 6.3.5/34
			-			12-2011	M-455561-02-1	KCA 6.3.5/35

N-EU northern Europe S-EU southern Europe
WG 50: wettable granule formulation containing 50% trifloxystrobin

Table 6.3.5- 3: Overall summary of residue data on grape covering AIR critical GAP

Application Rate	Region	Formulation	Crop	Sample material	n	Residue level (mg/kg) trifloxystrobin		
						Min.	Max.	STMR
3 applications at about 125 g/ha	N-EU	WG	Grape	Bunch or Berry	8	0.14	0.49	0.335
3 applications at about 125 g/ha	S-EU	WG	Grape	Bunch or Berry	9	0.11	0.51	0.180

N-EU northern Europe S-EU southern Europe n: number of trials

Field trials – northern Europe:

Report:	**KCA 6.3.5/32, [redacted]; 2013** ; M-456337-01-1
Title:	Determination of the residues of trifloxystrobin in/on grape after high or low volume spray application of Trifloxystrobin WG 50 in the field in northern France and Germany
Document No & Report No:	M-456337-01-1 11-2115
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.3.5/33, [redacted]; 2013 (amended)** ; M-453336-02-1
Title:	Determination of the residues of trifloxystrobin in/on grape after spray application and low-volume spray application of Trifloxystrobin WG 50 in the field in France (North) and Germany
Document No & Report No:	M-453336-02-1 12-2010
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Test system

In 2011 and 2012 eight trials were performed in the field in northern Europe in/on grapes with Trifloxystrobin WG 50 according to the use pattern supported within this dossier. The product was applied three times to grapes at application rates of 0.125 kg trifloxystrobin/ha. The treatments were performed with intervals of 10 days.
Berry samples were taken on day 14 and 21 after the last application in all trials, bunch samples were taken at day 0, 14 and 21 after last application in all trials and at day 7/8 and 10 in some of the trials.

Residues of trifloxystrobin (CGA 279202), its isomers CGA 331409, CGA 357261, CGA 357262, as well as the metabolite CGA 321113 and its isomer CGA 373466 were determined according to method 01313 or 01313/M001. The analytical methods were validated by recovery experiments prior to and during the analysis of the samples by spiking control samples. The limit of quantitation was 0.01 mg/kg for all analytes.

Findings

- Method performance: Overall mean recoveries at fortification levels of 0.01, 0.05 or 0.1, and 0.5 or 0.8 mg/kg per analyte were within the acceptable range of 70-110 %, RSD <20% as shown in Table 6.3.5-4, except for CGA 331409 with an overall mean recovery of 112% (berry) or 119% (bunch), which was accepted since the RSD was in line (6.5 or 5.9).

Table 6.3.5-4: Recoveries for trifloxystrobin, CGA321113, CGA 357261, CGA 357262, CGA 331409, CGA 373466 in/on grape

Report No.	Analyte	Sample Material	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
11-2115	Trifloxystrobin	grape, berry	0.01	109	109	-	0.01
			0.05	103	103	-	
			0.8	98; 100	99	-	
			Overall Recovery (n =4)		103	4.7	
	Trifloxystrobin	grape, bunch	0.01	116	116	-	0.01
			0.1	104, 106	105	-	
			0.8	102	102	-	
			Overall Recovery (n =4)		107	5.8	

Report No.	Analyte	Sample Material	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
11-2115	CGA 321113	grape, berry	0.01	108	108	-	0.01
			0.05	98	98	-	
			0.8	96; 98	97	-	
			Overall Recovery (n =4)		100	5.4	
	CGA 321113	grape, bunch	0.01	101	101	-	0.01
			0.1	96; 98	97	-	
			0.8	99	99	-	
			Overall Recovery (n =4)		99	2.1	
	CGA 357261	grape, berry	0.01	99	99	-	0.01
			0.05	99	99	-	
			0.8	97; 98	98	-	
			Overall Recovery (n =4)		98	1.0	
	CGA 357261	grape, bunch	0.01	109	109	-	0.01
			0.1	104; 105	105	-	
			0.8	101	101	-	
			Overall Recovery (n =4)		105	3.2	
	CGA 357262	grape, berry	0.01	112	112	-	0.01
			0.05	111	111	-	
			0.8	101; 102	102	-	
			Overall Recovery (n =4)		107	5.4	
	CGA 357262	grape, bunch	0.01	122	122	-	0.01
			0.1	109; 111	110	-	
			0.8	104	104	-	
			Overall Recovery (n =4)		112	6.8	
	CGA 331409	grape, berry	0.01	116	116	-	0.01
			0.05	120	120	-	
			0.8	104; 108	106	-	
			Overall Recovery (n =4)		112	6.5	
	CGA 331409	grape, bunch	0.01	128	128	-	0.01
			0.1	111; 118	115	-	
			0.8	117	117	-	
			Overall Recovery (n =4)		119	5.9	
	CGA 373466	grape, berry	0.01	96	96	-	0.01
			0.05	97	97	-	
			0.8	98; 100	99	-	
			Overall Recovery (n =4)		98	1.7	
	CGA 373466	grape, bunch	0.01	102	102	-	0.01
			0.1	99; 105	102	-	
			0.8	104	104	-	
			Overall Recovery (n =4)		103	2.6	

Report No.	Analyte	Sample Material	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
12-2010 12-2011	Trifloxystrobin	grape, bunch / berry	0.01	86; 94; 100; 100; 100; 109	98	7.8	0.01
			0.1	84; 91; 100; 106	95	10.2	
			0.5	81; 84; 93; 94	88	7.4	
			Overall Recovery (n =14)		94	9.1	
	CGA 321113	grape, bunch / berry	0.01	74; 80; 85; 96; 108	89	15.3	0.01
			0.1	80; 91; 107; 108	97	13.5	
			0.5	87; 89; 96; 97	92	5.4	
			Overall Recovery (n =13)		92	12.0	
	CGA 357261	grape, bunch / berry	0.01	90; 91; 98; 102; 105; 105	99	6.8	0.01
			0.1	86; 93; 91; 101	93	6.7	
			0.5	84; 97; 84; 86	88	7.1	
			Overall Recovery (n =14)		94	8.1	
	CGA 357262	grape, bunch / berry	0.01	83; 84; 91; 91; 93; 93	89	5.0	0.01
			0.1	91; 92; 93; 115	98	11.8	
			0.5	84; 87; 98; 100	92	8.6	
			Overall Recovery (n =14)		93	8.8	
	CGA 331409	grape, bunch / berry	0.01	61; 74; 96; 102; 106	88	22.0	0.01
			0.1	90; 96; 107; 116	102	11.3	
			0.5	79; 81; 90; 104	89	12.9	
			Overall Recovery (n =13)		92	16.6	
	CGA 373466	grape, bunch / berry	0.01	72; 86; 89; 102; 91; 94	89	11.2	0.01
			0.1	85; 105; 98; 103	98	9.2	
			0.5	95; 95; 99; 115	101	9.4	
			Overall Recovery (n =14)		95	10.9	

RSD = relative standard deviation n = number of tests

- Storage stability: The maximum storage period of deep-frozen samples was up to 426 days for all analytes and is covered by the storage stability studies.

- Residue results: In the northern European field trials the residues in berries or bunch of grapes at a PHI of 14 days ranged from 0.11 to 0.49 mg/kg for trifloxystrobin and were <0.01 to 0.044 mg/kg for CGA 321113. A maximum of CGA 321113 residues was found at up to 0.069 mg/kg at 21 days after last application.
Residues of CGA 357261 ranged between <0.01 and 0.066 mg/kg at day 14, and residues of CGA 357262 were between <0.01 and 0.035 mg/kg at day 14 after the last application. Residues of CGA 331409 were between <0.01 and 0.042, residues of CGA 373466 ranged between <0.01 and 0.012 mg/kg at day 14 after the last application.
- No residues above the LOQ of 0.01 mg/kg could be detected in any of the corresponding control samples.

Table 6.3.5-5: Application data and residues of trifloxystrobin and CGA 321113 in/ on grapes treated with Trifloxystrobin WG 50 in the field in northern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
11-2115 11-2115-01 11-2115-01-T GLP: yes 2011	Grape Sauvig-non; white variety	France F-[redacted] Europe, North	50 WG	3	0.125	0.0625	85	bunch of grapes	0* 0 8 10 **14** 21	0.13 0.16 0.19 0.16 0.19 0.12	0.01 <0.01 <0.01 <0.01 0.012 <0.01
								berry	**14** 21	0.11 0.097	<0.01 <0.01
11-2115 11-2115-02 11-2115-02-T GLP: yes 2011	Grape Gamay; red variety	France F-[redacted] Europe, North	50 WG	3	0.125	0.0625	85	bunch of grapes	0 **14** 21	0.73 0.42 0.36	0.020 0.031 0.019
								berry	**14** 21	0.32 0.26	0.021 0.022
11-2115 11-2115-03 11-2115-03-T GLP: yes 2011	Grape Dorn-felder; red variety	Germany D-[redacted] Europe, North	50 WG	3	0.125	0.0156	85	bunch of grapes	0* 0 7 10 **14** 21	0.27 0.51 0.42 0.35 0.38 0.32	0.021 0.014 0.019 0.019 0.025 0.028
								berry	**14** 21	0.36 0.22	0.024 0.021
11-2115 11-2115-04 11-2115-04-T GLP: yes 2011	Grape Müller Thurgau; white variety	Germany D-[redacted] Europe, North	50 WG	3	0.125	0.0156	85	bunch of grapes	0 **14** 21	0.52 0.49 0.34	0.011 0.044 0.049
								berry	**14** 21	0.35 0.49	0.032 0.069
12-2010 12-2010-01 12-2010-01-T GLP: yes 2012	Grape Gamay; red variety	France [redacted] Europe, North	50 WG	3	0.125	0.0625	83	bunch of grapes	0* 0 7 10 **14** 21	0.22 0.43 0.28 0.22 0.27 0.42	0.018 0.022 <0.01 0.014 0.010 <0.01
								berry	**14** 21	0.26 0.22	0.013 <0.01
12-2010 12-2010-02 12-2010-02-T GLP: yes 2012	Grape Sauvig-non; white variety	France [redacted] Europe, North	50 WG	3	0.125	0.0625	83	bunch of grapes	0 **14** 21	0.13 0.12 0.072	<0.01 <0.01 <0.01
								berry	**14** 21	0.14 0.11	<0.01 <0.01

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
12-2010 12-2010-03 12-2010-03-T GLP: yes 2012	Grape Müller-Thurgau; white variety	Germany [redacted] Europe, North	50 WG	3	0.125	0.0157	85	bunch of grapes	0* 0 7 10 **14** 21	0.18 0.30 0.30 0.41 0.28 0.29	0.013 0.010 0.014 0.012 0.015 0.016
								berry	**14** 21	0.28 0.24	0.015 0.015
12-2010 12-2010-04 12-2010-04-T GLP: yes 2012	Grape Dornfelder; red variety	Germany [redacted] Europe, North	50 WG	3	0.125	0.0157	85	bunch of grapes	0 **14** 21	0.26 0.14 0.18	0.015 0.015 0.011
								berry	**14** 21	0.27 0.13	0.018 0.014

FL: Formulation — No: number of applications
GS = growth stage (BBCH code) at last application — DALT = days after last treatment
* prior to last treatment

Table 6.3.5-6: Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in/ on grapes treated with Trifloxystrobin WG 50 in the field in northern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Portion analysed	DALT (days)	Residues CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
11-2115 11-2115-01 11-2115-01-T GLP: yes 2011	Grape Sauvignon; white variety	France F-[redacted] Europe, North	bunch of grapes	0* 0 8 10 **14** 21	0.013 0.013 0.016 0.017 0.021 0.015	<0.01 <0.01 <0.01 <0.01 0.011 <0.01	<0.01 <0.01 <0.01 0.010 0.013 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
			berry	**14** 21	0.012 0.013	<0.01 <0.01	0.010 <0.01	<0.01 <0.01
11-2115 11-2115-02 11-2115-02-T GLP: yes 2011	Grape Gamay; red variety	France F-[redacted] Europe, North	bunch of grapes	0 **14** 21	0.051 0.066 0.060	0.023 0.035 0.028	0.028 0.042 0.032	0.011 0.012 <0.01
			berry	**14** 21	0.044 0.044	0.023 0.025	0.027 0.028	<0.01 <0.01

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Residues Portion analysed	DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
11-2115 11-2115-03 11-2115-03-T GLP: yes 2011	Grape Dornfelder; red variety	Germany D-[redacted] Europe, North	bunch of grapes	0* 0 7 10 **14** 21	0.015 0.013 0.016 0.019 0.019 0.020	<0.01 <0.01 <0.01 <0.01 <0.01 0.010	0.010 <0.01 0.010 <0.01 0.013 0.015	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
			berry	**14** 21	0.017 0.016	<0.01 <0.01	0.011 0.013	<0.01 <0.01
11-2115 11-2115-04 11-2115-04-T GLP: yes 2011	Grape Müller Thurgau; white variety	Germany D-[redacted] Europe, North	bunch of grapes	0 **14** 21	0.024 0.044 0.038	0.010 0.019 0.019	0.014 0.026 0.025	<0.01 0.011 0.014
			berry	**14** 21	0.034 0.050	0.016 0.026	0.023 0.032	<0.01 0.019
12-2010 12-2010-01 12-2010-01-T GLP: yes 2012	Grape Gamay; red variety	France [redacted] Europe, North	bunch of grapes	0* 0 7 10 **14** 21	0.022 0.021 0.020 0.021 0.020 0.025	0.012 0.014 <0.01 0.011 <0.01 0.013	0.013 0.014 0.012 0.012 0.014 0.017	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
			berry	**14** 21	0.019 0.023	<0.01 0.011	0.013 0.011	<0.01 <0.01
12-2010 12-2010-02 12-2010-02-T GLP: yes 2012	Grape Sauvignon; white variety	France [redacted] Europe, North	bunch of grapes	0 **14** 21	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01
			berry	**14** 21	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01
12-2010 12-2010-03 12-2010-03-T GLP: yes 2012	Grape Müller-Thurgau; white variety	Germany [redacted] Europe, North	bunch of grapes	0* 0 7 10 **14** 21	0.012 0.015 0.026 0.026 0.024 0.035	<0.01 <0.01 <0.01 <0.01 <0.01 0.011	<0.01 <0.01 0.012 0.017 0.011 0.017	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
			berry	**14** 21	0.022 0.022	0.010 <0.01	0.014 0.012	<0.01 <0.01
12-2010 12-2010-04 12-2010-04-T GLP: yes 2012	Grape Dornfelder; red variety	Germany [redacted] Europe, North	bunch of grapes	0 **14** 21	0.010 0.012 0.012	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01
			berry	**14** 21	0.010 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01

DALT = days after last treatment * prior to last treatment

Field trials – southern Europe:

Report:	**KCA 6.3.5/34,** [redacted]**; 2013** ; M-454927-01-1
Title:	Determination of the residues of trifloxystrobin in/on grape after high or low-volume spray application of Trifloxystrobin WG 50 in the field in southern France, Spain, Italy and Portugal
Document No & Report No:	M-454927-01-1 11-2114
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.3.5/35,** [redacted]**; 2013 (amended)**; M-455561-02-1
Title:	Determination of the residues of trifloxystrobin in/on grape after spray of Trifloxystrobin WG 50 in the field in Italy, Greece and Spain
Document No & Report No:	M-455561-02-1 12-2011
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Test system

In 2011 and 2012 nine residue trials were performed in the field in southern Europe in/on grapes with Trifloxystrobin WG 50 according to the use pattern supported within this dossier. The product was applied three times to grapes at application rates of about 0.125 kg (up to 0.135) trifloxystrobin/ha. The treatments were performed with intervals of about 10 days (up to 20 days in one trial).
Berry samples were taken on day 14 (13) and 21 (20) after the last application in all trials, bunch samples were taken at day 0, 14 (13) and 21 after last application in all trials and at day 7 and 10/11 in some of the trials.

Residues of trifloxystrobin (CGA 279202), its isomers CGA 331409, CGA 357261, CGA 357262, as well as the metabolite CGA 321113 and its isomer CGA 373466 were determined according to method 01313 or 01313/M001. The analytical methods were validated by recovery experiments prior to and during the analysis of the samples by spiking control samples. The limit of quantitation was 0.01 mg/kg for all analytes.

Findings

- <u>Method performance</u>: Overall mean recoveries at fortification levels of 0.01, 0.1, 0.5 or 0.8 mg/kg for each analyte were within the acceptable range of 70-110 %, RSD <20% as shown in Table 6.3.5-7.

Table 6.3.5-7: Recoveries for trifloxystrobin, CGA321113, CGA 357261, CGA 357262, CGA 331409, CGA 373466 in/on grape

Report No.	Analyte	Sample Material	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
11-2114	Trifloxystrobin	grape, berry	0.01	91; 96; 100; 100	97	4.4	0.01
			0.1	97; 100; 102; 103; 105	101	3.0	
			0.8	100	100	-	
			Overall Recovery (n =10)		99	4.0	
	Trifloxystrobin	grape, bunch	0.01	93; 101, 103; 105	101	5.2	0.01
			0.1	98; 100; 100; 105; 110	103	5.5	
			0.8	105	105	-	
			Overall Recovery (n =10)		102	5.0	
	CGA 321113	grape, berry	0.01	84; 93; 98; 107	96	10.1	0.01
			0.1	93; 96; 98; 100; 101	98	3.3	
			0.8	96	96	-	
			Overall Recovery (n =10)		97	6.3	
	CGA 321113	grape, bunch	0.01	90; 96; 101; 107	99	7.3	0.01
			0.1	96; 98; 99; 99; 105	99	3.4	
			0.8	106	106	-	
			Overall Recovery (n =10)		100	5.3	
	CGA 357261	grape, berry	0.01	93; 99; 100; 100	98	3.4	0.01
			0.1	100; 104; 105; 106; 107	104	2.6	
			0.8	99	99	-	
			Overall Recovery (n =10)		101	4.2	
	CGA 357261	grape, bunch	0.01	97; 98; 101; 105	100	3.6	0.01
			0.1	100; 101, 104, 106; 107	104	2.9	
			0.8	101	101	-	
			Overall Recovery (n =10)		102	3.3	
	CGA 357262	grape, berry	0.01	93; 103; 109; 110	104	7.5	0.01
			0.1	102; 102; 105; 106; 108	105	2.5	
			0.8	104	104	-	
			Overall Recovery (n =10)		104	4.7	
	CGA 357262	grape, bunch	0.01	96; 99; 105; 113	103	7.3	0.01
			0.1	100; 102; 102; 107; 112	105	4.7	
			0.8	105	105	-	
			Overall Recovery (n =10)		104	5.3	

Report No.	Analyte	Sample Material	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
11-2114	CGA 331409	grape, berry	0.01	100; 104; 105; 105	104	2.3	0.01
			0.1	97; 102 103; 108; 110	104	4.9	
			0.8	103	103	-	
			Overall Recovery (n =10)		104	3.6	
	CGA 331409	grape, bunch	0.01	92; 100; 109, 111	103	8.5	0.01
			0.1	97; 101; 101; 109; 110	104	5.4	
			0.8	108	108	-	
			Overall Recovery (n =10)		104	6.2	
	CGA 373466	grape, berry	0.01	81; 95; 100; 106	96	11.2	0.01
			0.1	88; 92; 99; 99; 100	96	5.6	
			0.8	96	96	-	
			Overall Recovery (n =10)		96	7.4	
	CGA 373466	grape, bunch	0.01	91; 97; 99; 101	97	4.5	0.01
			0.1	93; 95; 95; 101; 107	98	5.9	
			0.8	106	106	-	
			Overall Recovery (n =10)		99	5.4	

RSD = relative standard deviation n = number of tests
For 12-2011 see Table 6.3.5-4

- Storage stability: The maximum storage period of deep-frozen samples was up to 385 days for all analytes and is covered by the storage stability studies.

- Residue results: In the southern European field trials the residues in berries or bunch of grapes at a PHI of 14 days ranged from 0.058 to 0.51 mg/kg for trifloxystrobin and were <0.01 to 0.034 mg/kg for CGA 321113.
Residues of CGA 357261 ranged between <0.01 and 0.044 mg/kg at day 14, and residues of CGA 357262 were between <0.01 and 0.015 mg/kg at day 14 after the last application. Residues of CGA 331409 were between <0.01 and 0.021 at day 14 after the last application, residues of CGA 373466 were below LOQ (<0.01 mg/kg) in all samples.

- No residues above the LOQ of 0.01 mg/kg could be detected in any of the corresponding control samples.

Table 6.3.5-8: Application data and residues of trifloxystrobin and CGA 321113 in/ on grapes treated with Trifloxystrobin WG 50 in the field in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
11-2114 11-2114-01 11-2114-01-T GLP: yes 2011	Grape Ugni blanc ; C5 - Vines, white variety	France F-[redacted] Europe, South	50 WG	3	0.125	0.0625	85	bunch of grapes	0* 0 7 11 **14** 21	0.20 0.31 0.24 0.21 0.22 0.19	0.027 0.023 0.023 0.029 0.034 0.034
								berry	**14** 21	0.16 0.19	0.025 0.030
11-2114 11-2114-02 11-2114-02-T GLP: yes 2011	Grape Bobal ; red variety	Spain E-[redacted] (Requena) Europe, South	50 WG	3	0.125	0.0125	85	bunch of grapes	0 **14** 21	0.088 0.11 0.12	<0.01 <0.01 <0.01
								berry	**14** 21	0.095 0.083	<0.01 <0.01
11-2114 11-2114-03 11-2114-03-T GLP: yes 2011	Grape Labrusco Graspa-rossa; red variety	Italy I-[redacted] (Bologna) Europe, South	50 WG	3	0.125	0.0125	85	bunch of grapes	0* 0 7 10 **14** 21	0.088 0.30 0.25 0.13 0.14 0.096	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
								berry	**14** 21	0.11 0.11	<0.01 <0.01
11-2114 11-2114-04 11-2114-04-T GLP: yes 2011	Grape fernão pires ; white variety	Portugal P-[redacted] Europe, South	50 WG	3	0.125	0.0250	83	bunch of grapes	0 **14** 21	0.64 0.32 0.39	<0.01 <0.01 <0.01
								berry	**14** 21	0.32 0.25	<0.01 <0.01
12-2011 12-2011-01 12-2011-01-T GLP: yes 2012	Grape Lambrusco Graspa-rossa; red variety	Italy [redacted] (Bologna) Europe, South	50 WG	3	0.125	0.0125	85	bunch of grapes	0* 0 7 10 **14** 21	0.13 0.22 0.25 0.24 0.099 0.13	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
								berry	**14** 21	0.18 0.090	<0.01 <0.01

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
12-2011 12-2011-02 12-2011-02-T GLP: yes 2012	Grape Lam-brusco di sorbara; red variety	Italy [redacted] Europe, South	50 WG	3	0.125	0.0125	85	bunch of grapes	0 **14** 21	0.89 0.34 0.19	<0.01 <0.01 <0.01
								berry	**14** 21	0.51 0.32	0.016 <0.01
12-2011 12-2011-03 12-2011-03-T GLP: yes 2012	Grape Victoria ; white table grape variety	Greece GR - [redacted] Europe, South	50 WG	3	0.125	0.0125	85	bunch of grapes	0* 0 7 10 **14** 21	0.080 0.24 0.17 0.071 0.064 0.019	0.010 0.012 0.010 <0.01 <0.01 <0.01
								berry	**14** 21	0.20 0.14	0.021 0.024
12-2011 12-2011-04 12-2011-04-T GLP: yes 2012	Grape Zalema; white variety	Spain [redacted] Europe, South	50 WG	3	0.125-0.135	0.0156-0.0158	89	bunch of grapes	0 **14** 20	0.21 0.085 0.12	<0.01 <0.01 <0.01
								berry	**14** 20	0.058 0.036	<0.01 <0.01
12-2011 12-2011-05 12-2011-05-T GLP: yes 2012	Grape Maca-beo; white variety	Spain [redacted] Europe, South	50 WG	3	0.125-0.131	0.0156-0.0156	85	bunch of grapes	0 **13** 21	0.18 0.13 0.071	<0.01 <0.01 <0.01
								berry	**13** 21	0.15 0.099	<0.01 <0.01

FL: Formulation No: number of applications
GS = growth stage (BBCH code) at last application DALT = days after last treatment
* prior to last treatment

Table 6.3.5-9: Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in/ on grapes treated with Trifloxystrobin WG 50 in the field in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Residues Portion analysed	DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
11-2114 11-2114-01 11-2114-01-T GLP: yes 2011	Grape Ugni blanc ; C5 - Vines, white variety	France F-[redacted] Europe, South	bunch of grapes	0* 0 7 11 **14** 21	0.016 0.015 0.016 0.016 0.015 0.018	0.015 0.014 0.012 0.015 0.015 0.018	0.019 0.016 0.016 0.020 0.021 0.025	<0.01 0.01 <0.01 <0.01 <0.01 <0.01
			berry	**14** 21	0.014 0.018	0.014 0.016	0.018 0.021	<0.01 <0.01
11-2114 11-2114-02 11-2114-02-T GLP: yes 2011	Grape Bobal ; red variety	Spain E-[redacted] (Requena) Europe, South	bunch of grapes	0 **14** 21	<0.01 0.014 0.019	<0.01 <0.01 <0.01	<0.01 <0.01 0.011	<0.01 <0.01 <0.01
			berry	**14** 21	0.017 0.020	0.011 <0.01	0.017 0.010	<0.01 <0.01
11-2114 11-2114-03 11-2114-03-T GLP: yes 2011	Grape Lam-brusco Graspa-rossa ; red variety	Italy I-[redacted] (Bologna) Europe, South	bunch of grapes	0* 0 7 10 **14** 21	0.011 0.010 0.019 0.014 0.015 0.011	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
			berry	**14** 21	<0.01 0.015	<0.01 0.014	<0.01 0.018	<0.01 <0.01
11-2114 11-2114-04 11-2114-04-T GLP: yes 2011	Grape fernão pires ; white variety	Portugal P-[redacted] Europe, South	bunch of grapes	0 **14** 21	0.020 0.021 0.035	<0.01 <0.01 0.011	0.013 0.013 0.018	<0.01 <0.01 <0.01
			berry	**14** 21	0.024 0.025	<0.01 <0.01	0.013 0.015	<0.01 <0.01
12-2011 12-2011-01 12-2011-01-T GLP: yes 2012	Grape Lam-brusco Graspa-rossa; red variety	Italy [redacted] (Bologna) Europe, South	bunch of grapes	0* 0 7 10 **14** 21	<0.01 <0.01 <0.01 0.011 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
			berry	**14** 21	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Residues Portion analysed	DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
12-2011 12-2011-02 12-2011-02-T GLP: yes 2012	Grape Lam-brusco di sorbara; red variety	Italy [redacted] Europe, South	bunch of grapes	0 **14** 21	0.032 0.025 0.012	0.010 0.010 <0.01	0.011 <0.01 <0.01	<0.01 <0.01 <0.01
			berry	**14** 21	0.044 0.018	0.013 <0.01	0.016 <0.01	<0.01 <0.01
12-2011 12-2011-03 12-2011-03-T GLP: yes 2012	Grape Victoria ; white table grape variety	Greece GR - [redacted] Europe, South	bunch of grapes	0* 0 7 10 **14** 21	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
			berry	**14** 21	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01
12-2011 12-2011-04 12-2011-04-T GLP: yes 2012	Grape Zalema; white variety	Spain [redacted] Europe, South	bunch of grapes	0 **14** 20	0.018 0.030 <0.01	<0.01 0.014 <0.01	<0.01 0.016 <0.01	<0.01 <0.01 <0.01
			berry	**14** 20	0.018 0.010	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01
12-2011 12-2011-05 12-2011-05-T GLP: yes 2012	Grape Maca-beo; white variety	Spain [redacted] Europe, South	bunch of grapes	0 **13** 21	<0.01 <0.01 0.023	<0.01 <0.01 0.012	<0.01 <0.01 0.011	<0.01 <0.01 <0.01
			berry	**13** 21	0.011 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01

DALT = days after last treatment * prior to last treatment

CA 6.3.8 Strawberry

Former Annex II dossier and Annex III dossiers

Strawberry was not a crop supported in the Annex II dossier.
The critical GAP for trifloxystrobin supported at the European level (northern and southern Europe, Annex III dossiers) relevant for the recent EU MRL (0.5 mg/kg, CR1004/2013) of trifloxystrobin consisted of up to 3 foliar spray applications at rates of up to 150 g a.s./ha trifloxystrobin and a PHI of 1 day.

Annex I renewal process/ New studies

The critical Good Agricultural Practice (cGAP) supported at the European level in the Annex I renewal (AIR) process consists of 2 foliar spray applications at 150 g a.s./ha trifloxystrobin in northern and southern Europe, with a minimum spray interval of 7 days and a PHI of 1 day.

Table 6.3.8-1: Summary of the critical GAP for the proposed uses of Trifloxystrobin WG 50

Crop	Region*	F, G or I**	Maximum Number of Applications	Minimum Application Interval (days)	Maximum Rate (g a.s./ha)	Minimum PHI (days)
Strawberry	EU-N	F	2	7	150	1
Strawberry	EU-S	F	2	7	150	1
Strawberry	EU-N EU-S	G	2	7	150	1

* EU-N northern Europe EU-S southern Europe ** F Field; G Greenhouse; I Indoor

Trials available to support the European GAPs relevant for Annex I renewal are summarised in Table 6.3.8-2 and Table 6.3.8-3.

Table 6.3.8-2: Residue trials conducted per geographical region and formulation

Region	Crop	Formulation	Number of Trials			Report No.	Document No.	Dossier Ref.
			Vegetation period 2011	Vegetation period 2012	Total			
Supplementary data								
N-EU field	Strawberry	WG 50	4	-	9	11-2128	M-457953-01-1	KCA 6.3.8/01
			-	5		12-2012	M-452140-04-1	KCA 6.3.8/02
S-EU field	Strawberry	WG 50	4	-	9	11-2129	M-457958-02-1	KCA 6.3.8/03
			-	5		12-2013	M-460009-01-1	KCA 6.3.8/04
N-EU S-EU greenhouse	Strawberry	WG 50	4	-	8	11-2120	M-456769-02-1	KCA 6.3.8/05
			-	4		12-2014	M-453332-02-1	KCA 6.3.8/06

N-EU northern Europe S-EU southern Europe
WG 50: wettable granule formulation containing 50% trifloxystrobin

Table 6.3.8-3: Overall summary of residue data on grape covering AIR critical GAP

Application Rate	Region	Formulation	Crop	Sample material	n	Residue level (mg/kg) trifloxystrobin		
						Min.	Max.	STMR
2 applications at about 150 g/ha	N-EU field	WG	Strawberry	Fruit	9	0.038	0.15	0.096
2 applications at about 150 g/ha	S-EU field	WG	Strawberry	Fruit	9	0.061	0.23	0.150
2 applications at about 150 g/ha	N-EU S-EU green-house	WG	Strawberry	Fruit	8	0.082	0.41	0.125

N-EU northern Europe S-EU southern Europe n: number of trials

Field trials – northern Europe:

Report:	**KCA 6.3.8/01, [redacted]; 2013** ; M-457953-01-1
Title:	Determination of the residues of trifloxystrobin in/on strawberry after spray application of Trifloxystrobin WG 50 in the field in Germany, northern France and Belgium
Document No & Report No:	M-457953-01-1 11-2128
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.3.8/02, [redacted]; [redacted]; 2013** ; M-452140-01-1
Title:	Determination of the residues of trifloxystrobin in/on strawberry after spraying of Trifloxystrobin WG 50 in the field in Germany, the Netherlands, France (North) and Belgium
Document No & Report No:	M-452140-01-1 12-2012
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Test system

In 2011 and 2012 nine trials were performed in the field in northern Europe in/on strawberries with Trifloxystrobin WG 50 according to the use pattern supported within this dossier. The product was applied two times to strawberries at application rates of 0.13 kg trifloxystrobin/ha. The treatments were performed with intervals of 7 days.
Fruit samples were taken on day 1 and 3 after the last application in all trials. Additional samples of fruit were taken at later time points in some trials.

Residues of trifloxystrobin (CGA 279202), its isomers CGA 331409, CGA 357261, CGA 357262, as well as the metabolite CGA 321113 and its isomer CGA 373466 were determined according to method 01313 or 01313/M001. The analytical methods were validated by recovery experiments prior to and during the analysis of the samples by spiking control samples. The limit of quantitation was 0.01 mg/kg for all analytes.

Findings

- Method performance: Overall mean recoveries at fortification levels of 0.01, 0.1 and 0.5 mg/kg per analyte were within the acceptable range of 70-110 %, RSD <20% as shown in Table 6.3.8-4.

Table 6.3.8-4: Recoveries for trifloxystrobin, CGA321113, CGA 357261, CGA 357262, CGA 331409, CGA 373466 in/on strawberry

Report No.	Analyte	Sample Material	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
11-2128	Trifloxystrobin	strawberry fruit	0.01	88; 95; 103	95	7.9	0.01
			0.1	94; 108	101	-	
			0.5	93	93	-	
			Overall Recovery (n =6)		97	7.5	
	CGA 321113	strawberry fruit	0.01	60; 77; 82	73	15.8	0.01
			0.1	85; 97	91	-	
			0.5	89	89	-	
			Overall Recovery (n =6)		82	15.4	
	CGA 357261	strawberry fruit	0.01	88; 91; 93	91	2.8	0.01
			0.1	97; 103	100	-	
			0.5	91	91	-	
			Overall Recovery (n =6)		94	5.1	
	CGA 357262	strawberry fruit	0.01	81; 95; 106	94	13.3	0.01
			0.1	94; 107	101	-	
			0.5	95	95	-	
			Overall Recovery (n =6)		96	9.9	
	CGA 331409	strawberry fruit	0.01	77; 93; 108	93	16.7	0.01
			0.1	101; 108	105	-	
			0.5	94	94	-	
			Overall Recovery (n =6)		97	12.1	
	CGA 373466	strawberry fruit	0.01	84; 84; 89	86	3.4	0.01
			0.1	84; 96	90	-	
			0.5	89	89	-	
			Overall Recovery (n =6)		88	5.4	
12-2012 12-2013 12-2014	Trifloxystrobin	strawberry fruit	0.01	85; 87; 95; 95; 96; 97; 98; 102; 111	96	8.0	0.01
			0.1	71; 84; 88; 89; 102; 107; 109	93	14.8	
			0.5	64; 79; 81; 84	77	11.6	
			Overall Recovery (n =20)		91	13.5	
	CGA 321113	strawberry fruit	0.01	70; 71; 72; 72; 73; 78; 86; 90; 97	79	12.5	0.01
			0.1	72; 76; 83; 83; 85; 86; 101	84	10.9	
			0.5	75; 77; 77; 79	77	2.1	
			Overall Recovery (n =20)		80	10.8	

Report No.	Analyte	Sample Material	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
12-2012 12-2013 12-2014	CGA 357261	strawberry fruit	0.01	75; 78; 79; 82; 82; 84; 85; 89; 92	83	6.5	0.01
			0.1	79; 80; 83; 83; 88; 91; 96	86	7.2	
			0.5	76; 76; 80; 82	79	3.8	
			Overall Recovery (n =20)		83	6.9	
	CGA 357262	strawberry fruit	0.01	70; 84; 85; 86; 91; 93; 94; 97; 118	91	14.2	0.01
			0.1	73; 75; 79; 83; 87; 97; 108	86	14.7	
			0.5	67; 74; 81; 84	77	9.9	
			Overall Recovery (n =20)		86	14.6	
	CGA 331409	strawberry fruit	0.01	70; 75; 76; 77; 78; 78; 81; 85; 96	80	9.3	0.01
			0.1	73; 75; 82; 83; 92; 92; 96	85	10.4	
			0.5	72; 74; 77; 78	75	3.7	
			Overall Recovery (n =20)		80	9.7	
	CGA 373466	strawberry fruit	0.01	72; 73; 75; 78; 78; 86; 87; 92; 95	82	10.3	0.01
			0.1	72; 74; 77; 78; 79; 79; 85	78	5.3	
			0.5	71; 72; 75; 80	75	5.4	
			Overall Recovery (n =20)		79	8.7	

RSD = relative standard deviation n = number of tests

- Storage stability: The maximum storage period of deep-frozen samples was up to 502 days for all analytes and is covered by the storage stability studies.

- Residue results: In the northern European field trials the residues at a PHI of 1 day ranged from 0.024 to 0.15 mg/kg for trifloxystrobin and were <0.01 to 0.018 mg/kg for CGA 321113 in strawberry fruit. A maximum of CGA 321113 residues was found at up to 0.027 mg/kg at later sampling dates. Residues of CGA 331409 were always below LOQ (<0.01 mg/kg), the same applies for CGA 373466, except in one trial at day 0, where 0.012 mg/kg were found. Residues of CGA 357261 ranged between <0.01 and 0.029 mg/kg at day 1 and residues of CGA 357262 were between <0.01 and 0.043 mg/kg at day 1 after the last application, nevertheless, this only applies for 2 trials, while in the other 7 trials residues were < LOQ in all samples.

- No residues above the LOQ of 0.01 mg/kg could be detected in any of the corresponding control samples.

Table 6.3.8-5: Application data and residues of trifloxystrobin and CGA 321113 in/ on strawberries treated with Trifloxystrobin WG 50 in the field in northern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
11-2128 11-2128-01 11-2128-01-T GLP: yes 2011	Straw-berry Lamba-da	Germany [redacted] Europe, North	50 WG	2	0.150	0.030	85	fruit	0* 0 **1** 3 7 10	0.031 0.10 0.067 0.070 0.047 0.032	0.01 0.012 <0.01 0.016 0.021 0.025
11-2128 11-2128-02 11-2128-02-T GLP: yes 2011	Straw-berry Matis	France [redacted] Europe, North	50 WG	2	0.150	0.025	85	fruit	0* 0 **1** 3 7 10	0.019 0.068 0.049 0.089 0.045 0.047	<0.01 <0.01 <0.01 0.012 0.013 0.024
11-2128 11-2128-03 11-2128-03-T GLP: yes 2011	Straw-berry Elsanta	Germany [redacted] Europe, North	50 WG	2	0.150	0.015	87	fruit	0 **1** 3	0.14 0.13 0.077	0.015 0.015 0.014
11-2128 11-2128-04 11-2128-04-T GLP: yes 2011	Straw-berry Lamba-da	Belgium [redacted] Europe, North	50 WG	2	0.150	0.020	87	fruit	0 **1** 3	0.12 0.082 0.096	0.010 <0.01 0.012
12-2012 12-2012-01 12-2012-01-T GLP: yes 2012	Straw-berry Elsanta	Germany [redacted] Europe, North	50 WG	2	0.150	0.025	87	fruit	0* 0 **1** 3 7 10	0.029 0.12 0.081 0.076 0.039 0.037	0.012 0.016 0.015 0.013 0.021 0.026
12-2012 12-2012-02 12-2012-02-T GLP: yes 2012	Straw-berry Sonata-	Nether-lands [redacted] Europe, North	50 WG	2	0.150	0.030	87	fruit	0 **1** **1** 3	0.042 0.038 0.024 0.015	<0.01 <0.01 <0.01 <0.01
12-2012 12-2012-03 12-2012-03-T GLP: yes 2012	Straw-berry Mathis-	France [redacted] Europe, North	50 WG	2	0.150	0.025	87	fruit	0* 0 **1** 3 7 10	0.040 0.091 0.14 0.095 0.059 0.037	0.013 0.010 0.018 0.026 0.025 0.027
12-2012 12-2012-04 12-2012-04-T GLP: yes 2012	Straw-berry Lamba-da	Belgium [redacted] Europe, North	50 WG	2	0.150	0.015	87	fruit	0* 0 **1** 3 7 10	0.039 0.14 0.15 0.080 0.069 0.035	0.014 0.015 0.016 0.017 0.021 0.012

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
12-2012 12-2012-05 12-2012-05-T GLP: yes 2012	Straw-berry Elsanta	Germany [redacted] Europe, North	50 WG	2	0.150	0.030	87	fruit	0 **1** 3 7	0.15 0.14 0.15 0.11	0.011 0.016 0.018 0.015

FL: Formulation No: number of applications
GS = growth stage (BBCH code) at last application DALT = days after last treatment
* prior to last treatment

Table 6.3.8-6: Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in/ on strawberries treated with Trifloxystrobin WG 50 in the field in northern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Portion analysed	DALT (days)	Residues CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
11-2128 11-2128-01 11-2128-01-T GLP: yes 2011	Straw-berry Lamba-da	Germany [redacted] Europe, North	fruit	0* 0 **1** 3 7 10	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
11-2128 11-2128-02 11-2128-02-T GLP: yes 2011	Straw-berry Matis	France [redacted] Europe, North	fruit	0* 0 **1** 3 7 10	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
11-2128 11-2128-03 11-2128-03-T GLP: yes 2011	Straw-berry Elsanta	Germany [redacted] Europe, North	fruit	0 **1** 3	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01
11-2128 11-2128-04 11-2128-04-T GLP: yes 2011	Straw-berry Lamba-da	Belgium [redacted] Europe, North	fruit	0 **1** 3	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01
12-2012 12-2012-01 12-2012-01-T GLP: yes 2012	Straw-berry Elsanta	Germany [redacted] Europe, North	fruit	0* 0 **1** 3 7 10	<0.01 0.041 0.029 0.024 <0.01 <0.01	0.012 0.056 0.043 0.036 0.019 0.011	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Residues Portion analysed	DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
12-2012 12-2012-02 12-2012-02-T GLP: yes 2012	Straw-berry Sonata; [redacted]	Nether-lands Europe, North	fruit	0 **1** **1** 3	0.016 0.010 <0.01 <0.01	0.021 0.016 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	0.012 <0.01 <0.01 <0.01
12-2012 12-2012-03 12-2012-03-T GLP: yes 2012	Straw-berry Mathis [redacted]	France Europe, North	fruit	0* 0 **1** 3 7 10	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
12-2012 12-2012-04 12-2012-04-T GLP: yes 2012	Straw-berry Lamba-da	Belgium [redacted] Europe, North	fruit	0* 0 **1** 3 7 10	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
12-2012 12-2012-05 12-2012-05-T GLP: yes 2012	Straw-berry Elsanta [redacted]	Germany Europe, North	fruit	0 **1** **1** 3	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01

DALT = days after last treatment * prior to last treatment

Field trials – southern Europe:

Report:	**KCA 6.3.8/03, [redacted]; 2013 (amended)**; M-457958-02-1
Title:	Determination of the residues of trifloxystrobin in/on strawberry after spray application of Trifloxystrobin WG 50 in the field in southern France, Spain and Italy
Document No & Report No:	M-457958-02-1 11-2129
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.3.8/04, [redacted]; [redacted]; [redacted]; 2013** ; M-460009-01-1
Title:	Determination of the residues of trifloxystrobin in/on strawberry after spray application of Trifloxystrobin WG 50 in Spain, Italy and Greece
Document No & Report No:	M-460009-01-1 12-2013
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed

GLP	yes

Test system

In 2011 and 2012 nine trials were performed in southern Europe in the field in/on strawberries with Trifloxystrobin WG 50 according to the use pattern supported within this dossier. The product was applied two times to strawberries at application rates of about 0.15 kg trifloxystrobin/ha. The treatments were performed with intervals of 7 - 9 days.
Fruit samples were taken on day 1 and 3 (2 or 4) after the last application in all trials. Additional samples of fruit were taken at later time points in some trials.

Residues of trifloxystrobin (CGA 279202), its isomers CGA 331409, CGA 357261, CGA 357262, as well as the metabolite CGA 321113 and its isomer CGA 373466 were determined according to method 01313 or 01313/M001. The analytical methods were validated by recovery experiments prior to and during the analysis of the samples by spiking control samples. The limit of quantitation was 0.01 mg/kg for all analytes.

Findings

- Method performance: Overall mean recoveries at fortification levels of 0.01, 0.1 and 0.5 mg/kg per analyte were within the acceptable range of 70-110 %, RSD ≤20% as shown in Table 6.3.8-7.

Table 6.3.8-7: Recoveries for trifloxystrobin, CGA321113, CGA 357261, CGA 357262, CGA 331409, CGA 373466 in/on strawberry

Report No.	Analyte	Sample Material	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
11-2129	Trifloxystrobin	strawberryfruit	0.01	97; 102; 112; 119	108	9.2	0.01
			0.1	101	101	-	
			0.5	100	100	-	
			Overall Recovery (n =6)		105	8.1	
	CGA 321113	strawberryfruit	0.01	94; 96; 97, 100	97	2.6	0.01
			0.1	100	100	-	
			0.5	91	91	-	
			Overall Recovery (n =6)		96	3.6	
	CGA 357261	strawberryfruit	0.01	95; 98; 110; 116	105	9.5	0.01
			0.1	104	104	-	
			0.5	98	98	-	
			Overall Recovery (n =6)		104	7.9	
	CGA 357262	strawberryfruit	0.01	88; 105; 109; 123	106	13.6	0.01
			0.1	106	106	-	
			0.5	99	99	-	
			Overall Recovery (n =6)		105	11.0	
	CGA 331409	strawberryfruit	0.01	95; 112; 114; 126	112	11.4	0.01
			0.1	105	105	-	
			0.5	104	104	-	
			Overall Recovery (n =6)		109	9.7	

Report No.	Analyte	Sample Material	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
11-2129	CGA 373466	strawberryfruit	0.01	90; 99; 104; 116	102	10.6	0.01
			0.1	100	100	-	
			0.5	91	91	-	
			Overall Recovery (n =6)		100	9.5	

RSD = relative standard deviation n = number of tests

For 12-2013 see Table 6.3.8-4

- Storage stability: The maximum storage period of deep-frozen samples was up to 528 days for all analytes and is covered by the storage stability studies.

- Residue results: In the southern European field trials the residues at a PHI of 1 day ranged from 0.054 to 0.23 mg/kg for trifloxystrobin and were <0.01 to 0.059 mg/kg for CGA 321113 in strawberry fruit. A maximum of CGA 321113 residues was found at up to 0.064 mg/kg at later sampling dates. Residues of CGA 357262 and CGA 331409 were always below LOQ (<0.01 mg/kg). Residues of CGA 373466 were <LOQ, except in two trials with 0.011 and 0.01 mg/kg at day 3 or 1 after the last application. Residues of CGA 357261 were below LOQ except in two trials (0.01 mg/kg at day 1 and 0.012 mg/kg at day 3).

- No residues above the LOQ of 0.01 mg/kg could be detected in any of the corresponding control samples.

Table 6.3.8-8: Application data and residues of trifloxystrobin and CGA 321113 in/ on strawberries treated with Trifloxystrobin WG 50 in the field in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
11-2129 11-2129-01 11-2129-01-T GLP: yes 2011	Straw-berry Dark select	France [redacted] Europe, South	50 WG	3	0.150	0.0188	85	fruit	0* 0 **1** 3 7 10	0.050 0.13 0.11 0.11 0.053 0.041	<0.01 <0.01 <0.01 0.019 0.013 0.015
11-2129 11-2129-02 11-2129-02-T GLP: yes 2011	Straw-berry Whitney	Spain [redacted] Europe, South	50 WG	2	0.150	0.0150	87	fruit	0* 0 **1** 3 7 11	0.12 0.24 0.20 0.17 0.079 0.053	0.029 0.022 0.025 0.045 0.043 0.025
11-2129 11-2129-03 11-2129-03-T GLP: yes 2011	Straw-berry Camela	Italy [redacted] Europe, South	50 WG	2	0.150	0.0250	87	fruit	0 **1** 3	0.26 0.20 0.17	0.028 0.026 0.038

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
11-2129 11-2129-04 11-2129-04-T GLP: yes 2011	Straw-berry Ventana	Italy [redacted] Europe, South	50 WG	2	0.150	0.0214	87	fruit	0 **1** 3	0.19 0.15 0.12	0.020 0.020 0.030
12-2013 12-2013-01 12-2013-01-T GLP: no 2012	Straw-berry Cama-rosa	Spain [redacted] Europe, South (altitude 148 m, mediterra-nean climate)	50 WG	2	0.150-0.158	0.0188	87	fruit	0* 0 **1** 3 7 10	0.028 0.29 0.23 0.17 0.070 0.043	0.022 0.026 0.026 0.019 0.027 0.019
12-2013 12-2013-02 12-2013-02-T GLP: no 2012	Straw-berry Halifa	Spain [redacted] Europe, South (altitude 968 m, mountain climate)	50 WG	2	0.15	0.0188	87	fruit	0 **1** 2	0.082 0.054 0.061	<0.01 <0.01 0.014
12-2013 12-2013-03 12-2013-03-T GLP: no 2012	Straw-berry Cama-rosa	Spain [redacted] Europe, South	50 WG	2	0.15	0.0188	87	fruit	0* 0 **1** 3 7 11	0.043 0.10 0.082 0.049 0.083 0.051	<0.01 <0.01 <0.01 <0.01 0.014 0.014
12-2013 12-2013-04 12-2013-04-T GLP: no 2012	Straw-berry Selva	Italy [redacted] Europe, South	50 WG	2	0.15	0.0150	87	fruit	0* 0 **1** 3 6 10	0.045 0.12 0.17 0.16 0.059 0.051	0.054 0.047 0.059 0.064 0.039 0.051
12-2013 12-2013-05 12-2013-05-T GLP: no 2012	Straw-berry Kama-roze	Greece [redacted] Europe, South	50 WG	2	0.15	0.0188	87	fruit	0 **1** 4	0.15 0.12 0.13	0.017 0.014 0.016

FL: Formulation No: number of applications
GS = growth stage (BBCH code) at last application DALT = days after last treatment
* prior to last treatment

Table 6.3.8-9: Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in/ on strawberries treated with Trifloxystrobin WG 50 in the field in southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Residues Portion analysed	DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
11-2129 11-2129-01 11-2129-01-T GLP: yes 2011	Straw-berry Dark select	France [redacted] Europe, South	fruit	0* 0 **1** 3 7 10	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
11-2129 11-2129-02 11-2129-02-T GLP: yes 2011	Straw-berry Witney	Spain [redacted] Europe, South	fruit	0* 0 **1** 3 7 11	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
11-2129 11-2129-03 11-2129-03-T GLP: yes 2011	Straw-berry Carmela	Italy [redacted] Europe, South	fruit	0 **1** 3	<0.01 <0.01 0.012	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 0.011
11-2129 11-2129-04 11-2129-04-T GLP: yes 2011	Straw-berry Ventana	Italy [redacted] Europe, South	fruit	0 **1** 3	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01
12-2013 12-2013-01 12-2013-01-T GLP: no 2012	Straw-berry Cama-rosa	Spain [redacted] Europe, South (altitude 148 m, mediterra-nean climate)	fruit	0* 0 **1** 3 7 10	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
12-2013 12-2013-02 12-2013-02-T GLP: no 2012	Straw-berry Halifa	Spain [redacted] Europe, South (altitude 968 m, mountain climate)	fruit	0 **1** 2	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01
12-2013 12-2013-03 12-2013-03-T GLP: no 2012	Straw-berry Cama-rosa	Spain [redacted] Europe, South	fruit	0* 0 **1** 3 7 11	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
12-2013 12-2013-04 12-2013-04-T GLP: no 2012	Straw-berry Selva	Italy [redacted] Europe, South	fruit	0* 0 **1** 3 6 10	<0.01 <0.01 0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 0.01 0.01 <0.01 <0.01

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Residues Portion analysed	DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
12-2013 12-2013-05 12-2013-05-T GLP: no 2012	Straw-berry Kama-roze	Greece [redacted] Europe, South	fruit	0 **1** 4	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01

DALT = days after last treatment * prior to last treatment

Greenhouse trials:

Report:	**KCA 6.3.8/05, [redacted]; 2013 (amended)**; M-456769-02-1
Title:	Determination of the residues of trifloxystrobin in/on strawberry after spray application of Trifloxystrobin WG 50 in the greenhouse in Spain, Italy, Portugal and Greece
Document No & Report No:	M-456769-02-1 11-2120
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.3.8/06, [redacted]; 2013 (amended)**; M-453332-02-1
Title:	Determination of the residues of trifloxystrobin in/on strawberry after spraying of Trifloxystrobin WG 50 in the greenhouse in Belgium, France (North) and Germany
Document No & Report No:	M-453332-02-1 12-2014
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Test system

In 2011 and 2012 eight trials were performed in the greenhouse in Europe in/on strawberries with Trifloxystrobin WG 50 according to the use pattern supported within this dossier. The product was applied two times to strawberries at application rates of 0.15 kg trifloxystrobin/ha. The treatments were performed with intervals of 7 days.
Fruits samples were taken on day 1 and 3 after the last application in all trials. Additional samples of fruit were taken at later time points in some trials.

Residues of trifloxystrobin (CGA 279202), its isomers CGA 331409, CGA 357261, CGA 357262, as well as the metabolite CGA 321113 and its isomer CGA 373466 were determined according to method 01313 or 01313/M001. The analytical methods were validated by recovery experiments prior

to and during the analysis of the samples by spiking control samples. The limit of quantitation was 0.01 mg/kg for all analytes.

In one trial (12-2014-02) plants were cultivated in the field covered with plastic tube instead of cultivation in a classical greenhouse. Nevertheless, since the plants were covered by plastic during the whole trial period from first application till sampling and since the sampling was done only 1 and 3 days after last application, no differences to strawberries grown in a completely closed system are expected.

Findings

- Method performance: Overall mean recoveries at fortification levels of 0.01, 0.1 and 0.5 mg/kg per analyte were within the acceptable range of 70-110 %, RSD <20% as shown in Table 6.3.8-10.

Table 6.3.8-10: Recoveries for trifloxystrobin, CGA321113, CGA 357261, CGA 357262, CGA 331409, CGA 373466 in/on strawberry

Report No.	Analyte	Sample Material	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
11-2120	Trifloxystrobin	strawberry fruit	0.01	80; 84; 93	86	7.8	0.01
			0.1	88; 91; 92; 92	91	2.1	
			0.5	88	88	-	
			Overall Recovery (n =8)		89	5.1	
	CGA 321113	strawberry fruit	0.01	72; 72; 87	77	11.2	0.01
			0.1	82; 82; 84; 87	84	2.8	
			0.5	82	82	-	
			Overall Recovery (n =8)		81	7.3	
	CGA 357261	strawberry fruit	0.01	77; 82; 82	80	3.6	0.01
			0.1	84; 84; 84; 86	85	1.2	
			0.5	86	86	-	
			Overall Recovery (n =8)		83	3.5	
	CGA 357262	strawberry fruit	0.01	75; 76; 91	81	11.1	0.01
			0.1	89; 93; 93; 100	94	4.9	
			0.5	91	91	-	
			Overall Recovery (n =8)		89	9.8	
	CGA 331409	strawberry fruit	0.01	72; 84; 90	82	11.2	0.01
			0.1	93; 94; 96; 99	96	2.8	
			0.5	100	100	-	
			Overall Recovery (n =8)		91	10.1	
	CGA373466	strawberry fruit	0.01	68; 74; 75	72	5.2	0.01
			0.1	80; 81; 85; 85	83	3.2	
			0.5	88	88	-	
			Overall Recovery (n =8)		80	8.5	

RSD = relative standard deviation n = number of tests

For 12-2014 see Table 6.3.8-4

- Storage stability: The maximum storage period of deep-frozen samples was up to 513 days for all analytes and is covered by the storage stability studies.

- Residue results: In the greenhouse trials the residues at a PHI of 1 day ranged from 0.076 to 0.41 mg/kg for trifloxystrobin in strawberry fruit. Residues of CGA 321113 were between <0.01 and 0.015 mg/kg at day 1 after last application and up to 0.032 at later samplings.
Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 were always below the LOQ (<0.01 mg/kg).

- No residues above the LOQ of 0.01 mg/kg could be detected in any of the corresponding control samples.

Table 6.3.8-11: Application data and residues of trifloxystrobin and CGA 321113 in/ on strawberries treated with Trifloxystrobin WG 50 in the greenhouse

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
11-2120 11-2120-01 11-2120-01-T GLP: yes 2011	Straw-berry Splen-dor	Spain [redacted] Europe, South, G	50 WG	2	0.150	0.0188	87	fruit	0* 0 **1** 3 7 10	0.15 0.35 0.41 0.30 0.28 0.16	0.015 0.013 0.015 0.013 0.032 0.031
11-2120 11-2120-02 11-2120-02-T GLP: yes 2011	Straw-berry Ventana	Italy [redacted] Europe, South, G	50 WG	2	0.150	0.0225	85	fruit	0 **1** 3	0.10 0.06 0.12	<0.01 <0.01 0.015
11-2120 11-2120-03 11-2120-03-T GLP: yes 2011	Straw-berry Cama-rrosa; hanging variety	Portugal [redacted] Europe, South, G	50 WG	2	0.150	0.0200	85	fruit	0* 0 **1** 3 7 10	0.11 0.31 0.27 0.23 0.23 0.14	<0.01 <0.01 <0.01 0.010 0.015 0.017
11-2120 11-2120-04 11-2120-04-T GLP: yes 2011	Straw-berry Cama-rosa	Greece [redacted] Europe, South, G	50 WG	2	0.150	0.0150	85	fruit	0 **1** 3	0.14 0.13 0.12	<0.01 0.010 0.016
12-2014 12-2014-01 12-2014-01-T GLP: yes 2012	Straw-berry Dar-select; hanging variety	Belgium [redacted] Europe, North, G	50 WG	2	0.150	0.0150	87	fruit	0* 0 **1** 3 7 10	0.038 0.076 0.076 0.082 0.056 0.053	<0.01 <0.01 <0.01 0.017 0.029 0.025
12-2014 12-2014-02 12-2014-02-T GLP: yes 2012	Straw-berry Cigaline	France [redacted] Europe, North, (G)	50 WG	2	0.150	0.0250	87	fruit	0 **1** 3	0.10 0.090 0.096	<0.01 <0.01 0.014
12-2014 12-2014-03 12-2014-03-T GLP: yes 2012	Straw-berry Clery	Germany [redacted] Europe, North, G	50 WG	2	0.150	0.0300	87	fruit	0* 0 **1** 3 7 10	0.037 0.067 0.091 0.080 0.045 0.032	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
12-2014 12-2014-04 12-2014-04-T GLP: yes 2012	Straw-berry Dar-select	Germany [redacted] Europe, North, G	50 WG	2	0.150	0.0150	87	fruit	0 **1** 3	0.16 0.12 0.12	<0.01 0.012 0.028

FL: Formulation No: number of applications
GS = growth stage (BBCH code) at last application DALT = days after last treatment
* prior to last treatment.

Table 6.3.8-12: Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in/ on strawberries treated with Trifloxystrobin WG 50 in the greenhouse

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Residues Portion analysed	DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
11-2120 11-2120-01 11-2120-01-T GLP: yes 2011	Straw-berry Splen-dor	Spain [redacted] Europe, South, G	fruit	0* 0 **1** 3 7 10	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
11-2120 11-2120-02 11-2120-02-T GLP: yes 2011	Straw-berry Ventana	Italy [redacted] Europe, South, G	fruit	0 **1** 3	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01
11-2120 11-2120-03 11-2120-03-T GLP: yes 2011	Straw-berry Cama-rrosa; hanging variety	Portugal [redacted] Europe, South, G	fruit	0* 0 **1** 3 7 10	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
11-2120 11-2120-04 11-2120-04-T GLP: yes 2011	Straw-berry Cama-rosa	Greece GR-[redacted] Europe, South, G	fruit	0 **1** 3	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01
12-2014 12-2014-01 12-2014-01-T GLP: yes 2012	Straw-berry Dar-select; hanging variety	Belgium [redacted] Europe, North, G	fruit	0* 0 **1** 3 7 10	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
12-2014 12-2014-02 12-2014-02-T GLP: yes 2012	Straw-berry Cigaline	France [redacted] Europe, North, G	fruit	0 **1** 3	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01
12-2014 12-2014-03 12-2014-03-T GLP: yes 2012	Straw-berry Clery	Germany [redacted] Europe, North, G	fruit	0* 0 **1** 3 7 10	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
12-2014 12-2014-04 12-2014-04-T GLP: yes 2012	Straw-berry Dar-select	Germany [redacted] Europe, North, G	fruit	0 **1** 3	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01

DALT = days after last treatment * prior to last treatment.

CA 6.4 Feeding studies

Data/information on livestock feeding studies were reviewed during the peer review under Directive 91/414/EEC and considered to be acceptable. For further information, please refer to the Annex II, Section 6, Point 6.4.
In addition to the ruminant feeding study formerly submitted, a poultry study is available and summarised below, although it can already be concluded from the poultry metabolism study, that no residues above LOQ are expected in poultry commodities.

CA 6.4.1 Poultry

A three-level (1.5 mg/kg, 4.5 mg/kg and 15 mg/kg) poultry feeding study was conducted with trifloxystrobin in the USA in 1998.

Report:	**KCA 6.4.1/01, [redacted], C.G.; 1999** ; M-036568-01-1
Title:	CGA-279202 - Magnitude of the residues in poultry meat and eggs
Document No & Report No:	M-036568-01-1 243-98
Guidelines:	OPPTS 860.1480 Residue Chemistry Test Guidelines - Meat/Milk/Poultry/Eggs
GLP	yes

Materials and methods

A poultry feeding study was conducted with white leghorn laying hens using technical-grade trifloxystrobin (CGA 279202) treated feed. Three treatment groups consisting of 15 animals per group plus the control animals were used. Treatment rates were 1.5 mg/kg (1X), 4.5 mg/kg (3X), and 15.0 mg/kg (10X). Animals were kept on diet for 28 days. Eggs were sampled throughout the treatment period, before dosing (day 0) and on dose-days 1, 3, 7, 14, 21, and 28. The hens were sacrificed on day 29 (20-24 hours after the last treated feed was removed) and samples of muscle (breast and thigh), skin plus attached fat, peritoneal fat, and liver were obtained.

Treated feed was sampled and analysed to demonstrate dose conformity and stability. Eggs and poultry tissues were analysed using analytical method AG 659A, which determines CGA 279202 and its acid metabolite CGA 321113 by gas chromatography using nitrogen/phosphorus detection (NPD) following an acetonitrile:water extraction and solvent partition plus a C18 solid phase extraction cartridge cleanup. The screening level employed for all samples analysed was 0.02 mg/kg for each analyte.

Findings / Conclusion

Feed samples taken at each week indicated that the nominal dosages were achieved and that the test substance was stable upon storage at room temperature and under freezer conditions in poultry feed. Mean recoveries from the 10X dosing level were 105% for eggs and 79% for tissues for trifloxystrobin and 90% for eggs and 87% for tissues for CGA 321113.
No detectable residues (<0.02 mg/kg) of CGA 279202 or CGA 321113 were found in eggs, skin, muscle, fat and liver samples at the exaggerated 10X feeding level, see Table 6.4.1-1 below. Analyses of egg and tissue samples from hens dosed with 1X and 3X were not performed, since no residues were detected at the highest treatment rate.

Based on results of this study, no residues are anticipated in poultry and it is in principle not necessary to establish tolerances in eggs and poultry tissues.

Table 6.4.1-1: Residues found in laying hens dosed with CGA 279202 for 28 days

		Maximum Residues Found (mg/kg)					
		Feeding Level (mg/kg)					
	Dose	1.5 mg/kg (1X)		4.5 mg/kg (3X)		15.0 mg/kg (10X)	
Substrate	Day	CGA 279202	CGA 321113	CGA 279202	CGA 321113	CGA 279202	CGA 321113
Eggs	0	-	-	-	-	<0.02	<0.02
	1	-	-	-	-	<0.02	<0.02
	3	-	-	-	-	<0.02	<0.02
	7	-	-	-	-	<0.02	<0.02
	14	-	-	-	-	<0.02	<0.02
	21	-	-	-	-	<0.02	<0.02
	28	-	-	-	-	<0.02	<0.02
Skin plus attached fat	28-29	-	-	-	-	<0.02	<0.02
Peritoneal Fat	28-29	-	-	-	-	<0.02	<0.02
Muscle (breast plus thigh)	28-29	-	-	-	-	<0.02	<0.02
Liver	28-29	-	-	-	-	<0.02	<0.02

CA 6.4.2 Ruminants

The magnitude of trifloxystrobin residues in ruminants was already investigated in a feeding study with lactating cows evaluated during the peer review under Directive 91/414/EEC. For further information, please refer to the Annex II, Section 4, Point 6.4.

CA 6.4.3 Pigs

No specific feeding study has been conducted with pigs as the metabolic pattern in ruminants does not differ significantly from that in the rat. This point is therefore adequately covered by the cattle feeding study, which was already evaluated during the peer review under Directive 91/414/EEC.

CA 6.4.4 Fish

So far no official working document exists on fish feeding studies. No feeding study for fish was conducted with trifloxystrobin.
Pomefruit, grape and strawberry fruit are not considered relevant for the formulation of aquaculture diets.

CA 6.5 Effects of processing

Some processing data were already submitted in the Annex II dossier for trifloxystrobin. Further data is given below.

CA 6.5.1 Nature of the residue

The parameter which is most likely to affect the nature of the residue during processing operations is hydrolysis, because processes like heating would generally inactivate enzymes present in the substrate, leaving primarily hydrolysis as a degradation mechanism. Hence, a study was conducted to investigate the hydrolytic degradation of trifloxystrobin under representative conditions of processing.

Trifloxystrobin was hydrolytically stable under conditions of pasteurisation (90°C, pH 4 for 20 min) and showed minor degradation of 2.6 % under conditions of baking, brewing and boiling (100°C, pH 5 for 60 min). Only under conditions of sterilisation (120°C, pH 6 for 20 min) a more pronounced degradation of 22.5 % was observed. The main degradate observed (2 % at pH 5 and 20.8 % at pH 6) was CGA 321113.

The respective study ([redacted], 2000) was already submitted in the updated Annex II dossier. Since this study cannot be found in the CRD evaluation, it is summarised again below.

Report:	**KCA 6.5/01, [redacted], 2000 (already submitted)** ; M-047519-01-1
Title:	Hydrolysis of [Glyoxyl-phenyl-U-14C]-CGA 279202 under processing conditions
Document No & Report No:	M-047519-01-1 00MO02
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products food and feed
GLP	yes

Materials and methods

The study describes the degradation behaviour of radioactive [Glyoxyl-phenyl-U-14C]-trifloxystrobin in buffered water. The experiment was carried out under laboratory conditions, which were representative for processing operations of raw agricultural commodities (RAC) like pasteurisation, baking and sterilisation.

The test systems were incubated at three representative sets of conditions: 90°C, pH 4 for 20 min. (pasteurisation), 100°C, pH 5 for 60 min. (baking, brewing and boiling) and 120°C, pH 6 for 20 min. (sterilisation). The samples were analysed by HPLC and thin-layer chromatography. The content of radioactivity was determined by liquid scintillation counting.

Findings

Trifloxystrobin was hydrolytically stable under conditions of pasteurisation (90°C, pH 4 for 20 min) and showed minor degradation of 2.6 % under conditions of baking, brewing and boiling (100°C, pH 5 for 60 min). Only under conditions of sterilisation (120°C, pH 6 for 20 min) a more pronounced degradation of 22.5 % was observed. The main degradate observed (2 % at pH 5 and 20.8 % at pH 6) was CGA 321113.

Conclusion

The results demonstrate that trifloxystrobin will be partially hydrolysed to CGA 321113 under conditions representative for sterilisation (pH 6, 120°C). In these cases, the nature of the residue in the processed agricultural commodities may be partly different from that found in raw agricultural commodities. Under conditions representative for pasteurisation, (pH 4, 90°C) and baking, brewing and boiling (pH 5, 100°C) trifloxystrobin was found to be stable and the nature of the residue can be considered as being identical to that in the raw agricultural commodity.

CA 6.5.2 Distribution of the residue in peel and pulp

The distribution of the residue in peel and pulp is not relevant for the supported crops, since the peel is edible and usually the entire fruit is eaten by consumers.

CA 6.5.3 Magnitude of residues in processed commodities

Relevant information on pomefruit and grapes was already given in the Annex II dossiers, Section 6, chapter 6.3 (residues) and 6.5 (processing). Further data are summarised below, including strawberry processing trials.

Pomefruit:

Summary Annex II studies

Apples from some European residue trials were subjected to small scale processing (reported in the residue trial chapter 6.3) No concentration of parent residues was observed from apples having 0.03 - 0.44 mg/kg initial residues to dried fruits, puree, juice (<0.02 - 0.04 mg/kg) or pomace (0.33 mg/kg). For CGA 321113, the initial residues were low and no transfer of residues from fruits to processed commodities was observed.

In an US study (Campbell, 1997) residues found were predominantly CGA 279202, with CGA 321113 being detected only in the wet apple pomace. The results show that the residues are not transferred to the juice, but remain in the wet pomace, resulting in a considerable concentration.

Supplementary studies

Report:	**KCA 6.5.3/02, [redacted]; 2000 (also filed: KCA 6.3.1/31)**, M-024932-01-1
Title:	Residue study with CGA 279202 in or on apples in France (north)
Document No & Report No:	M-024932-01-1 2007/99
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

A processing trial was conducted in on apples with trifloxystrobin in 1999 in northern France. Trifloxystrobin WG 50 was applied three times to apple trees at rates of 0.15 kg/ha active substance trifloxystrobin. The treatments were performed at intervals of 10 days with the last application 14 days prior to the expected date of harvest.
Samples were taken 14 days after the last treatment. Residues were determined in the raw agricultural commodity (fruit), juice and puree.

For processing into juice the fruits were mixed, and the juice filtered and pasteurised. For processing into puree the fruits were cooked and mixed.

The results of the trials are summarised in Table 6.5.3-1 as well as in greater detail on the attached Tier I summary forms.

Findings / conclusion:

- Method performance: modified method AG 659 was used to determine residues of trifloxystrobin and CGA 321113 in apple processed matrices.
- Storage stability: The maximum storage period of deep-frozen samples (processed commodities) for trifloxystrobin and its metabolite was 211 days.

- Processing results:
Residues of trifloxystrobin in apple fruit (raw agricultural commodity, RAC) were 0.03 and 0.02 mg/kg, those of CGA 321113 were <0.02 mg/kg. Residues of trifloxystrobin were below LOQ (<0.01

mg/kg) in juice and 0.04 mg/kg in puree. Residues of CGA 321113 were below LOQ (<0.01 mg/kg) in juice and puree.

The transfer factors with respect to trifloxystrobin were <0.4 (calculation <0.01/0.025 mg/kg) for juice and 1.6 for puree. No transfer factors were calculated for CGA 321113, as no residues at or above LOQ were found neither in the RAC, nor in the processed commodity. According to this trial, a concentration of trifloxystrobin is seen in puree, while no accumulation and no residues were observed in juice.

Report:	**KCA 6.5.3/03, [redacted]; 2000 (also filed: KCA 6.3.1/34)** ; M-136411-01-1
Title:	Residue study with CGA 279202 in or on apples in Switzerland
Document No & Report No:	M-136411-01-1 2125/99
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Materials and methods

A processing trial was conducted in/on apples with trifloxystrobin in 1999 in Switzerland. Trifloxystrobin WG 50 was applied three times to apple trees at exaggerated rates of 0.375 kg/ha active substance trifloxystrobin. The treatments were performed at intervals of 10-12 days with the last application 14 days prior to the expected date of harvest.
Samples were taken 14 days after the last treatment. Residues were determined in the raw agricultural commodity (fruit), washed fruit, juice, wet pomace, dry pomace, and puree.

For processing into juice the fruits were washed, sliced, pressed and the juice pasteurized. For processing into puree the fruits were washed, cooked, filtered and pasteurised.
The results of the trials are summarised in Table 6.5.3-1 as well as in greater detail on the attached Tier I summary forms.

Findings / Conclusion

- Method performance: modified method AG 659 was used to determine residues of trifloxystrobin and CGA 321113 in apple processed matrices.
- Storage stability: The maximum storage period of deep-frozen samples processed commodities for trifloxystrobin and its metabolite was 258 days.

- Processing results:
Residues of trifloxystrobin in apple fruit (RAC) were 0.25 and 0.35 mg/kg, those of CGA 321113 were <0.01 mg/kg. Residues of trifloxystrobin ranged from 0.20 to 0.35 mg/kg in fruit washed, were at 0.0088 or 0.0080 mg/kg in juice before pasteurisation and ranged between 0.0026 and 0.0034 in juice after pasteurisation. Trifloxystrobin residues in wet pomace were at 1.18 or 1.28 mg/kg, between 5.0 and 6.1 mg/kg in dry pomace and between 0.036 and 0.056 mg/kg in pasteurised puree. Residues of CGA 321113 were <0.01 mg/kg in washed fruit and puree, <0.002 mg/kg in juice (before and after pasteurisation), 0.046 and 0.052 mg/kg in wet pomace and between 0.94 and 1.5 in dry pomace.

The transfer factors with respect to trifloxystrobin were between 0.63 and 1.24 for washed fruit, between 0.010 and 0.014 for pasteurised juice, between 20 and 24 for dry pomace and between 0.10 and 0.16 for pasteurised puree.

No transfer factors were calculated for CGA 321113 in washed fruit and juice, as no residues at or above LOQ were found neither in the RAC, nor in the processed commodity. In wet and dry pomace an accumulation of residues of CGA 321113 was observed.
According to this trial, a concentration of trifloxystrobin and CGA 321113 is seen in wet and dry pomace, while no accumulation was observed in juice or puree.

Table 6.5.3-1: Results from processing studies on apples

Portion analysed	PHI (d)	Residues of trifloxystrobin		Residues of CGA 321113		*Report No.*
		residue values (mg/kg)	transfer factor	residue values (mg/kg)	transfer factor	Study no
fruit	14	0.03 0.02 mean: 0.25	n.a.	<0.02 <0.02	n.a.	*2007/99*
juice	14	<0.01 <0.01	0.4	<0.01 <0.01	n.c.	
puree	14	0.04 0.04	1.6	<0.01 <0.01	n.c.	
fruit	14	0.25	n.a.	< 0.01	n.a.	*2125/99*
fruit, washed	14	0.31 0.31 0.25 0.20	1.24 1.24 1.00 0.80	<0.01 <0.01 <0.01 <0.01	n.c.	
juice after pasteurisation	14	0.0026 0.0032 0.0034 0.0034	0.010 0.013 0.014 0.014	<0.002 <0.002 <0.002 <0.002	n.c.	
wet pomace	14	1.18 1.28	4.72 5.12	0.052 0.046	>5.2 >4.6	
dry pomace	14	6.1 5.1 5.1 5.0	24 20 20 20	1.5 0.94 0.99 0.96	>150 >94 >99 >96	
fruit	14	0.35	n.a.	< 0.01	n.a.	2125/99
fruit, washed	14	0.22 0.27 0.35 0.30	0.67 0.77 1.00 0.86	<0.01 <0.01 <0.01 <0.01	n.c.	
puree	14	0.056 0.044 0.036 0.038	0.16 0.13 0.10 0.11	<0.01 <0.01 <0.01 <0.01	n.c.	

n.a.: not applicable n.c.: not calculated (residues <LOQ in RAC and processed commodity)

Grape:

Summary Annex II studies

Grapes from some European trials were processed to make must or wine (reported in the residue trial chapter 6.3). No concentration of parent residues was observed from grapes having 0.06 - 2.3 mg/kg initial residues to must (2.5 mg/kg) or wine (<0.02 - 0.25 mg/kg). For CGA 321113, the initial residues were low (0.03 - 0.14 mg/kg) and no transfer of residues from fruits to processed commodities was observed.

Supplementary studies

Report:	**KCA 6.5.3/04, [redacted], [redacted]; 2009 (amended)**; M-357708-02-1
Title:	Determination of the residues of AE C656948 and Trifloxystrobin in/on grape after spraying and spraying, low-volume of AEC656948 & CGA279202 SC 500 in the field in France (South) and Italy
Document No & Report No:	M-357708-02-1 08-2204 (field part to 08-3204)
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.5.3/05, [redacted], [redacted]; 2010**; M-384844-01-1
Title:	Determination of the residues of AE C656948 and trifloxystrobin in/on grape and the processed fractions (must; pomace, grape; wine at bottling and wine at first taste test) after spraying of AE C656948 & CGA279202 SC 500 in the field in France (South) and Italy
Document No & Report No:	M-384844-01-1 08-3204 (processing part)
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Materials and methods

In 2008 two processing trials were conducted in/on grapes with trifloxystrobin in southern Europe. A trifloxystrobin SC formulation was applied two times to apple trees at rates of 0.05 kg/ha active substance trifloxystrobin. The treatments were performed at intervals of 14-15 days with the last application 14 days prior to the expected date of harvest.

Samples were taken 14 days after the last treatment (21 days in one trial). Residues were determined in the raw agricultural commodity (bunch), in must, pomace and wine. In the trial where processing trials were taken at day 21 after last application, while RAC samples were taken at day 14, the results of must were taken as RAC value for calculation of the transfer factors, as in the other trials bunch and must showed the same residues at day 14.

For processing into must the bunches were crushed, for processing into wine the must was treated with flash vacuum-expansion, pressed after fermentation, clarified and filtered.

The results of the trials are summarised in Table 6.5.3-2 as well as in greater detail in the Tier I summary forms.

Findings / Conclusion

- Method performance: method 01013 was used to determine residues of trifloxystrobin and CGA 321113 in grape processed matrices.

- Storage stability: The maximum storage period of deep-frozen samples (processed commodities) for trifloxystrobin and its metabolite was 348 days.

- Processing results:
Residues of trifloxystrobin in bunch of grapes (raw agricultural commodity, RAC) were 0.02 mg/kg, those of CGA 321113 were <0.01 mg/kg. Residues of trifloxystrobin were below LOQ (<0.01 mg/kg) in wine, 0.02 mg/kg in must and 0.10 in pomace. Residues of CGA 321113 were below LOQ (<0.01 mg/kg) in wine and must, and at 0.01 mg/kg in pomace.

The transfer factors with respect to trifloxystrobin were 1.0 for must, 5.0 and 5.9 for pomace and <0.1 and <0.5 for wine. No transfer factors were calculated for CGA 321113, as no residues at or above LOQ were found neither in the RAC, nor in the processed commodity, except in one trial in pomace at LOQ. According to these trials, a concentration of trifloxystrobin is seen in pomace, while no accumulation was observed in must and wine.

Report:	**KCA 6.5.3/06, [redacted], T.P.; 1998** ; M-104033-01-1
Title:	CGA 279202 - Magnitude of the residue in or on grapes
Document No & Report No:	M-104033-01-1 110440
Guidelines:	EPA guideline No: 860.1500, 860.1520
GLP	yes

Materials and methods

In 1996 six processing trials (2 locations) were performed in the USA with Trifloxystrobin 50 WG in/on grapes. Grapes were sprayed with the test substance using three treatment regimes. Treatment 1 (1-fold rate) consisted of six foliar spray applications at a rate of 0.1408 kg a.s./ha. Treatment 2 consisted of six applications at the 3-fold rate (0.4225 kg a.s./ha) and treatment 3 of six applications at the 5-fold rate (0.7042 kg a.s./ha). Samples for processing were taken 14 days after the last application.

For juice production the grapes were crushed, the pulp collected and the stems discarded. The pulp was heated, filtered and pasteurised.

Raisins were processed in the field by air drying according to normal agricultural practices for 17 or 26 days.

The results of the trials are summarised in Table 6.5.3-2 as well as in greater detail in the Tier I summary forms.

Findings / Conclusion

- Method performance: method AG 659 was used to determine residues of trifloxystrobin and CGA 321113 in grape processed matrices.

- Storage stability: The maximum storage period of deep-frozen samples (processed commodities) for trifloxystrobin and its metabolite was 212 days.

- Processing results: Residues of trifloxystrobin in bunch of grapes (RAC) were between 0.24 and 2.2 mg/kg, those of CGA 321113 were between <0.02 and 0.080 mg/kg. Residues of trifloxystrobin ranged from 0.034 to 0.34 mg/kg in juice, and were at 0.12, 0.16, 1.5, or 1.2 in raisin. Residues of CGA 321113 were between <0.02 mg/kg in juice and either <0.02 or at 0.16 and 0.23 mg/kg.

The transfer factors with respect to trifloxystrobin were between 0.072 and 0.18 for juice, and 0.50, 0.67, 2.5 or 2.0 for raisin.

No transfer factors were calculated for CGA 321113 in trial USA-OW-FR-415-96, as no residues at or above LOQ were found neither in the RAC, nor in the processed commodity. In trial USA-02-FR-025-96 an accumulation of residues of CGA 321113 in raisin was observed.

According to these trials, no accumulation was observed in juice, while a concentration of trifloxystrobin and CGA 321113 may be seen in raisin.

Table 6.5.3-2: Results from processing studies on grapes

Portion analysed	PHI (d)	Residues of trifloxystrobin		Residues of CGA 321113		*Report No./* Trial no (g as/ha)
		residue values (mg/kg)	transfer factor	residue values (mg/kg)	transfer factor	
bunch	14	0.02	n.a.	<0.01	n.a.	*08-3204* *08-3204-01*
must	14	0.02	1.0	<0.01	n.c.	
pomace	14	0.10	5.0	0.01	>1	
wine at bottling	14	<0.01	<0.5	<0.01	n.c.	
wine at 1st taste test	14	<0.01	<0.5	<0.01	n.c.	
bunch / must	21	0.07	n.a.	<0.01	n.a.	*08-3204* *08-3204-02*
must	21	0.07	1.0	<0.01	n.c.	
pomace	21	0.41	5.9	<0.01	n.c.	
wine at bottling	21	<0.01	<0.1	<0.01	n.c.	
wine at 1st taste test	21	<0.01	<0.1	<0.01	n.c.	
bunch	14	0.24	n.a.	<0.02	n.a.	*110440* *USA-OW-FR-415-96-A*
raw juice	14	0.034	0.14	<0.02	n.c.	
juice	14	0.035	0.15	<0.02	n.c.	
raisin	14	0.12 0.16	0.50 0.67	<0.02 <0.02	n.c.	

Portion analysed	PHI (d)	Residues of trifloxystrobin		Residues of CGA 321113		*Report No./*
		residue values (mg/kg)	transfer factor	residue values (mg/kg)	transfer factor	*Trial no. (g as/ha)*
bunch	14	0.87	n.a.	0.022	n.a.	*110440* *USA-OW-FR-415-96-B*
raw juice	14	0.087	0.10	<0.02	n.c.	
juice	14	0.16	0.18	<0.02	n.c.	
bunch	14	1.1	n.a.	0.023	n.a.	*110440* *USA-OW-FR-415-96-C*
raw juice	14	0.14	0.13	<0.02	n.c.	
juice	14	0.15	0.14	<0.02	n.c.	
bunch	14	0.60	n.a.	0.066	n.a.	*110440* *USA-02-FR-025-96-A*
raw juice	14	0.082	0.14	0.023	0.35	
juice	14	0.057	0.095	<0.02	<0.30	
raisin	14	1.5 1.2	2.5 2.0	0.16 0.23	2.4 3.5	
bunch	14	1.8	n.a.	0.080	n.a.	*110440* *USA-02-FR-025-96-B*
raw juice	14	0.18	0.1	0.029	0.36	
juice	14	0.13	0.072	0.023	0.29	
bunch	14	2.2	n.a.	0.078	n.a.	*110440* *USA-02-FR-025-96-C*
raw juice	14	0.31	0.14	0.035	0.45	
juice	14	0.34	0.15	0.038	0.49	

n.a.: not applicable n.c.: not calculated (residues <LOQ in RAC and processed commodity)

Strawberry:

Supplementary studies

Report:	**KCA 6.5.3/07, [redacted]; 2003** ; M-086063-01-1
Title:	Determination of residues of Trifloxystrobin and CGA 321113 in/on strawberry (fruit washed, preserve, washings, jam) following spray application of Flint 50 WG in the field in Northern France and Germany
Document No & Report No:	M-086063-01-1 RA-3038/02
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Materials and methods

Two processing trials were conducted in/on strawberry with trifloxystrobin in 2002 in northern France and Germany. Trifloxystrobin WG 50 was applied three times to strawberry plants at a product rate of 0.5 kg/ha and 600 L water per ha, corresponding to a spray concentration of 0.083% and 0.25 kg/ha

active substance trifloxystrobin. The treatments were performed at intervals of 7 days with the last application 3 days prior to the expected date of harvest.

Samples were taken 3 days after the last treatment. Residues were determined in the raw agricultural commodity (fruit), washed fruit, washings (wash water), preserve and jam. The processing of washed fruits, washing water and jam simulated household practice. The processing of strawberry preserve simulated industrial practice at laboratory scale. Processed samples were stored deep frozen until analysis.

Washed fruit samples were prepared by washing strawberry fruits in standing water under slow movement. The stalks were removed where necessary.

For the preparation of preserves, washed fruits were filled into preserving cans and sugar solution was added. The strawberry preserves were pasteurised at approx. 90 °C.

Samples of jam were prepared by washing fruits in standing water under slow movement and removing the stalks where necessary. Part of the fruits was minced with a mixer, the other part was cut with a knife into small pieces. Gelly agent was added and the mixture heated to 98 - 100°C for about 3 minutes.

The results of the trials are summarised in Table 6.5.3-3 as well as in greater detail in the Tier I summary forms.

Findings / Conclusion

- Method performance: Method 00742/E001 was used to determine residues of trifloxystrobin and CGA 321113 in strawberry processed matrices.

- Storage stability: The maximum storage period of deep-frozen samples for trifloxystrobin and its metabolite was 152 days.

- Processing results: Residues of trifloxystrobin in strawberry fruit (RAC) were 0.12 and 0.15 mg/kg, those of CGA 321113 were <0.02 and 0.03 mg/kg. Residues of trifloxystrobin ranged from 0.07 to 0.14 mg/kg for fruit washed, from 0.02 to 0.05 mg/kg for washings (washing water), from 0.03 to 0.06 mg/kg for preserve and from 0.06 to 0.13 mg/kg for jam. Residues of CGA 321113 ranged from <0.02 to 0.04 mg/kg for fruit washed. They were <0.02 mg/kg for washings and preserve and <0.02 or 0.02 mg/kg in jam.

The transfer factors with respect to trifloxystrobin were between 0.6 and 0.9 for washed fruit, between 0.1 and 0.4 for washings, 0.3 or 0.4 for preserve and between 0.5 and 0.9 for jam. Regarding the metabolite, transfer factors could only be calculated for one trial, where residues above the LOQ were determined. They were 1.0 or 1.3 for washed fruit and 0.7 or <0.7 for all other materials. Thus no accumulation of trifloxystrobin or CGA 321113 was observed.

Table 6.5.3-3: Results from processing studies on strawberries

Portion analysed	PHI (d)	Residues of trifloxystrobin		Residues of CGA 321113		*Report No./* Trial no:
		residue values (mg/kg)	transfer factor	residue values (mg/kg)	transfer factor	
fruit	3	0.15	n.a.	0.03	n.a.	*RA-3038/02* 0188-02
fruit, washed	3	0.14 0.12	0.9 0.8	0.04 0.03	1.3 1.0	
preserve	3	0.06 0.05	0.4 0.3	< 0.02 < 0.02	<0.7 <0.7	
jam	3	0.11 0.13	0.7 0.9	0.02 0.02	0.7 0.7	
washing water	3	0.02 0.03	0.1 0.2	< 0.02 < 0.02	<0.7 <0.7	
fruit	3	0.12	n.a.	< 0.02	n.a.	*RA-3038/02* 0191-02
fruit, washed	3	0.08 0.07	0.7 0.6	< 0.02 < 0.02	n.c. n.c.	
preserve	3	0.03 0.03	0.3 0.3	< 0.02 < 0.02	n.c. n.c.	
jam	3	0.07 0.06	0.6 0.5	0.02 0.02	n.c. n.c.	
washing water	3	0.05 0.04	0.4 0.3	< 0.02 0.02	n.c. n.c.	

n.a.: not applicable n.c.: not calculated (residues <LOQ in RAC and processed commodity)

Report:	**KCA 6.5.3/08, [redacted]; 2013 (amended);** M-464835-02-1
Title:	Determination of the residues of trifloxystrobin in/on strawberry and the processed fractions (fruit, washed; washings; preserve and jam) after spray application of Trifloxystrobin WG 50 in the field in the Netherlands and Germany
Report No.: Document No.:	12-3012 M-464835-02-1
Guidelines	Regulation No. 1107/2009 of the European Parliamanet and of the Council, EC guidance document 7029/VI/95 and 7035/VI/95, OECD Guideline 508
GLP	Yes

Materials and methods

Two processing trials were conducted in/on strawberry with trifloxystrobin in 2012 in the Netherlands and Germany. Trifloxystrobin WG 50 was applied two times to strawberry plants at a product rate of 0.3 kg/ha and 500 L water per ha, corresponding to 0.15 kg/ha active substance trifloxystrobin. The treatments were performed at intervals of 7 days with the last application 1 day prior to the expected date of harvest.

Samples were taken 1 day after the last treatment. Residues were determined in the raw agricultural commodity (fruit), washed fruit, washing, preserve and jam. The processing simulated industrial practice at laboratory scale.

For the preparation of preserves, washed fruits were pasteurised at a max. temperature of 86-88 °C for 15-22 minutes.

Samples of jam were prepared by washing fruits and pureeing them, then heating the received pulp for 4 minutes at 100°C.

Samples of jam and preserve were deep frozen until analysis. The results of the trials are summarised in Table 6.5.3-4 as well as in greater detail in the Tier I summary forms.

Findings / Conclusion

- Method performance: Method 01313/M001 was used to determine residues of trifloxystrobin, CGA 321113, CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in strawberry fruit and processed matrices.
- Storage stability: The maximum storage period of deep-frozen samples for trifloxystrobin and its isomers / metabolites was 356 days.

- Processing results: Residues of trifloxystrobin in strawberry fruit (RAC) were 0.031 and 0.145 mg/kg, those of CGA 321113 were <0.01 and 0.017 mg/kg. Residues of trifloxystrobin were <0.01 or 0.038 mg/kg in jam, and <0.01 or 0.025 in preserve. Residues of CGA 321113 were below LOQ (<0.01 mg/kg) in jam and preserve.

No residues (i.e. residues <LOQ) were detected in the RAC and the processed commodities for CGA 331409 and CGA 373466. Therefore no transfer factors could be calculated for these two analytes. For CGA 357261 and CGA 357262 residues were below LOQ in the processed commodities and at 0.01 or 0.013 in strawberry fruit (RAC) in one of the trials, while below LOQ in the other trial.

The transfer factors with respect to trifloxystrobin were <0.3 or 0.3 for jam and <0.3 or 0.2 for preserve. Regarding the metabolite CGA 321113 transfer factors could only be calculated for one trial, where residues above the LOQ were determined. They were <0.6 for jam and preserve. Thus no accumulation of trifloxystrobin and its isomers / metabolites was observed.

Table 6.5.3-4: Results from processing studies on strawberries

Portion analysed	PHI (d)	Residues of trifloxystrobin		Residues of CGA 321113		Residues of CGA 357261		*Report No.*
		residue values (mg/kg)	transfer factor	residue values (mg/kg)	transfer factor	residue values (mg/kg)	transfer factor	Trial No.
fruit	1	0.031	n.a.	<0.01	n.a.	0.010	n.a.	*12-3012*
fruit, washed	1	0.014	0.5	<0.01	n.c.	<0.01	<1	12-3012-01 12-2012-02
washings	1	<0.01	<0.3	<0.01	n.c.	<0.01	<1	
jam	1	<0.01	<0.3	<0.01	n.c.	<0.01	<1	
preserve	1	<0.01	<0.3	<0.01	n.c.	<0.01	<1	
fruit	1	0.145	n.a.	0.017	n.a.	<0.01	n.a.	*12-3012*
fruit, washed	1	0.060	0.4	0.014	0.8	<0.01	n.c.	12-3012-02 12-2012-05
washings	1	0.029	0.2	<0.01	<0.6	<0.01	n.c.	
jam	1	0.038	0.3	<0.01	<0.6	<0.01	n.c.	
preserve	1	0.025	0.2	<0.01	<0.6	<0.01	n.c.	

Portion analysed	PHI (d)	Residues of CGA 357262		Residues of CGA 331409		Residues of CGA 373466		*Report No./*
		residue values (mg/kg)	transfer factor	residue values (mg/kg)	transfer factor	residue values (mg/kg)	transfer factor	Trial No.
fruit	1	0.013	n.a.	<0.01	n.a.	<0.01	n.a.	*12-3012*
fruit, washed	1	<0.01	<0.8	<0.01	n.c.	<0.01	n.c.	12-3012-01 12-2012-02
washings	1	<0.01	<0.8	<0.01	n.c.	<0.01	n.c.	
jam	1	<0.01	<0.8	<0.01	n.c.	<0.01	n.c.	
preserve	1	<0.01	<0.8	<0.01	n.c.	<0.01	n.c.	
fruit	1	<0.01	n.a.	<0.01	n.a.	<0.01	n.a.	*12-3012*
fruit, washed	1	<0.01	n.c.	<0.01	n.c.	<0.01	n.c.	12-3012-02 12-2012-05
washings	1	<0.01	n.c.	<0.01	n.c.	<0.01	n.c.	
jam	1	<0.01	n.c.	<0.01	n.c.	<0.01	n.c.	
preserve	1	<0.01	n.c.	<0.01	n.c.	<0.01	n.c.	

n.a.: not applicable n.c.: not calculated (residues <LOQ in RAC and processed commodity)

CA 6.6 Residues in rotational crops

Strawberries may be grown in rotation with other crops, while pomefruit and grape are permanent crops and therefore not relevant for crop rotation.
No supplementary studies are required for this submission. All European studies have previously been submitted and evaluated. Please refer to the Annex II dossier, Section 6, Point 6.6 (succeeding crops).

CA 6.6.1 Metabolism in rotational crops

Two confined rotational crop studies were conducted in Europe ([redacted]; 1998; M-038288-02-1 ; [redacted]; 1997; M-038296-01-1). For details please refer to the Annex II dossier, Section 6, Point 6.6 (succeeding crops).

The occurrence of trifloxystrobin residues in rotational crops was investigated in lettuce, radish and wheat. The data on metabolism and distribution of trifloxystrobin in succeeding crops demonstrate that the metabolism of the active substance in rotational crops is similar to the pathway observed in primary crops. Thus, the same residue definition applies.

Based on the results from the metabolism studies in rotational crops, which were performed with a higher application rate (0.5 kg a.s./ha) than the intended seasonal application rate on the representative crops under consideration (max. 0.375 kg a.s./ha/season), and application to bare soil (interception of trifloxystrobin by the plants is expected in practice), relevant residue levels are unlikely to occur in rotational crops provided that the compound is used according to the intended GAPs for the representative uses.

CA 6.6.2 Magnitude of residues in rotational crops

Since the results of the metabolism in rotational crop studies did not indicate that significant accumulation of residues occurs through soil uptake into food or feed commodities, field studies in rotational crops are not required.

CA 6.7 Proposed residue definitions and maximum residue levels

CA 6.7.1 Proposed residue definitions

In the peer review under Directive 91/414/EEC the metabolism of trifloxystrobin in plant was investigated in cereals, fruits and fruiting vegetables. The metabolism in these crop groups was found to proceed according to similar pattern and it was concluded that the metabolite CGA 321113 is not of toxicological concern. Therefore the plant residue definitions for enforcement and risk assessment were set as parent trifloxystrobin only.

In some recently published EFSA reasoned opinions EFSA proposed to consider the inclusion of metabolite CGA 321113 in the residue definition for the risk assessment for plant commodities based on the findings of metabolism studies in root and tuber vegetables (not available at the peer review under Directive 91/414/EEC) and since in some commodities the metabolite CGA 321113 occurred at higher levels than parent trifloxystrobin in the field trials.
In recent Reasoned Opinions EFSA recommended to investigate the possible changes in the risk assessment residue definition for trifloxystrobin in the framework of the MRL review under Article 12 of Regulation (EC) No 396/2005.

For enforcement EFSA concluded that the metabolism of trifloxystrobin is sufficiently addressed and the plant residue definition for enforcement established in Regulation (EC) 396/2005 and confirmed by the peer review is trifloxystrobin.

In the peer review under Directive 91/414/EEC the metabolism of trifloxystrobin in animal was evaluated and the residue definitions for enforcement and risk assessment were set as the sum of parent trifloxystrobin and metabolite CGA 321113.

Table 6.7.1- 1: EU residue definitions

Matrices	Residue definition		Reference
Food of plant origin	Enforcement (Monitoring)	Trifloxystrobin	Peer review under Directive 91/414/EEC EFSA Reasoned Opinions 2009 - 2013
	Risk assessment	Trifloxystrobin	Peer review under Directive 91/414/EEC
	Risk assessment (Proposal)	Sum of trifloxystrobin and its metabolite CGA 321113, expressed as trifloxystrobin	Proposal in EFSA Reasoned Opinions 2009 - 2013
Food of animal origin	Enforcement (Monitoring) Risk assessment	Sum of trifloxystrobin and its metabolite CGA 321113	Peer review under Directive 91/414/EEC Commission Regulations amending Annexes II and III to Regulation (EC) No 396/2005
	Enforcement (Monitoring) Risk assessment	Sum of trifloxystrobin and its metabolite CGA 321113, expressed as trifloxystrobin	EFSA Reasoned Opinions 2009 - 2013

Bayer CropScience proposes to keep the residue definitions as proposed by EFSA, i.e. trifloxystrobin for plant enforcement and the sum of trifloxystrobin and its acid metabolite CGA 321113 for animal (enforcement and risk assessment) and for plant risk assessment.

New residue data were compiled including analysis of the photo-isomers of trifloxystrobin and the mono-acid metabolite.
The parent isomers CGA 357261 (ZE isomer), CGA 357262 (ZZ isomer) and CGA 331409 (EZ isomer) show low individual occurrence and remain well below the residue levels of parent CGA 279202 (EE isomer). In strawberry residues of CGA 331409 were always below LOQ.
Occurrence of CGA 373466 (ZE-isomer of the mono-acid metabolite) above LOQ is rare. In pome fruit residues of CGA 373466 were always below LOQ.
A summary of the residue results of the isomers of parent trifloxystrobin and of CGA 321113 and its isomer CGA 373466 in the representative crops is given in Table 6.7.1-2. Further information on additional crops can be found in chapter 6.10.

The toxicological characterisation of the trifloxystrobin photo-isomers (ZE, EZ, ZZ) shows that an additional toxicological impact of these compounds is not expected. In fact the isomers (ZE, EZ, ZZ) of the parent compound trifloxystrobin exhibit significantly lower toxicity.
The ZE-isomer is non-toxic (LD50 >2000 mg/kg, no clinical signs) after acute oral exposure.

The isomers are subjected to genotoxicity (bacterial reverse mutation, clastogenicity and numerical chromosome aberration) and comparative in vitro testing (inhibition of mitochondrial respiration and cytotoxicity). No evidence of genotoxicity is found.
The comparative *in vitro* tests reveal a clear ranking of the isomers in terms of cytotoxicity and their inhibiting potential on mitochondrial respiration. The photo-isomers in comparison to trifloxystrobin are at least 35 times less cytotoxic in rat hepatocytes. Trifloxystrobin potentially inhibits the mitochondrial respiration at nanomolar concentrations whereas the photo-isomers are more than one order of magnitude less active.
In addition, a very similar metabolic pathway for trifloxystrobin and its photo-isomers can be assumed with the initial degrading of the methoxyacrylate toxophor by hydrolysis to the respective less toxic metabolites.
CGA 373466 is acutely non-toxic (LD50 > 2000 mg/kg bw). It does not possess a genotoxic potential and is less toxic compared to trifloxystrobin in the comparative in vitro assays. Furthermore, 4-week dietary exposure was well tolerated up to the highest dose tested with a NOAEL of 209/235 mg/kg bw.

Therefore, the photo-isomers are considered to be toxicologically adequately investigated and uncritical for human health and it is not considered necessary to include the isomers in the residue definition for risk assessment (see supplementary dossier CA Section 5.8.1).

Table 6.7.1-2: Summary of residue results on isomers , representative crops

Report No.	Dossier ref.	Crop	No. of trials		CGA 279202 [mg/kg]	CGA 321113 [mg/kg]	CGA 357261 [mg/kg]	CGA 357262 [mg/kg]	CGA 331409 [mg/kg]	CGA 373466 [mg/kg]
11-2117 11-2116	M-457963-01-1 M-457957-01-1	Apple / Pear	8	Residues at PHI (14 d)	0.044 – 0.17 (all trials >LOQ)	<0.01 (0 trials >LOQ)	<0.01 – 0.068 (6 trials >LOQ)	<0.01 – 0.031 (4 trials >LOQ)	<0.01 – 0.036 (4 trials >LOQ)	<0.01 (0 trials >LOQ)
				Residues at all dates	<0.01 – 0.28 (all trials >LOQ)	<0.01 (0 trials >LOQ)	<0.01 – 0.068 (6 trials >LOQ)	<0.01 – 0.031 (4 trials >LOQ)	<0.01 – 0.036 (4 trials >LOQ)	<0.01 (0 trials >LOQ)
11-2115 12-2010 11-2114 12-2011	M-456337-01-1 M-453336-02-1 M-454927-01-1 M-455561-02-1	Grape	17	Residues at PHI (14 d)	0.058 – 0.51 (all trials >LOQ)	<0.01 – 0.044 (10 trials >LOQ)	<0.01 – 0.066 (14 trials >LOQ)	<0.01 – 0.035 (8 trials >LOQ)	<0.01 – 0.042 (11 trials >LOQ)	<0.01 – 0.012 (2 trials >LOQ)
				Residues at all dates	0.036 – 0.89 (all trials >LOQ)	<0.01 – 0.069 (10 trials >LOQ)	<0.01 – 0.066 (16 trials >LOQ)	<0.01 – 0.035 (13 trials >LOQ)	<0.01 – 0.042 (13 trials >LOQ)	<0.01 – 0.019 (2 trials >LOQ)
11-2128 12-2012 11-2129 12-2013 11-2120 12-2014	M-457953-01-1 M-452140-01-1 M-457958-02-1 M-460009-01-1 M-456769-02-1 M-453332-02-1	Straw-berry	26	Residues at PHI (1 d)	0.024 – 0.41 (all trials >LOQ)	<0.01 – 0.059 (all trials >LOQ)	<0.01 – 0.029 (3 trials >LOQ)	<0.01 – 0.043 (2 trials > LOQ)	<0.01 (0 trials >LOQ)	<0.01 – 0.01 (1 trial > LOQ)
				Residues at all dates	0.015 – 0.41 (all trials >LOQ)	<0.01 – 0.064 (all trials >LOQ)	<0.01 – 0.041 (4 trials > LOQ)	<0.010 – 0.056 (2 trials > LOQ)	<0.01 (0 trials >LOQ)	<0.01 – 0.012 (3 trials > LOQ)

LOQ: 0.01 mg/kg PHI: pre-harvest interval

CA 6.7.2 Proposed MRLs and justification of the acceptability of the levels proposed

Maximum Residue Limits (MRLs) for trifloxystrobin were set at European Level under several Commission Regulations, the latest one being Commission Regulation 1004/2013 of 15 October 2013 amending Annexes II and III of Regulation EC 396/2005. In the process of the MRL review program under Article 12 of the Regulation 396/2005, summaries from relevant field residue trials were provided to UK CRD and other countries. The Article 12 review was under evaluation when this dossier was compiled.

The trifloxystrobin EU MRLs for the crops supported within this dossier and the MRLs for animal matrices, as published in Commission Regulation 1004/2013 of 15 October 2013 amending Annex II of Regulation (EC) No. 396/2005, are summarised in Table 6.7.2-1.

Table 6.7.2-1: EU-MRLs for trifloxystrobin as given in Commission Regulation 1004/2013 (Annex II to Regulation 396/2005)

Code number	Groups and examples of individual products to which the MRLs apply	Trifloxy-strobin **
130000	(iii) Pome fruit	0.5
150000	(v) Berries & small fruit	
151000	(a) Table and wine grapes	5
152000	(b) Strawberries	0.5
1000000	10. PRODUCTS OF ANIMAL ORIGIN-TERRESTRIAL ANIMALS	
1010000	(i) Meat, preparations of meat, offals, blood, animal fats fresh chilled or frozen, salted, in brine, dried or smoked or processed as flours or meals other processed products such as sausages and food preparations based on these	0.04 (*)
1020000	(ii) Milk and cream, not concentrated, not containing added sugar or sweetening matter, butter and other fats derived from milk cheese and curd	0.02 (*)
1030000	(iii) Birds' eggs, fresh preserved or cooked Shelled eggs and egg yolks fresh, dried, cooked by steaming or boiling in water, moulded, frozen or otherwise preserved whether or not containing added sugar or sweetening matter	0.04 (*)

(*) indicates lower limit of analytical determination
** for products of animal origin: the sum of trifloxystrobin and its metabolite (E, E)-methoxyimino- {2-[1-(3-trifluoromethyl-phenyl)-ethylideneamino-oxymethyl]-phenyl}-acetic acid (CGA 321113)

Calculation of the Maximum Residue Limits based on the European use pattern and residue trials as submitted within this dossier was done according to the OECD calculator and is presented below for pomefruit, grape and strawberry.

Table 6.7.2-2: Summary of MRLs and MRL proposals for trifloxystrobin

Crop	EU MRL (mg/kg) as in CR 1004/2013	Result of MRL calculation (mg/kg) based on new EU trials submitted within this dossier and OECD calculator	Codex MRL (mg/kg) as adopted in 2006	MRL proposal (mg/kg) to cover EU use and import
Pomefruit	0.5	0.3	0.7	0.5 or 0.7
Grape	5	1	3	3
Strawberry	0.5	0.2	0.2 *	1

* as proposed in JMPR Evaluation 2012

Pome fruit - Trifloxystrobin

For trifloxystrobin a MRL proposal of 0.3 mg/kg for pome fruit would be appropriate based on the new EU trials carried out according to the use pattern as supported within the present supplementary dossier. The southern European use pattern is the critical GAP relevant for MRL calculation, since the application rate for southern Europe is higher than for northern Europe.

Nevertheless, since US residue data evaluated by JMPR in 2004 and still covering the recent US use pattern of trifloxystrobin in/on pomefruit show trifloxystrobin residues up to 0.37 mg/kg (see also 6.7.3), it is proposed to maintain the recent EU MRL of 0.5 mg/kg (as in CR 1004/2013) for trifloxystrobin in/on pomefruit or to take into account the Codex MRL of 0.7 mg/kg.

Table 6.7.2-3: Calculation of MRL proposal according to OECD calculator; northern European trials

No.	Crop	Days after application	Residue value (mg/kg)	Plot No. [1]/ Study No.	No. of applic.	FL-Type	Product	Country	Area of applic.
1	Apple	14	<0.02	0079-03 / RA-2170/03	3	WG 64	Trifloxystro-bin & Captan WG 64	Belgium	F
2	Pear	13	0.05	0183-04 / RA-2046/04	3	WG 68.8	Tolylfluanid & Trifloxystro-bin WG 68.8	United Kingdom	F
3	Pear	14	0.09	0184-04 / RA-2046/04	3	WG 68.8	Tolylfluanid & Trifloxystro-bin WG 68.8	Germany	F
4	Apple	14	0.03	0182-04 / RA-2044/04	3	WG 68.8	Tolylfluanid & Trifloxystro-bin WG 68.8	Nether-lands	F
5	Apple	14	0.05	0124-06 / RA-2006/06	3	WG 75	Tebuconazole & Trifloxystro-bin WG 75	Germany	F
6	Apple	14	0.05	SWZ-2125-99A / 2125/99	3	WG 50	Trifloxystro-bin WG 50	Switzer-land	F
7	Apple	14	0.04	SWZ-2124-99 / 2124/99	3	WG 50	Trifloxystro-bin WG 50	Switzer-land	F
8	Apple	14	0.04	NIE-2109-99 / 2109/99	3	WG 50	Trifloxystro-bin WG 50	Nether-lands	F
9	Apple	14	0.03	FRA-2007-99 / 2007/99	3	WG 50	Trifloxystro-bin WG 50	France	F
10	Pear	14	0.11	11-2117-02-T / 11-2117	3	WG 50	Trifloxystro-bin WG 50	France	F
11	Pear	14	0.057	11-2117-03-T / 11-2117	3	WG 50	Trifloxystro-bin WG 50	Germany	F
12	Apple	14	0.044	11-2117-04-T / 11-2117	3	WG 50	Trifloxystro-bin WG 50	United Kingdom	F
13	Apple	15	0.058	11-2117-01-T / 11-2117	3	WG 50	Trifloxystro-bin WG 50	Germany	F

[1] as given in the Tier 1 summaries
value no. 10 is an outlier for t1

Results (Apple;Pear)

Total number of data (n)	13	Standard deviation (SD)	0.025
Lowest residue	0.02	Percentage of censored data	8
Highest residue	0.11	Number of non-censored data	12
Median residue	0.050	Correction factor for censoring (CF)	0.949
Mean	0.051		

Proposed MRL estimate

Highest residue	0.11
Mean + 4 SD	0.150
CF x 3 mean	0.146
Unrounded MRL	0.150
Rounded MRL	0.15

Table 6.7.2-4: Calculation of MRL proposal according to OECD calculator; southern European trials

No.	Crop	Days after application	Residue value (mg/kg)	Plot No. [1]/ Study No.	No. of applic.	FL-Type	Product	Country	Area of applic.
1	Apple	14	0.055	11-2116-01-T / 11-2116	3	WG 50	Trifloxystro-bin WG 50	France	F
2	Apple	14	0.15	11-2116-02-T / 11-2116	3	WG 50	Trifloxystro-bin WG 50	Portugal	F
3	Pear	14	0.12	11-2116-03-T / 11-2116	3	WG 50	Trifloxystro-bin WG 50	Italy	F
4	Pear	14	0.17	11-2116-04-T / 11-2116	3	WG 50	Trifloxystro-bin WG 50	Spain	F
5	Apple	14	0.12	0083-03 / RA-2171/03	3	WG 64	Trifloxystrobin & Captan WG 64	Portugal	F
6	Apple	14	0.02	0186-04 / RA-2045/04	3	WG 68.8	Tolylfluanid & Trifloxystro-bin WG 68.8	Italy	F
7	Apple	14	0.05	0187-04 / RA-2045/04	3	WG 68.8	Tolylfluanid & Trifloxystrobin WG 68.8	Spain	F
8	Pear	14	0.07	0188-04 / RA-2047/04	3	WG 68.8	Tolylfluanid & Trifloxystrobin WG 68.8	Italy	F
9	Pear	13	0.10	0189-04 / RA-2047/04	3	WG 68.8	Tolylfluanid & Trifloxystrobin WG 68.8	Spain	F

[1] as given in the Tier 1 summaries

Results (Pear;Apple)

Total number of data (n)	9	Standard deviation (SD)	0.050
Lowest residue	0.02	Percentage of censored data	0
Highest residue	0.17	Number of non-censored data	9
Median residue	0.100	Correction factor for censoring (CF)	1.000
Mean	0.095		

Proposed MRL estimate

Highest residue	0.17
Mean + 4 SD	0.294
CF x 3 mean	0.285
Unrounded MRL	0.294
Rounded MRL	0.3

Grape - Trifloxystrobin

For trifloxystrobin a MRL proposal of 1 mg/kg for grape would be appropriate based on the new EU trials carried out according to the use pattern as supported within the present supplementary dossier.

Nevertheless, since US and Canadian residue data evaluated by JMPR in 2004 and still covering the recent US or Canadian use pattern of trifloxystrobin in/on grape show trifloxystrobin residues up to 2.2 mg/kg (see also 6.7.3), it is proposed to consider also the recent Codex MRL of 3 mg/kg for trifloxystrobin in/on grape.

Table 6.7.2-5: Calculation of MRL proposal according to OECD calculator; northern European trials

No.	Crop	Days after application	Residue value (mg/kg)	Plot No. [1]/ Study No.	No. of applic.	FL-Type	Product	Country	Area of applic.
1	Grape	14	0.19	11-2115-01-T / 11-2115	3	WG 50	Trifloxystrobin WG 50	France	F
2	Grape	14	0.42	11-2115-02-T / 11-2115	3	WG 50	Trifloxystrobin WG 50	France	F
3	Grape	14	0.38	11-2115-03-T / 11-2115	3	WG 50	Trifloxystrobin WG 50	Germany	F
4	Grape	14	0.49	11-2115-04-T / 11-2115	3	WG 50	Trifloxystrobin WG 50	Germany	F
5	Grape	21*	0.42	12-2010-01-T / 12-2010	3	WG 50	Trifloxystrobin WG 50	France	F
6	Grape	14	0.14	12-2010-02-T / 12-2010	3	WG 50	Trifloxystrobin WG 50	France	F
7	Grape	21*	0.29	12-2010-03-T / 12-2010	3	WG 50	Trifloxystrobin WG 50	Germany	F
8	Grape	20*	0.18	12-2010-04-T / 12-2010	3	WG 50	Trifloxystrobin WG 50	Germany	F

[1] as given in the Tier 1 summaries

** day 21 results reported and used for calculation since higher than day 14 results

Results (Grape)

Total number of data (n)	8	Standard deviation (SD)	0.132
Lowest residue	0.14	Percentage of censored data	0
Highest residue	0.49	Number of non-censored data	8
Median residue	0.335	Correction factor for censoring (CF)	1.000
Mean	0.314		

Proposed MRL estimate

Highest residue	0.49
Mean + 4 SD	0.842
CF x 3 mean	0.941
Unrounded MRL	0.941
Rounded MRL	1

Table 6.7.2-6: Calculation of MRL proposal according to OECD calculator; southern European trials

No.	Crop	Days after application	Residue value (mg/kg)	Plot No. / Study No.	No. of applic.	Fl. Type	Product	Country	Area of applic.
1	Grape	14	0.22	11-2114-01-T / 11-2114	3	WG 50	Trifloxystrobin WG 50	France	F
2	Grape	21*	0.12	11-2114-02-T / 11-2114	3	WG 50	Trifloxystrobin WG 50	Spain	F
3	Grape	14	0.14	11-2114-03-T / 11-2114	3	WG 50	Trifloxystrobin WG 50	Italy	F
4	Grape	21*	0.39	11-2114-04-T / 11-2114	3	WG 50	Trifloxystrobin WG 50	Portugal	F
5	Grape	14	0.18	12-2011-01-T / 12-2011	3	WG 50	Trifloxystrobin WG 50	Italy	F
6	Grape	14	0.51	12-2011-02-T / 12-2011	3	WG 50	Trifloxystrobin WG 50	Italy	F
7	Grape	14	0.20	12-2011-03-T / 12-2011	3	WG 50	Trifloxystrobin WG 50	Greece	F
8	Grape	20*	0.12	12-2011-04-T / 12-2011	3	WG 50	Trifloxystrobin WG 50	Spain	F
9	Grape	14	0.15	12-2011-05-T / 12-2011	3	WG 50	Trifloxystrobin WG 50	Spain	F

[1] as given in the Tier 1 summaries

* day 20/21 results reported and used for calculation since higher than day 14 results

Results (Grape)

Total number of data (n)	9	Standard deviation (SD)	0.135
Lowest residue	0.12	Percentage of censored data	0
Highest residue	0.51	Number of non-censored data	9
Median residue	0.180	Correction factor for censoring (CF)	1.000
Mean	0.226		

Proposed MRL estimate

Highest residue	0.51
Mean + 4 SD	0.766
CF x 3 mean	0.677
Unrounded MRL	0.766
Rounded MRL	0.8

Strawberry - Trifloxystrobin

A total of 26 residue trials conducted on strawberry in Europe are available: 9 trials from field use in the northern European zone, 9 trials from field use in the southern European zone and 8 trials from greenhouse use. The MRL calculation was done separately with the field trials and the indoor trials; the indoor trials showing the highest results (HR 0.41 mg/kg).

Based on the indoor trials for trifloxystrobin a MRL of 0.7 mg/kg for strawberry is proposed based on the new EU trials carried out according to the use pattern as supported within the present supplementary dossier.

Nevertheless, since US residue data evaluated by JMPR in 2012 show trifloxystrobin residues up to 0.50 mg/kg (see also 6.7.3), it proposed to consider also the proposed Codex MRL of 1 mg/kg for trifloxystrobin in/on strawberry.

Table 6.7.2-7: Calculation of MRL proposal according to OECD calculator; greenhouse trials

No.	Crop	Days after application	Residue value (mg/kg)	Plot No. [1]/ Study No.	No. of applic.	FL-Type	Product	Country	Area of applic.
1	Strawberry	1	0.091	12-2014-03-T / 12-2014	2	WG 50	Trifloxystrob in WG 50	Germany	G
2	Strawberry	3*	0.082	12-2014-01-T / 12-2014	2	WG 50	Trifloxystrob in WG 50	Belgium	G
3	Strawberry	3*	0.096	12-2014-02-T / 12-2014	2	WG 50	Trifloxystrob in WG 50	France	G
4	Strawberry	1	0.12	12-2014-04-T / 12-2014	2	WG 50	Trifloxystrob in WG 50	Germany	G
5	Strawberry	1	0.13	11-2120-04-T / 11-2120	2	WG 50	Trifloxystrob in WG 50	Greece	G
6	Strawberry	1	0.16	11-2120-02-T / 11-2120	2	WG 50	Trifloxystrob in WG 50	Italy	G
7	Strawberry	1	0.41	11-2120-01-T / 11-2120	2	WG 50	Trifloxystrob in WG 50	Spain	G
8	Strawberry	1	0.27	11-2120-03-T / 11-2120	2	WG 50	Trifloxystrob in WG 50	Portugal	G

[1] as given in the Tier 1 summaries

* day 3 results reported and used for calculation since higher than day 1 results

Results (Strawberry)

Total number of data (n)	8	Standard deviation (SD)	0.114
Lowest residue	0.082	Percentage of censored data	0
Highest residue	0.41	Number of non-censored data	8
Median residue	0.125	Correction factor for censoring (CF)	1.000
Mean	0.170		

Proposed MRL estimate

Highest residue	0.41
Mean + 4 SD	0.627
CF x 3 mean	0.510
Unrounded MRL	0.627
Rounded MRL	0.7

Table 6.7.2-8: Calculation of MRL proposal according to OECD calculator; northern European trials

No.	Crop	Days after application	Residue value (mg/kg)	Plot No. [1]/ Study No.	No. of applic.	FL-Type	Product	Country	Area of applic.
1	Strawberry	1	0.13	11-2128-03-T / 11-2128	2	WG 50	Trifloxystrob in WG 50	Germany	F
2	Strawberry	3*	0.096	11-2128-04-T / 11-2128	2	WG 50	Trifloxystrob in WG 50	Belgium	F
3	Strawberry	3*	0.089	11-2128-02-T / 11-2128	2	WG 50	Trifloxystrob in WG 50	France	F
4	Strawberry	3*	0.070	11-2128-01-T / 11-2128	2	WG 50	Trifloxystrob in WG 50	Germany	F
5	Strawberry	1	0.15	12-2012-04-T / 12-2012	2	WG 50	Trifloxystrob in WG 50	Belgium	F
6	Strawberry	1	0.14	12-2012-03-T / 12-2012	2	WG 50	Trifloxystrob in WG 50	France	F
7	Strawberry	1	0.038	12-2012-02-T / 12-2012	2	WG 50	Trifloxystrob in WG 50	Nether-lands	F
8	Strawberry	1	0.15	12-2012-05-T / 12-2012	2	WG 50	Trifloxystrob in WG 50	Germany	F
9	Strawberry	1	0.081	12-2012-01-T / 12-2012	2	WG 50	Trifloxystrob in WG 50	Germany	F

[1] as given in the Tier 1 summaries

* day 3 results reported and used for calculation since higher than day 1 results

Results (Strawberry)

Total number of data (n)	9	Standard deviation (SD)	0.040
Lowest residue	0.038	Percentage of censored data	0
Highest residue	0.15	Number of non-censored data	9
Median residue	0.096	Correction factor for censoring (CF)	1.000
Mean	0.105		

Proposed MRL estimate

Highest residue	0.15
Mean + 4 SD	0.263
CF x 3 mean	0.315
Unrounded MRL	0.315
Rounded MRL	0.4

Table 6.7.2-9: Calculation of MRL proposal according to OECD calculator; southern European trials

No.	Crop	Days after application	Residue value (mg/kg)	Plot No. [1]/ Study No.	No. of applic.	FL-Type	Product	Country	Area of applic.
1	Strawberry	1	0.11	11-2129-01-T / 11-2129	2	WG 50	Trifloxystrob in WG 50	France	F
2	Strawberry	1	0.20	11-2129-03-T / 11-2129	2	WG 50	Trifloxystrob in WG 50	Italy	F
3	Strawberry	1	0.20	11-2129-02-T / 11-2129	2	WG 50	Trifloxystrob in WG 50	Spain	F
4	Strawberry	1	0.15	11-2129-04-T / 11-2129	2	WG 50	Trifloxystrob in WG 50	Italy	F
5	Strawberry	7*	0.083	12-2013-03-T / 12-2013	2	WG 50	Trifloxystrob in WG 50	Spain	F
6	Strawberry	1	0.23	12-2013-01-T / 12-2013	2	WG 50	Trifloxystrob in WG 50	Spain	F
7	Strawberry	2*	0.061	12-2013-02-T / 12-2013	2	WG 50	Trifloxystrob in WG 50	Spain	F
8	Strawberry	1	0.17	12-2013-04-T / 12-2013	2	WG 50	Trifloxystrob in WG 50	Italy	F
9	Strawberry	4*	0.13	12-2013-05-T / 12-2013	2	WG 50	Trifloxystrob in WG 50	Greece	F

[1] as given in the Tier 1 summaries

* day 7, 2 or 4 results reported and used for calculation since higher than day 1 results

Results (Strawberry)

Total number of data (n)	9	Standard deviation (SD)	0.057
Lowest residue	0.061	Percentage of censored data	0
Highest residue	0.23	Number of non-censored data	9
Median residue	0.150	Correction factor for censoring (CF)	1.000
Mean	0.148		

Proposed MRL estimate

Highest residue	0.23
Mean + 4 SD	0.377
CF x 3 mean	0.445
Unrounded MRL	0.445
Rounded MRL	0.5

CA 6.7.3 Proposed MRLs and justification of the acceptability of the levels proposed for imported products (import tolerance)

Trifloxystrobin was evaluated by JMPR (Joint Meeting on Pesticide Residues) in 2004 and 2012, including the crops / crop groups pomefruit, grape and strawberry.
Since Codex MRLs are higher than the MRLs calculated based on submitted EU trials and GAPs supported within this dossier, the relevant JMPR evaluations are summarised below and it is requested to take into account these Codex MRLs and the uses in non-EU countries.
The Meeting agreed that the residue definition for enforcement purposes for plant commodities should be trifloxystrobin *per se* and that the residue definition for consideration of dietary intake should consist of the parent compound plus CGA321113 (expressed as trifloxystrobin equivalents).

Pome fruit

A Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group (JMPR) was held in Rome in 2004.
Trifloxystrobin was on the agenda for a first review, including the use in/on pomefruit, and the meeting estimated a MRL of 0.7 mg/kg for this crop group.
An excerpt of the JMPR evaluation 2004 is given below:

Pome fruit

Trials were conducted on apple and pear in Australia, Canada, Europe, South Africa and the USA.

The Meeting agreed to combine the data sets on apples and pears from two trials in Australia 42 trials in Europe, four trials in South Africa and 26 trials in Canada and the USA. The residue concentrations of trifloxystrobin *per se*, in ranked order, were: 0.03 (two), < 0.04, 0.04 (five), 0.05 (six), 0.06 (three), 0.07 (eight), 0.08 (three), 0.09 (four), 0.10 (five), 0.11 (two), 0.12 (three), 0.13 (four), 0.14 (three), 0.15 (two), 0.16 (two), 0.17 (three), 0.18 (three), 0.19 (three), 0.20, 0.21 (two), 0.22, 0.23, 0.24, 0.26, 0.30, 0.31, 0.37 (two) and 0.44 mg/kg. The residue concentrations of the sum of trifloxystrobin and CGA321113 expressed as trifloxystrobin, in ranked order, were: 0.03 (two), < 0.04, 0.04 (five), 0.05 (five), 0.06 (four), 0.07 (eight), 0.08 (three), 0.09 (four), 0.10 (four), 0.11 (two), 0.12 (three), 0.13 (three), 0.14 (three), 0.15 (three), 0.16 (two), 0.17 (two), 0.18 (two), 0.19 (four), 0.20 (two), 0.21 (three), 0.22, 0.23, 0.24, 0.26, 0.30, 0.31, 0.37, 0.41 and 0.44 mg/kg.

The Meeting estimated a maximum residue level of 0.7 mg/kg and an STMR value of 0.11 mg/kg for residues of trifloxystrobin in pome fruit.

(Pesticide residues in food 2004; EVALUATIONS 2004; PART I – RESIDUES. Joint FAO/WHO Meeting on Pesticide Residues. World Health Organization. Food and Agriculture Organization of the United Nations. Rome, 2005. Page 1361-1362)

While the highest residue of 0.44 mg/kg for trifloxystrobin was found in a trial conducted in Europe with 10 applications at 0.075 kg as/ha, 0.37 mg/kg is the highest residue found for trifloxystrobin in the non-European trials. This US trial is still valid to support the recent US use pattern of trifloxystrobin in/on pomefruit.
Therefore it proposed to maintain the recent EU MRL of 0.5 mg/kg (CR 35/2013) for trifloxystrobin in/on pomefruit or to consider the Codex MRL of 0.7 mg/kg.

Grape

A Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group (JMPR), was held in Rom in 2004.
Trifloxystrobin was on the agenda for a first review, including the use in/on grapes, and the meeting estimated a MRL of 3 mg/kg for grapes.
An excerpt of the JMPR evaluation 2004 is given below:

Berries and small fruit

Trials on *grape* were conducted in Australia, Canada, France, Germany, Greece, Italy, South Africa, Spain, Switzerland and the USA.

In summary, the residue levels of trifloxystrobin *per se* in 39 trials in Australia, Europe, South Africa, Canada and the USA, in ranked order were < 0.02, 0.03, 0.04 (five), 0.05, 0.06 (three), 0.08 (two), 0.09 (three), 0.11 (two), 0.13 (two), 0.14 (two), 0.16, 0.17, 0.18, 0.21, 0.24, 0.26, 0.27, 0.28 (two), 0.29 (two), 0.33, 0.36, 0.61, 0.62, 1.1 and 2.2 mg/kg. The residue concentrations of the sum of trifloxystrobin and CGA321113 expressed as trifloxystrobin were < 0.02, 0.04 (four), 0.05 (three), 0.06 (two), 0.07, 0.08, 0.09 (three), 0.11 (two), 0.13, 0.14, 0.15, 0.16 (three), 0.17, 0.21, 0.22, 0.26, 0.28 (two), 0.29, 0.30, 0.33, 0.36, 0.38 (two), 0.63, 0.64, 1.2 and 2.2 mg/kg.

The Meeting estimated a maximum residue level of 3 mg/kg and an STMR of 0.15 mg/kg for residues in grapes.

(Pesticide residues in food 2004; EVALUATIONS 2004; PART I - RESIDUES, Joint FAO/WHO Meeting on Pesticide Residues. World Health Organization. Food and Agriculture Organization of the United Nations. Rome, 2005. Page 1363-1364)

Since US and Canadian residue data evaluated by JMPR in 2004 are still covering the recent US or Canadian use patterns of trifloxystrobin in/on grape and show trifloxystrobin residues up to 2.2 mg/kg, it proposed to consider the recent Codex MRL of 3 mg/kg for trifloxystrobin in/on grape also for Europe.

Strawberry

A Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group (JMPR) was held at FAO Headquarters, Rome (Italy), in 2012. Trifloxystrobin was on the agenda for some crops including the review of strawberry. While in the JMPR meeting of 2004 a use of trifloxystrobin in Switzerland was observed, which resulted in a Codex MRL of 0.2 mg/kg, the meeting in 2012 evaluated the additional uses of trifloxystrobin in/on strawberry in Australia and the USA, resulting in a MRL proposal of 1 mg/kg.
An excerpt of the JMPR evaluation 2012 is given below:

Strawberry

Based on the Swiss GAP (3 × 0.25 kg ai/ha, PHI 14 days) and five European supervised trials, the 2004 JMPR estimated a maximum residue level of 0.2 mg/kg and an STMR of 0.1 mg/kg.

The 2012 Meeting received additional residue data from the USA and Australia. The Australian trials were carried out with 3 × 0.2 kg ai/ha and did not match the GAP (3 × 0.15 kg ai/ha, PHI 1 day). The registered GAP in the USA is 6 × 0.11 kg ai/ha and a 0-day PHI. In eight trials matching GAP conditions, the residue levels of trifloxystrobin *per se* were (n=8): 0.10, 0.19, 0.20, 0.28, 0.30, 0.44, 0.47 and 0.50 mg/kg. The residue concentrations of the sum of trifloxystrobin and CGA 321113 were: 0.23, 0.23, 0.27, 0.31, 0.36, 0.47, 0.51 and 0.56 mg/kg.

The Meeting estimated a maximum residue level of 1 mg/kg and an STMR of 0.335 mg/kg for trifloxystrobin in strawberries to replace the former recommendation.

(Pesticide residues in food 2012; EVALUATIONS 2012; PART I – RESIDUES. Joint FAO/WHO Meeting on Pesticide Residues. World Health Organization, Food and Agriculture Organization of the United Nations. Rome, 2013. Page 2052)

Since US residue data evaluated by JMPR in 2012 show trifloxystrobin residues up to 0.50 mg/kg and the OECD MRL calculator leads to a proposed MRL of 1 mg/kg, it is proposed to consider this Codex MRL of 1 mg/kg for trifloxystrobin in/on strawberry also for Europe.

CA 6.8 Proposed safety intervals

Pre-harvest interval (in days) for each relevant crop

According to the proposed uses for Trifloxystrobin WG 50, the proposed pre-harvest interval is 14 days for pomefruit and grapes and 1 day for strawberries.

Re-entry period (in days) for livestock, to areas to be grazed

It is not relevant to define a re-entry period for livestock after use trifloxystrobin in/on pomefruit, grape and strawberry, since these crops are not intended to be grazed by livestock.

Re-entry period for man to crops, buildings or spaces treated

Trifloxystrobin is applied in crops which may require re-entry activities shortly after application. Exposure estimates which are made under the conservative assumption that foliar dislodgeable residues do not degrade, show that the potential worker exposure is within the established AOEL, independent from the use or non-use of protective clothing. Nevertheless, it is considered as a general rule that treated areas should not be entered before spray deposit on leave surfaces has dried, unless protective clothing is worn.

Withholding period (in days) for animal feedingstuffs

Not relevant, no use as feeding stuff before harvest.

Waiting period before sowing or planting crop to be protected

There are no restrictions or waiting periods to avoid phytotoxic effects on succeeding crops following the use of trifloxystrobin.

Waiting period (in days) before sowing or planting succeeding crops

Based on the available data (cf. CA 6.6), no waiting periods beyond normal agricultural practice are proposed for succeeding crop to be planted.

CA 6.9 Estimation of the potential and actual exposure through diet and other sources

The Acceptable Daily Intake (ADI) and the Acute Reference Dose (ARfD) for trifloxystrobin as published in SANCO/4339/2000 of April 2003, are summarised in the table below.

Table 6.9-1: EU endpoints of trifloxystrobin

Active substance	End-Point	Value (mg/kg bw/day)	Study	Safety factor	Reference
Trifloxystrobin	Acceptable Daily Intake (ADI)	0.1	2-year rat study	100	SANCO/4339/2000
	Acute Reference Dose (ARfD)	none allocated	not necessary due to low acute toxicity of trifloxystrobin	-	SANCO/4339/2000

Chronic (long-term) dietary exposure calculation

The consumer risk assessment was performed with revision 2 of the EFSA Pesticide Residues Intake Model (PRIMo). The calculation of the long-term exposure (chronic exposure) is based on the mean consumption data representative for 22 national diets collected from MS surveys plus 1 regional and 4 cluster diets from the WHO GEMS Food database; for the acute exposure assessment the most critical large portion consumption data from 19 national diets collected from MS surveys is used.

For the chronic exposure calculations for grape the median residue value of trifloxystrobin derived from the trials submitted within this dossier is used, multiplied by the respective conversion factor from enforcement to risk assessment. For pomefruit and strawberry the median residues of the sum of trifloxystrobin and CGA 321113 are used as published in the JMPR evaluation 2004 and 2012, as these values are higher than the ones resulting from the trials submitted within this dossier.

Table 6.9-2: Summary of median residue levels

crop	Region	EU STMR TFS [mg/kg]	Conversion factor (median)	EU STMR (sum of trifloxystrobin + CGA 321113) [mg/kg]	Codex STMR trifloxystrobin + CGA 321113 [mg/kg]
Pomefruit	northern Europe	0.05	1	0.05	**0.11**
	southern Europe	0.10	1	0.10	
Grape	northern Europe	0.335	1.1	**0.37**	0.15
	southern Europe	0.18	1.1	0.20	
Strawberry	indoor	0.125	1.1	0.14	**0.335**
	northern Europe	0.096	1.1	0.11	
	southern Europe	0.15	1.1	0.17	

For certain other crops assessed in previously issued EFSA reasoned opinions (EFSA, 2009 to 2013) the median residue values and the respective conversion factors are used if applicable. For the remaining commodities of plant and animal origin, the existing MRLs as established in Annexes II and III of Regulation (EC) No 396/2005 (Commission Regulation (EU) No 1004/2013 of 15 October 2013 and No 508/2011 of 24 May 2011) were used as input values.

Although in case of the MRLs, the metabolite CGA 321113 is not included, the calculation using the MRL represents a worst case, since the MRL values are generally higher than the median residue of trifloxystrobin multiplied by the conversion factor to accommodate for residues of metabolite CGA 321113.

The input values used for the chronic dietary exposure calculation are summarised in Table 6.9-3.

Table 6.9-3: Input values for the consumer dietary exposure assessment

Commodity	Input value (mg/kg)	Comment
Pomefruit	0.11	Median residue Trifloxystrobin and CGA321113 JMPR / Codex
Grape	0.37	Median residue Trifloxystrobin EU trials dossier * CF 1.1
Strawberry	0.335	Median residue Trifloxystrobin and CGA321113 JMPR / Codex
Horseradish	0.04	Median residue * CF 1.6 EFSA Journal 2013;11(8):3349
Parsley root	0.04	Median residue * CF 1.6 EFSA Journal 2013;11(8):3349
Beans with pods	0.2	Median residue EFSA Journal 2013;11(4):3199
Spring onion	0.04	Median residue * CF 2.6 EFSA Journal 2012;10(9):2873
Globe artichoke	0.07	Median residue EFSA Journal 2012;10(9):2873
Aubergines	0.08	Median residue EFSA Journal 2011;9(11):1973
Blueberries	0.78	Median residue EFSA Scientific Report (2009) 273,1-27 (the median residue given in the report is 0.63 mg/kg, while the calculation of the reported residue values leads to a median of 0.78 mg/kg)
Brussels sprouts	0.13	Median residue * CF 1.3 EFSA Scientific Report (2009) 273,1-27
Head cabbage	0.05	Median residue * CF 1.7 EFSA Scientific Report (2009) 273,1-27
Lettuce, scarole, herbs	5.5	Median residue EFSA Scientific Report (2009) 273,1-27
Celery	0.1	Median residue * CF 1.3 EFSA Scientific Report (2009) 273,1-27
Swedes, turnip, salsify, parsnip	0.02	Median residue * CF 2.0 EFSA Scientific Report (2009) 314,1-27
Kale, Chinese cabbage	0.66	Median residue EFSA Journal 2010;8(6):1648
Other commodities of plant and animal origin	MRL	see Commission Regulation 1004/2013 of 15 October 2013 and 508/2011 of 24 May 2011

Conversion Factor (CF) only used for calculation if > 1, i.e. if residues of CGA 321113 above LOQ

The calculated exposure was then compared with the toxicological reference value derived for trifloxystrobin (see Table 6.9-1). The results of the intake calculation are presented in Table 6.9-4.

No long-term consumer intake concerns were identified for any of the European diets incorporated in the EFSA PRIMo. The total calculated intake values accounted up to 9.1 % of the ADI (WHO cluster diet B).

It can be concluded that the proposed and existing uses of trifloxystrobin will not result in a consumer exposure exceeding the toxicological reference value and therefore trifloxystrobin is unlikely to pose a consumer health risk.

Table 6.9-4: Trifloxystrobin EFSA model (2.0), TMDI (based on MRLs as published in CR 1004/2013 and CR 508/2011 or the input values as given in Table 6.9-3, and an ADI of 0.1 mg/kg body weight/day)

trifloxystrobin	
Status of the active substance:	Code no.
LOQ (mg/kg bw):	proposed LOQ
Toxicological end points	
ADI (mg/kg bw/day): 0,1	ARfD (mg/kg bw):
Source of ADI:	Source of ARfD:
Year of evaluation:	Year of evaluation:

Chronic risk assessment

TMDI (range) in % of ADI
minimum - maximum
1 9

No of diets exceeding ADI:

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
9,1	WHO Cluster diet B	2,0	Lettuce	1,5	Tomatoes	0,8	Table and wine grapes	
8,0	DE child	1,4	Pome fruit	1,4	Citrus fruit	0,9	Passion fruit	
7,4	NL child	1,2	Citrus fruit	1,1	Scarole (broad-leaf endive)	0,7	Pome fruit	
6,5	IE adult	0,8	Citrus fruit	0,8	Passion fruit	0,8	Other leafy brassica	
5,5	ES adult	2,9	Lettuce	0,4	Citrus fruit	0,4	Tomatoes	
5,5	ES child	2,3	Lettuce	0,7	Citrus fruit	0,5	Tomatoes	
5,3	WHO regional European diet	2,1	Lettuce	0,6	Tomatoes	0,2	Citrus fruit	
4,9	FR toddler	1,2	Beans (with pods)	0,8	Milk and cream,	0,7	Citrus fruit	
4,5	IT adult	2,1	Lettuce	0,6	Tomatoes	0,4	Peaches	
4,5	WHO cluster diet E	0,7	Table and wine grapes	0,5	Lettuce	0,3	Scarole (broad-leaf endive)	
4,2	IT kids/toddler	1,6	Lettuce	0,7	Tomatoes	0,3	Peaches	
4,1	WHO Cluster diet F	1,7	Lettuce	0,3	Tomatoes	0,3	Citrus fruit	
4,1	UK Toddler	1,1	Sugar beet (root)	0,7	Citrus fruit	0,4	Milk and cream,	
3,8	NL general	0,7	Lettuce	0,6	Scarole (broad-leaf endive)	0,6	Citrus fruit	
3,7	FR all population	1,5	Table and wine grapes	0,5	Lettuce	0,2	Tomatoes	
3,4	DK child	0,8	Lettuce	0,3	Pome fruit	0,3	Cucurbits - edible peel	
3,4	WHO cluster diet D	0,5	Tomatoes	0,4	Herbs	0,3	Wheat	
3,2	UK Infant	0,8	Milk and cream,	0,5	Sugar beet (root)	0,4	Citrus fruit	
3,0	SE general population 90th percentile	0,4	Citrus fruit	0,4	Tomatoes	0,2	Milk and cream,	
3,0	FR infant	0,8	Beans (with pods)	0,5	Milk and cream,	0,3	Citrus fruit	
2,9	PT General population	1,0	Table and wine grapes	0,4	Tomatoes	0,4	Peaches	
2,7	UK vegetarian	0,8	Lettuce	0,3	Table and wine grapes	0,3	Citrus fruit	
2,5	UK Adult	0,6	Lettuce	0,4	Table and wine grapes	0,2	Tomatoes	
1,8	FI adult	0,4	Lettuce	0,3	Citrus fruit	0,2	Tomatoes	
1,7	DK adult	0,5	Table and wine grapes	0,2	Tomatoes	0,1	Pome fruit	
1,5	LT adult	0,3	Lettuce	0,3	Tomatoes	0,2	Pome fruit	
1,4	PL general population	0,4	Tomatoes	0,3	Pome fruit	0,1	Table and wine grapes	

Acute Reference Dose (ARfD) and acute (short-term) dietary exposure calculation

As trifloxystrobin is not acutely toxic, no Acute Reference Dose (ARfD) was set. Therefore, no calculation of the Short-Term Intake (acute risk assessment) is performed.

CA 6.10 Other studies

Residue data on trifloxystrobin isomers

In addition to residue trial data available for the representative crops pome fruit, grape and strawberry, residue trials are available for trifloxystrobin and its isomers and CGA 321113 and its isomer for further crops. Although these trials may not cover the critical GAPs for these crops in Europe, the data are summarised within this chapter to provide further information on the ratio of trifloxystrobin to the isomers and the residue levels of the isomers of trifloxystrobin and CGA 321113.

Further residue trials including isomer analysis will become available in future and may be submitted on request when available.

Table 6.10-1: Residue trials conducted per geographical region and vegetation period

Type of formulation**	Crop	Use pattern TFS	Region*	No. of trials			Report No.	Dossier ref.
				2009	2010	2012		
Trifloxystrobin WG 50	Olive	2x60 g/ha; BBCH 75-81	S-EU	4	-	-	09-2015 in MR-11/044	M-421645-02-1
AE C656948 & Trifloxystrobin SC 500	Hops	2x150 g/ha	N-EU	5	-	-	09-2076 in MR-11/044	M-421645-02-1
Tebuconazole & Trifloxystrobin WG 75	Brussels sprouts	3x100 g/ha	S-EU	2	-	-	09-2135 in MR-11/044	M-421645-02-1
Tebuconazole & Trifloxystrobin WG 75	Head cabbage	3x100 g/ha	S-EU	2	-	-	09-2136 in MR-11/044	M-421645-02-1
Trifloxystrobin WG 50	Hops	2 x 625 g/ha	N-EU	-	1	-	10-2174	M-443126-02-1
Trifloxystrobin WG 50	Cucumber	3x187.5 g/ha	N-EU	-	4	-	10-2179	M-441575-01-1
Trifloxystrobin WG 50	Cucumber	3x187.5 g/ha	S-EU	-	4	-	10-2180	M-438321-02-1
Trifloxystrobin WG 50	Cucumber	3x94 g/ha*m	indoor	-	4	-	10-2181	M-438698-02-1
Tebuconazole & Trifloxystrobin WG 75	Broccoli-/Cauliflower	2x90 g/ha	N-EU	-	-	4	12-2068	M-457379-01-1
Tebuconazole & Trifloxystrobin WG 75	Broccoli /Cauliflower	2x100 g/ha	S-EU	-	-	4	12-2069	M-457394-01-1

TFS: trifloxystrobin

* N-EU: Northern Europe; S-EU: Southern Europe

** WG 50: Wettable Granule formulation containing 50% trifloxystrobin

WG 75: Wettable Granule formulation containing 50% tebuconazole and 25% trifloxystrobin

SC 500: Suspension Concentrate containing 250 g/L of AE C656948 and 250 g/L of trifloxystrobin

Table 6.10-2: Summary of residue results on isomers

Report No.	Dossier ref.	Crop	No. of trials	CGA 279202 [mg/kg]	CGA 321113 [mg/kg]	CGA 357261 [mg/kg]	CGA 357262 [mg/kg]	CGA 331409 [mg/kg]	CGA 373466 [mg/kg]
09-2015 in MR-11/044	M-421645-02-1	Olive	4	up to 0.10 at PHI	<LOQ to 0.01	<LOQ to 0.02	<LOQ	<LOQ to 0.02	<LOQ
09-2076 in MR-11/044, 10-2174	M-421645-02-1 M-443126-02-1	Hops, green cone	6	up to 0.88 at day 14 or later	up to 0.31 at day 14 or later	0.01 to <0.05	<LOQ	0.02 to <0.05	<LOQ
		Hops, dried cone	6	up to 2.1 at day 14 or later	up to 0.71 at day 14 or later	0.05 to 0.07	<LOQ	<0.05 to 0.08	0.05 to 0.08
09-2135 in MR-11/044	M-421645-02-1	Brussels sprouts	2	<LOQ at PHI	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
09-2136 in MR-11/044	M-421645-02-1	Head cabbage	2	<LOQ at PHI	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10-2179 10-2180 10-2181	M-441575-01-1 M-438321-02-1 M-438698-02-1	Cucumber	12	<LOQ to 0.06 at PHI	<LOQ to 0.02	<LOQ	<LOQ to 0.03	<LOQ	<LOQ
12-2068 12-2069	M-457379-01-1 M-457394-01-1	Broccoli/ Cauliflower	8	<LOQ to 0.011 at PHI	<LOQ to 0.014 at PHI	<LOQ to 0.02	<LOQ	<LOQ	<LOQ

LOQ: 0.01 mg/kg, except for hops (0.01 or 0.05 mg/kg) PHI: pre-harvest interval

Supplementary trials on olive, hops, Brussels sprouts, Savoy cabbage and white cabbage:

Report:	**KCA 6.10.01, [redacted]; 2012 (amended)**; M-421645-02-1
Title:	Determination of the residues of Trifloxystrobin, CGA 357261, CGA 357262, CGA 331409, CGA 321113, and CGA 373466 in/on materials of plant origin by HPLC-MS/MS
Document No. & Report No:	M-421645-02-1 MR-11/044 (P 652 11 5503)
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A section 8 residues in or on treated products, food and feed
GLP	yes

Test system

Olive: In 2009 four trials were performed in southern Europe in/on olive with trifloxystrobin WG 50. The product was applied two times to olive trees at application rates of 0.06 kg trifloxystrobin/ha. The treatments were performed at growth stage BBCH 75 and BBCH 81.
Fruit samples were taken on day 21 after the last application in all trials. In two trials, additional samples of fruit were taken at earlier or later time points.

Hops: In 2009 five trials were conducted in northern Europe with a trifloxystrobin SC mixture formulation. The product was applied twice at rates of 0.15 kg trifloxystrobin/ha (in trial -01 the first

application was overdosed: 0.161 kg/ha). The treatments were performed with a spray interval of 14-17 days and an intended PHI of 21 days.
Samples were taken at day 7, 13/14, 21 and 28 after last application, except in trial -01, where the plot was by mistake harvested completely before day 21, so that only samples up to day 14 could be taken.

Brussels sprouts: In 2009 two trials were performed in southern Europe in on Brussels sprouts with a trifloxystrobin WG mixture formulation. The product was applied three times at rates of 0.1 to 0.11 kg trifloxystrobin/ha, a spray interval of 21-26 days, and a pre-harvest interval of 21 days.
Samples were taken 21 days after the last application and in one trial additionally on day 7, 13 and 27 after last application.

Cabbages: In 2009 one trial was performed in white cabbage and one trial in Savoy cabbage with a trifloxystrobin WG mixture formulation. The product was applied three times at rates of 0.1 kg trifloxystrobin/ha, a spray interval of 20-22 days, and a pre-harvest interval of 21 days.
Samples were taken 21 days after the last application and in one trial additionally on day 7, 15 and 29 after last application.

(Please note that Field data of the respective studies are available in separate studies and may be submitted on request. Please note that report MR-11/044 also includes data on grape and strawberry, which are not summarised below, since these two crops are already summarised in chapter 6.3.5 and 6.3.8 with the relevant use pattern.)

Residues of trifloxystrobin, its isomers CGA 357261, CGA 357262, CGA 331409, its metabolite CGA 321113 and isomer CGA 373466 were determined according to method 01313. The analytical method was validated by recovery experiments prior to and during the analysis of the samples by spiking control samples with all analytes. The limit of quantitation was 0.01 in all cases, except for hops (0.05 mg/kg).

Findings

- Method performance: Overall mean recoveries at fortification levels between 0.01 and 1.0 mg/kg were within the acceptable range of 70-110 %, RSD <20%.
- Storage stability: The maximum storage period of deep-frozen samples was up to 725 days for trifloxystrobin, CGA 321113, CGA 357261, CGA 357262, CGA 331409 and CGA 373466 and is covered by the storage stability studies.

- Residue results:

Olive: Residues of trifloxystrobin at day 21 (PHI) after last application ranged between 0.03 and 0.10 mg/kg. Residues of CGA 321113 were at or below LOQ. Residues of CGA 357262 and CGA 373466 were always below LOQ. Residues of CGA 331409 and CGA 357261 were below LOQ in three trials, and up to 0.02 mg/kg in one trial.

Hops: Residues of trifloxystrobin at day 20/21 (PHI) after last application in kiln-dried cones ranged between 0.14 and 0.74 mg/kg. Residues of CGA 321113 ranged between 0.06 and 0.53 mg/kg. Residues of CGA 357262 were always below LOQ (<0.05 mg/kg). Residues of CGA 331409 were <0.05 mg/kg or up to 0.08 mg/kg throughout all trials and dates / sample materials. Residues of CGA 357261 were <0.05 mg/kg or up to 0.07 mg/kg throughout all trials and dates / sample materials. Residues of CGA 373466 were <0.05 mg/kg in all trials except in one trial, where samples up to 0.08 mg/kg were detected in kiln-dried cone.

Brussels sprouts: At day 21 after the last application (PHI) residues were <LOQ for all analytes. At the other sampling dates only trifloxystrobin was detected, but none of the other isomers / metabolites.

Cabbages: At day 21 after the last application (PHI) residues were <LOQ for all analytes. At the other sampling dates only trifloxystrobin was detected, but none of the other isomers / metabolites.

Table 6.10-3: Application data and residues of trifloxystrobin and CGA 321113 in/ on olive treated with Trifloxystrobin WG formulation

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
09-2015 09-2015-01 MR-11/044 GLP: yes 2009	Olive Arbe-quina	Spain [redacted] Europe, South	50 WG	2	0.060	0.0060	75 81	fruit	0* 0 7 14 **21** 28	<0.01 0.09 0.11 0.11 0.10 0.09	<0.01 <0.01 <0.01 <0.01 0.01 0.01
09-2015 09-2015-02 MR-11/044 GLP: yes 2009	Olive Nocella-ra etnea	Italy [redacted] Europe, South	50 WG	2	0.060	0.0060	75 81	fruit	0 **21**	0.10 0.03	<0.01 <0.01
09-2015 09-2015-03 MR-11/044 GLP: yes 2009	Olive Galega	Portugal [redacted] Europe, South	50 WG	2	0.060	0.0060	75 81	fruit	0* 0 7 14 **21** 28	0.01 0.09 0.07 0.05 0.03 0.02	<0.01 <0.01 0.01 0.01 0.01 0.01
09-2015 09-2015-04 MR-11/044 GLP: yes 2009	Olive Mega-ron	Greece [redacted] Europe, South	50 WG	2	0.060	0.0060	75 81	fruit	0 **21**	0.14 0.06	<0.01 <0.01

FL: Formulation No: number of applications
GS = growth stage (BBCH code) at application DALT = days after last treatment
* prior to last treatment.

Table 6.10-4: Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in/ on olive treated with Trifloxystrobin WG formulation

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Residues Portion analysed	DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
09-2015 09-2015-01 MR-11/044 GLP: yes 2009	Olive Arbe-quina	Spain [redacted] Europe, South	fruit	0* 0 1 14 **21** 28	<0.01 <0.01 0.01 0.02 0.01 0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 0.01 0.01 0.02	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
09-2015 09-2015-02 MR-11/044 GLP: yes 2009	Olive Nocella-ra etnea	Italy [redacted] Europe, South	fruit	0 **21**	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01
09-2015 09-2015-03 MR-11/044 GLP: yes 2009	Olive Galega	Portugal [redacted] Europe, South	fruit	0* 0 1 14 **21** 28	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
09-2015 09-2015-04 MR-11/044 GLP: yes 2009	Olive Mega-ron	Greece [redacted] Europe, South	fruit	0 **21**	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01

DALT = days after last treatment * prior to last treatment.

Table 6.10-5: Application data and residues of trifloxystrobin and CGA 321113 in/ on hops treated with Trifloxystrobin SC formulation

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Trifloxystrobin (mg/kg)	CGA 321113 (mg/kg)
09-2076 09-2076-01 MR-11/044 GLP: yes 2009	Hop Strissel-spalt	France [redacted] Europe, North	500 SC	2	0.15-0.161	0.0068-0.0083	77	cone, green	0*	0.05	<0.05
									0	1.2	0.08
									7	0.26	0.10
									13	0.16	0.09
								cone, kiln-dried	14	1.2	0.53
09-2076 09-2076-02 MR-01/044 GLP: yes 2009	Hop Mag-num	Germany [redacted] Europe, North	500 SC	2	0.15	0.0075	79	cone, green	0*	0.05	0.08
									0	0.93	0.20
									7	0.61	0.33
									14	0.13	0.15
									20	0.37	0.29
									28	0.28	0.31
								cone, kiln-dried	14	n.a.	n.a.
									20	0.52	0.53
									28	0.63	0.71
09-2076 09-2076-03 MR-11/044 GLP: yes 2009	Hop Herku-les	Germany [redacted] Europe, North	500 SC	2	0.15	0.0063	87	cone, green	0*	0.22	0.08
									0	1.4	0.21
									7	0.35	0.13
									14	0.14	0.10
									21	0.13	0.08
									28	0.71	0.08
								cone, kiln-dried	14	0.26	0.10
									21	0.21	0.13
									28	1.3	0.13
09-2076 09-2076-04 MR-11/044 GLP: yes 2009	Hop Perle	Germany [redacted] Europe, North	500 SC	2	0.15	0.0063	79	cone, green	0*	0.10	<0.05
									0	1.8	0.07
									7	0.95	0.19
									14	0.46	0.15
									21	0.31	0.12
									28	0.17	0.07
								cone, kiln-dried	14	1.2/0.10**	0.32
									21	0.74	0.23
									28	0.44	0.13
09-2076 09-2076-05 MR-11/044 GLP: yes 2009	Hop Haller-tauer Mittel-früh	Germany [redacted] Europe, North	500 SC	2	0.15	0.0071	83	cone, green	0*	0.46	0.11
									0	0.76	0.21
									7	0.36	0.10
									14	0.21	0.07
									21	0.09	<0.05
									28	0.13	<0.05
								cone, kiln-dried	14	0.26	0.14
									21	0.14	0.06
									28	0.24	0.08

FL: Formulation No: number of applications
GS = growth stage (BBCH code) at last application DALT = days after last treatment
* prior to last treatment ** residue in control n.a. = not analysed (sample nor available)

Table 6.10-6: Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in/ on hops treated with Trifloxystrobin SC formulation

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Residues Portion analysed	DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
09-2076 09-2076-01 MR-11/044 GLP: yes 2009	Hop Strissel-spalt	France [redacted] Europe, North	cone, green	0*	<0.05	<0.05	<0.05	<0.05
				0	<0.05	<0.05	<0.05	<0.05
				7	<0.05	<0.05	<0.05	<0.05
				13	<0.05	<0.05	<0.05	<0.05
			cone, kiln-dried	14	0.07	<0.05	0.08	<0.05
09-2076 09-2076-02 MR-01/044 GLP: yes 2009	Hop Mag-num	Germany [redacted] Europe, North	cone, green	0*	<0.05	<0.05	<0.05	<0.05
				0	<0.05	<0.05	<0.05	<0.05
				7	<0.05	<0.05	<0.05	<0.05
				14	<0.05	<0.05	<0.05	<0.05
				20	<0.05	<0.05	<0.05	<0.05
				28	<0.05	<0.05	<0.05	<0.05
			cone, kiln-dried	14	n.a.	n.a.	n.a.	n.a.
				20	<0.05	<0.05	0.05	0.05
				28	0.05	<0.05	0.06	0.08
09-2076 09-2076-03 MR-11/044 GLP: yes 2009	Hop Herku-les	Germany [redacted] Europe, North	cone, green	0*	<0.05	<0.05	<0.05	<0.05
				0	<0.05	<0.05	<0.05	<0.05
				7	<0.05	<0.05	<0.05	<0.05
				14	<0.05	<0.05	<0.05	<0.05
				21	<0.05	<0.05	<0.05	<0.05
				28	<0.05	<0.05	<0.05	<0.05
			cone, kiln-dried	14	<0.05	<0.05	<0.05	<0.05
				21	<0.05	<0.05	<0.05	<0.05
				28	<0.05	<0.05	<0.05	<0.05
09-2076 09-2076-04 MR-11/044 GLP: yes 2009	Hop Perle	Germany [redacted] Europe, North	cone, green	0*	<0.05	<0.05	<0.05	<0.05
				0	<0.05	<0.05	<0.05	<0.05
				7	<0.05	<0.05	<0.05	<0.05
				14	<0.05	<0.05	<0.05	<0.05
				21	<0.05	<0.05	<0.05	<0.05
				28	<0.05	<0.05	<0.05	<0.05
			cone, kiln-dried	14	<0.05	<0.05	0.05	<0.05
				21	<0.05	<0.05	<0.05	<0.05
				28	<0.05	<0.05	<0.05	<0.05
09-2076 09-2076-05 MR-11/044 GLP: yes 2009	Hop Haller-tauer Mittel-frih	Germany [redacted] Europe, North	cone, green	0*	<0.05	<0.05	<0.05	<0.05
				0	<0.05	<0.05	<0.05	<0.05
				7	<0.05	<0.05	<0.05	<0.05
				14	<0.05	<0.05	<0.05	<0.05
				21	<0.05	<0.05	<0.05	<0.05
				28	<0.05	<0.05	<0.05	<0.05
			cone, kiln-dried	14	<0.05	<0.05	<0.05	<0.05
				21	<0.05	<0.05	<0.05	<0.05
				28	<0.05	<0.05	<0.05	<0.05

DALT = days after last treatment * prior to last treatment

Table 6.10-7: Application data and residues of trifloxystrobin and CGA 321113 in/ on Brussels sprouts treated with Trifloxystrobin WG formulation

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
09-2135 09-2135-01 MR-11/044 GLP: yes 2009	Brussels sprouts Mezzo nano.	Italy [redacted] Europe, South	75 WG	3	0.100	0.0143	45	sprout	0* 0 7 13 **21** 27	<0.02 0.09 0.03 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
09-2135 09-2135-02 MR-11/044 GLP: yes 2009	Brussels sprouts F1 : Oliver	Spain [redacted] Europe, South	75 WG	3	0.100-0.110	0.0143-0.0166	46	sprout	0 **21**	0.07 <0.01	<0.01 <0.01

FL: Formulation No: number of applications
GS = growth stage (BBCH code) at last application DALT = days after last treatment
* prior to last treatment.

Table 6.10-8: Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in/ on Brussels sprouts treated with Trifloxystrobin WG formulation

GLP Year	Crop Variety	Country	Portion analysed	DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
09-2135 09-2135-01 MR-11/044 GLP: yes 2009	Brussels sprouts Mezzo nano	Italy [redacted] Europe, South	sprout	0* 0 7 13 **21** 27	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
09-2135 09-2135-02 MR-11/044 GLP: yes 2009	Brussels sprouts F1 : Oliver	Spain [redacted] Europe, South	sprout	0 **21**	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01

DALT = days after last treatment * prior to last treatment

Table 6.10-9: Application data and residues of trifloxystrobin and CGA 321113 in/ on cabbages treated with Trifloxystrobin WG formulation

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Trifloxystrobin (mg/kg)	CGA 321113 (mg/kg)
09-2136 09-2136-01 MR-11/044 GLP: yes 2009	Cabbage, white Premiere	Spain [redacted] Europe, South	75 WG	3	0.10	0.020-0.033	45	head	0* 0 7 15 **21** 29	<0.01 0.08 0.02 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
09-2136 09-2136-02 MR-11/044 GLP: yes 2009	Cabbage, Savoy Savoy king	Italy [redacted] Europe, South	75 WG	3	0.10	0.017	45	head	0 **21**	0.36 <0.01	<0.01 <0.01

FL: Formulation No: number of applications
GS = growth stage (BBCH code) at last application DALT = days after last treatment
* prior to last treatment.

Table 6.10-10: Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in/ on cabbages treated with Trifloxystrobin WG formulation

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Residues					
			Portion analysed	DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
09-2136 09-2136-01 MR-11/044 GLP: yes 2009	Cabbage, white Premiere	Spain [redacted] Europe, South	head	0* 0 7 15 **21** 29	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
09-2136 09-2136-02 MR-11/044 GLP: yes 2009	Cabbage, Savoy Savoy king	Italy [redacted] Puglia Europe, South	head	0 **21**	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01

DALT = days after last treatment * prior to last treatment.

Supplementary trials on hops:

Report:	**KCA 6.10/02, [redacted], [redacted]; 2012 (amended)**; M-443126-02-1
Title:	Determination of the residues of trifloxystrobin in/on hop after spraying of Trifloxystrobin WG 50 in the field in Germany
Document No & Report No:	M-443126-02-1 10-2174
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Test system

In 2010 one trial was performed in northern Europe in/on hops with Trifloxystrobin WG 50. The product was applied two times to hops at application rates of 0.625 kg trifloxystrobin/ha. The treatments were performed with a spray interval of 10 days and a PHI of 13 days.
Cone samples were taken on day 13 after the last application.
Residues of trifloxystrobin, its isomers CGA 357261, CGA 357262, CGA 331409, its metabolite CGA 321113 and isomer CGA 373466 were determined according to method 01313. The analytical method was validated by recovery experiments prior to and during the analysis of the samples by spiking control samples with all analytes. The limit of quantitation was 0.01 for green cone and 0.05 mg/kg for kiln-dried cone.

Findings

- Method performance: Overall mean recoveries at fortification levels between 0.01 and 0.5 mg/kg were within the acceptable range of 70-110 %.
- Storage stability: The maximum storage period of deep-frozen samples was up to 370 days for trifloxystrobin, CGA 321113, CGA 357261, CGA 357262, CGA 331409 and CGA 373466 and is covered by the storage stability studies.

- Residue results:
In kiln-dried hops residues of trifloxystrobin (2.1 mg/kg) and CGA 321113 (0.18 mg/kg) only were found. All other analytes were below LOQ (<0.05 mg/kg).
In green cone residues of CGA 357262 and CGA 373466 were below LOQ (<0.01 mg/kg), residues of trifloxystrobin were 0.88 mg/kg, residues of CGA 321113 were at 0.05 mg/kg, residues of CGA 331409 were at 0.02 mg/kg, and residues of CGA 357261 were at 0.01 mg/kg.

Table 6.10-11: Application data and residues of trifloxystrobin and CGA 321113 in/ on hops treated with Trifloxystrobin WG formulation

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
10-2174 10-2174-02 10-2127-02-T GLP: yes 2010	Hop Mag-num	Germany [redacted] Europe, North	50 WG	2	0.625	0.021	75	cone, green	13	0.88	0.05
								cone, kiln-dried	13	2.1	0.18

FL: Formulation No: number of applications
GS = growth stage (BBCH code) at application DALT = days after last treatment

Table 6.10-12: Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in/ on hops treated with Trifloxystrobin WG formulation

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Residues					
			Portion analysed	DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
10-2174 10-2174-02 10-2127-02-T GLP: yes 2010	Hop Mag-num	Germany [redacted] Europe, North	cone, green	13	0.01	<0.01	0.02	<0.01
			cone, kiln-dried	13	<0.05	<0.05	<0.05	<0.05

DALT = days after last treatment * prior to last treatment

Supplementary trials on cucumber:

Report:	**KCA 6.10/03; [redacted]; 2012** ; M-441575-01-1
Title:	Determination of the residues of trifloxystrobin in/on cucumber after spraying of Trifloxystrobin WG 50 in the field in France (North), Germany and Belgium
Document No & Report No:	M-441575-01-1 10-2179
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed Guidance doc. 7029/VI/95 rev. 5
GLP	yes

Report:	**KCA 6.10/04, [redacted], [redacted]; 2012 (amended)**; M-438321-02-1
Title:	Determination of the residues of trifloxystrobin in/on cucumber after spraying of Trifloxystrobin WG 50 in the field in France (South), Spain and Italy
Document No & Report No:	M-438321-02-1 10-2180
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed Guidance doc. 7029/VI/95 rev. 5
GLP	yes

Report:	**KCA 6.10/05, [redacted], [redacted]; 2012 (amended)**; M-438698-02-1
Title:	Determination of the residues of trifloxystrobin in/on cucumber after spraying of Trifloxystrobin WG 50 in the greenhouse in Spain, Italy, France (South) and the Netherlands
Document No & Report No:	M-438698-02-1 10-2181
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed Guidance doc. 7029/VI/95 rev. 5
GLP	yes

Test system

In 2010 residue trials were performed in northern and southern Europe in the field and in the greenhouse in/on cucumber with Trifloxystrobin WG 50. The product was applied three times to cucumber plants at application rates of 0.1875 kg trifloxystrobin/ha (field use), or about 0.094 kg trifloxystrobin per ha and meter crop height (indoor use), with a maximum of 0.188 kg a.s./ha. The treatments were performed with intervals of about 7 days. (In one trial a fourth application was conducted because of heavy rainfall after the third application.)
Fruit samples were taken on day 3 (2) after the last application in all trials. Additional samples of fruit were taken at earlier or later time points in some of the trials.

Residues of trifloxystrobin, its isomers CGA 357261, CGA 357262, CGA 331409, its metabolite CGA 321113 and isomer CGA 373466 were determined according to method 01313. The analytical method was validated by recovery experiments prior to and during the analysis of the samples by spiking control samples with all analytes. The limit of quantitation was 0.01 in all cases.

Findings

- Method performance: Overall mean recoveries at fortification levels between 0.01 and 1.0 mg/kg were within the acceptable range of 70-110 %, RSD <20%.

- Storage stability: The maximum storage period of deep-frozen samples was up to 483 days for trifloxystrobin, CGA 321113, CGA 357261, CGA 357262, CGA 331409 and CGA 373466 and is covered by the storage stability studies.

- Residue results:
Residues of trifloxystrobin at day 3 (2) after last application ranged between <0.01 and 0.06 mg/kg. Residues of CGA 321113 and CGA 357262 were between <0.01 and 0.02 mg/kg. Residues of CGA 331409, CGA 357261 and CGA 373466 were always below LOQ.

Table 6.10-13: Application data and residues of trifloxystrobin and CGA 321113 in/ on cucumber in the field in northern and southern Europe treated with Trifloxystrobin WG 50

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
10-2179 10-2179-01 10-2179-01-T GLP: yes 2010	Cucum-ber Ceto (gerkhin)	France [redacted] Europe, North	50 WG	3	0.1875	0.0375	74	fruit	0* 0 1 **2** 6	<0.01 0.03 0.02 0.01 <0.01	<0.01 0.01 0.01 <0.01 0.01
10-2179 10-2179-02 10-2179-02-T GLP: yes 2010	Cucumber Melody (gherkin)	Germany [redacted] Europe, North	50 WG	4	0.1875	0.0313	85	fruit	0 **3**	0.04 <0.01	<0.01 <0.01
10-2179 10-2179-03 10-2179-03-T GLP: yes 2010	Cucumber Pepinova	Belgium [redacted] Europe, North	50 WG	3	0.1875	0.0188	74	fruit	0* 0 **3**	<0.01 0.04 0.06	<0.01 <0.01 <0.01
10-2179 10-2179-04 10-2179-04-T GLP: yes 2010	Cucumber Melody (gherkin)	Germany [redacted] Europe, North	50 WG	3	0.1875	0.0313	71	fruit	0 1 **3** 7	0.02 0.03 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01
10-2180 10-2180-01 10-2180-01-T GLP: yes 2010	Cucumber Raider	France [redacted] Europe, South	50 WG	3	0.1875	0.0313	89	fruit	0* 0 1 **3** 7	<0.01 0.03 0.03 0.04 0.01	<0.01 <0.01 <0.01 <0.01 <0.01
10-2180 10-2180-02 10-2180-02-T GLP: yes 2010	Cucumber Llano-verde	Spain [redacted] Europe, South	50 WG	3	0.1875	0.0234	73	fruit	0 **3**	0.04 0.01	<0.01 <0.01
10-2180 10-2180-03 10-2180-03-T GLP: yes 2010	Cucumber Bellissi-ma	Italy [redacted] Europe, South	50 WG	3	0.1875	0.0313	77	fruit	0* 0 1 **3** 7	0.01 0.23 0.09 0.06 <0.01	<0.01 <0.01 0.02 0.02 <0.01
10-2180 10-2180-04 10-2180-04-T GLP: yes 2010	Cucumber N 1504	Italy [redacted] Europe, South	50 WG	3	0.1875	0.0234	73	fruit	0 **3**	0.10 0.01	<0.01 <0.01

FL: Formulation No: number of applications
GS = growth stage (BBCH code) at application DALT = days after last treatment
* prior to last treatment.

Table 6.10-14: Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in/ on cucumber in the field in northern and southern Europe treated with Trifloxystrobin WG 50

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Portion analysed	Residues DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
10-2179 10-2179-01 10-2179-01-T GLP: yes 2010	Cucumber Ceto (gerkhin)	France [redacted] Europe, North	fruit	0* 0 1 **2** 6	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 0.01 0.01 0.02 0.03	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01
10-2179 10-2179-02 10-2179-02-T GLP: yes 2010	Cucumber Melody (gherkin)	Germany [redacted] Europe, North	fruit	0 **3**	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01
10-2179 10-2179-03 10-2179-03-T GLP: yes 2010	Cucumber Pepinova	Belgium [redacted] Europe, North	fruit	0* 0 **3**	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01	<0.01 <0.01 <0.01
10-2179 10-2179-04 10-2179-04-T GLP: yes 2010	Cucumber Melody (gherkin)	Germany [redacted] Europe, North	fruit	0 1 **3** 7	<0.01 <0.01 <0.01 <0.01	0.02 0.02 0.02 <0.01	<0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01
10-2180 10-2180-01 10-2180-01-T GLP: yes 2010	Cucumber Raider Cucumber	France [redacted] Europe, South	fruit	0* 0 1 **3** 7	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01
10-2180 10-2180-02 10-2180-02-T GLP: yes 2010	Cucumber Llano-verde	Spain [redacted] Europe, South	fruit	0 **3**	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01
10-2180 10-2180-03 10-2180-03-T GLP: yes 2010	Cucumber Bellissima	Italy [redacted] Europe, South	fruit	0* 0 1 **3** 7	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 0.01 0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01
10-2180 10-2180-04 10-2180-04-T GLP: yes 2010	Cucumber N 1304	Italy [redacted] Europe, South	fruit	0 **3**	<0.01 <0.01	0.01 0.01	<0.01 <0.01	<0.01 <0.01

DALT = days after last treatment * prior to last treatment.

Table 6.10-15: Application data and residues of trifloxystrobin and CGA 321113 in/ on cucumber in the greenhouse treated with Trifloxystrobin WG 50

GLP Year	Crop Variety	Country	FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
10-2181 10-2181-01 10-2181-01-T GLP: yes 2010	Cucumber Alanis	Spain [redacted] Europe, South, Green-house	50 WG	3	0.188 0.094 kg/ha*m	0.0125	85	fruit	0* 0 1 **3** 7	0.02 0.09 0.06 0.03 0.02	<0.01 <0.01 <0.01 <0.01 <0.01
10-2181 10-2181-02 10-2181-02-T GLP: yes 2010	Cucumber Marinda F1	Italy [redacted] Europe, South, Green-house	50 WG	3	0.188 0.094 kg/ha*m	0.0125	75	fruit	0 **3**	0.06 0.05	<0.01 <0.01
10-2181 10-2181-03 10-2181-03-T GLP: yes 2010	Cucumber Colum-bia	France [redacted] Europe, South, Green-house	50 WG	3	0.1316-0.1645 0.094 kg/ha*m	0.0125	69	fruit	0* 0 1 **3** 7	0.01 0.08 0.05 0.05 0.01	<0.01 0.01 <0.01 <0.01 <0.01
10-2181 10-2181-04 10-2181-04-T GLP: yes 2010	Cucumber Cratos	Nether-lands [redacted] Europe, North, Green-house	50 WG	3	0.047-0.159 0.088-0.094 kg/ha*m	0.0125	88	fruit	0 **3**	0.04 0.01	<0.01 <0.01

FL: Formulation No: number of applications
GS = growth stage (BBCH code) at application DALT = days after last treatment
* prior to last treatment.

Table 6.10-16: Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in/ on cucumber in the greenhouse treated with Trifloxystrobin WG 50

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Residues Portion analysed	DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
10-2181 10-2181-01 10-2181-01-T GLP: yes 2010	Cucumber Alanis	Spain [redacted] Europe, South, Green-house	fruit	0* 0 1 3 7	<0.01 <0.01 <0.01 <0.01 <0.01	0.01 0.01 0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01
10-2181 10-2181-02 10-2181-02-T GLP: yes 2010	Cucumber Marinda F1	Italy [redacted] Europe, South, Green-house	fruit	0 3	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01
10-2181 10-2181-03 10-2181-03-T GLP: yes 2010	Cucumber Columbia	France [redacted] Europe, South, Green-house	fruit	0* 0 1 3 7	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01
10-2181 10-2181-04 10-2181-04-T GLP: yes 2010	Cucumber Cratofs	Nether-lands [redacted] Europe, North, Green-house	fruit	0 3	<0.01 <0.01	0.02 0.01	<0.01 <0.01	<0.01 <0.01

DALT = days after last treatment * prior to last treatment

Supplementary trials on broccoli and cauliflower:

Report:	**KCA 6.10/06, [redacted], [redacted]; 2013** ; M-457379-01-1
Title:	Determination of the residues of tebuconazole and trifloxystrobin in/on broccoli and cauliflower after spray application of Tebuconazole & Trifloxystrobin WG 75 in the field in Germany, France (North) and Belgium
Document No & Report No:	M-457379-01-1 12-2068
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.10/07, [redacted], [redacted]; 2013** ; M-457394-01-1
Title:	Determination of the residues of tebuconazole and trifloxystrobin in/on broccoli and cauliflower after spray application of Tebuconazole & Trifloxystrobin WG 75 in the field in France (South)
Document No & Report No:	M-457394-01-1 12-2069
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Test system

In 2012 residue trials were performed in northern and southern Europe in the field in/on broccoli and cauliflower with Tebuconazole + Trifloxystrobin WG 75, a formulation containing 50% tebuconazole and 25% trifloxystrobin. The product was applied two times to broccoli or cauliflower at application rates of 0.09 (northern Europe) or 0.1 kg trifloxystrobin/ha (southern Europe). The treatments were performed with spray intervals of 13-14 days (northern Europe) or 19-21 days (southern Europe).
Curd samples were taken on day 21 (19-21) after the last application in all trials. In some trials, additional samples of were taken at earlier or later time points.

Residues of trifloxystrobin, its isomers CGA 357261, CGA 357262, CGA 373466, its metabolite CGA 321113 and isomer CGA 373466 were determined according to method 01313/M001. The analytical method was validated by recovery experiments prior to and during the analysis of the samples by spiking control samples with all analytes. The limit of quantitation was 0.01 in all cases.

Findings

- Method performance: Overall mean recoveries at fortification levels between 0.01 and 2.0 mg/kg were within the acceptable range of 70-110 %, RSD <20%, except for CGA 321113 and CGA 373466 in whole plant without root with an overall mean recovery of 69%, which was accepted due to low overall RSD values (4.9 and 7.3%).

- Storage stability: The maximum storage period of deep-frozen samples was up to 281 days for trifloxystrobin, CGA 321113, CGA 357261, CGA 357262, CGA 331409 and CGA 373466 and is covered by the storage stability studies.

- Residue results:

Residues of trifloxystrobin in curd at day 19-21 after last application ranged between <0.01 and 0.011 mg/kg. Residues of CGA 321113 were between < 0.01 and 0.014 mg/kg at day 19-21. Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 were below LOQ at day 19-21 after last application.

Table 6.10-17: Application data and residues of trifloxystrobin and CGA 321113 in/ on broccoli and cauliflower treated with a Trifloxystrobin WG formulation in the field in northern and southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
12-2068 12-2068-01 12-2068-01-T GLP: yes 2012	Broccoli Mara-thon	Germany [redacted] Europe, North	75 WG	2	0.090	0.030	29	whole plant without roots curd	0* 0 7 15 **21** 28	0.022 1.4 0.045 <0.01 <0.01 <0.01	<0.01 <0.01 0.015 <0.01 <0.01 <0.01
12-2068 12-2068-02 12-2068-02-T GLP: yes 2012	Broccoli Monaco Hybride	France [redacted] Europe, North	75 WG	2	0.090	0.015	43	whole plant without roots curd	0 **21**	0.62 <0.01	<0.01 <0.01
12-2068 12-2068-03 12-2068-03-T GLP: yes 2012	Cauli-flower Free-mont	Germany [redacted] Europe, North	75 WG	2	0.090	0.030	19	whole plant without roots curd	0* 0 7 14 **21** 28	<0.01 0.96 0.099 <0.01 <0.01 <0.01	<0.01 <0.01 0.015 <0.01 <0.01 <0.01
12-2068 12-2068-04 12-2068-04-T GLP: yes 2012	Cauli-flower Ameri-go SG 5619	Belgium [redacted] Europe, North	75 WG	2	0.090	0.016	41	whole plant without roots curd	0 **21**	0.64 <0.01	0.018 0.011
12-2069 12-2069-01 12-2069-01-T GLP: yes 2012	Broccoli Iroman	France [redacted] Europe, South	75 WG	2	0.100	0.0167	41	whole plant without roots curd	0* 0 7 13 **20** 27	<0.01 0.85 0.025 <0.01 <0.01 <0.01	<0.01 0.032 0.010 <0.01 <0.01 <0.01
12-2069 12-2069-02 12-2069-02-T GLP: yes 2012	Broccoli Steel	France [redacted] Europe, South	75 WG	2	0.100	0.0125	41	whole plant without roots curd	0 **19**	0.89 0.011	0.019 <0.01

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
12-2069 12-2069-03 12-2069-03-T GLP: yes 2012	Cauli-flower Nautilus	France [redacted] Europe, South	75 WG	2	0.100	0.0167	41	whole plant without roots curd	0* 0 7 14 **20** 28	0.014 0.56 0.17 <0.01 <0.01 <0.01	<0.01 0.022 0.025 <0.01 <0.01 <0.01
12-2069 12-2069-04 12-2069-04-T GLP: yes 2012	Cauli-flower Frémont F1	France [redacted] Europe, South	75 WG	2	0.100	0.0167	41	whole plant without roots curd	0 **21**	0.67 0.01	0.027 0.014

FL: Formulation No: number of applications
GS = growth stage (BBCH code) at application DALT = days after last treatment
* prior to last treatment

Table 6.10-18: Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in/ on broccoli and cauliflower treated with a Trifloxystrobin WG formulation in the field in northern and southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Residues Portion analysed	DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
12-2068 12-2068-01 12-2068-01-T GLP: yes 2012	Broccoli Mara-thon	Germany [redacted] Europe, North	whole plant without roots curd	0* 0 7 15 **21** 28	<0.01 0.021 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
12-2068 12-2068-02 12-2068-02-T GLP: yes 2012	Broccoli Monaco Hybride	France [redacted] Europe, North	whole plant without roots curd	0 **21**	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01
12-2068 12-2068-03 12-2068-03-T GLP: yes 2012	Cauli-flower Freeont	Germany [redacted] Europe, North	whole plant without roots curd	0* 0 7 14 **21** 28	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01
12-2068 12-2068-04 12-2068-04-T GLP: yes 2012	Cauli-flower Ameri-go SG 5619	Belgium [redacted] Europe, North	whole plant without roots curd	0 **21**	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Residues Portion analysed	DALT (days)	CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
12-2069 12-2069-01 12-2069-01-T GLP: yes 2012	Broccoli Iroman	France [redacted] Europe, South	whole plant	0*	<0.01	<0.01	<0.01	<0.01
			without roots	0	0.012	<0.01	<0.01	<0.01
				7	<0.01	<0.01	<0.01	<0.01
			curd	13	<0.01	<0.01	<0.01	<0.01
				20	<0.01	<0.01	<0.01	<0.01
				27	<0.01	<0.01	<0.01	<0.01
12-2069 12-2069-02 12-2069-02-T GLP: yes 2012	Broccoli Steel	France [redacted] Europe, South	whole plant	0	<0.01	<0.01	<0.01	<0.01
			without roots					
			curd	19	<0.01	<0.01	<0.01	<0.01
12-2069 12-2069-03 12-2069-03-T GLP: yes 2012	Cauli-flower Nautilus	France [redacted] Europe, South	whole plant	0*	<0.01	<0.01	<0.01	<0.01
			without roots	0	<0.01	<0.01	<0.01	<0.01
				7	<0.01	<0.01	<0.01	<0.01
			curd	14	<0.01	<0.01	<0.01	<0.01
				20	<0.01	<0.01	<0.01	<0.01
				28	<0.01	<0.01	<0.01	<0.01
12-2069 12-2069-04 12-2069-04-T GLP: yes 2012	Cauli-flower Frémont F1	France [redacted] Europe, South	whole plant	0	<0.01	<0.01	<0.01	<0.01
			without roots					
			curd	21	<0.01	<0.01	<0.01	<0.01

DALT = days after last treatment * prior to last treatment

Residue data on cereals

On request of CRD (UK), residue data in/on cereals are submitted within this dossier in order to be able to address dietary burden for livestock and the dietary risk for consumers.

The critical EU GAP for the northern region is summarised below.

Table 6.10-19: Summary of recent critical use pattern of trifloxystrobin in/on wheat and barley in northern Europe

Crop	Region	F, G, I	Mode of application	Maximum Number of Applications	Maximum rate trifloxystrobin (g a.s./ha)	Minimum PHI (days)
Wheat	EU-N	F	Foliar treatment - spraying	2	250	35
Barley	EU-N	F	Foliar treatment - spraying	2	250	35

EU-N: northern Europe F Field, G Greenhouse, I Indoor

Residue trials on wheat and barley have been submitted with the Annex II dossier of trifloxystrobin and have been evaluated in the peer review under Directive 91/414. The trials submitted with the Annex II dossier and covering the above mentioned use pattern for northern Europe, are summarised in Table 6.10-20. Since these residue reports have been previously evaluated at European level, detailed summaries of the studies are not provided again in this document in order to avoid duplication of work and only a brief summary is given below. Further trials with a PHI of 42 or more days are not considered below.

Table 6.10-20: Residue trials and residue levels on wheat and barley as submitted with the Annex II dossier and covering a northern European use pattern of 2 applications at 250 g a.s./ha and a PHI of 35 days

Crop	Region	FL Type	GAP Dose rate (g TFS/ha) Timing of last application	commodities	Residue level TFS (mg/kg)	Residue level CGA 321113 (mg/kg)	Report-No.
wheat	EU-N	EC	2 x 187.5, BBCH 71, PHI 34 d	grain straw	<0.02 0.73	<0.02 0.21	gr31196 M-037187-01-1
wheat	EU-N	EC	2 x 187.5, BBCH 71, PHI 35 d	grain straw	<0.02 0.07	<0.02 <0.05	gr3195 (gr42395) M-037237-02-1
wheat	EU-N	EC	2 x 187.5, BBCH 65, PHI 36 d	grain straw	<0.02 0.35	<0.02 0.07	gr3195 (gr12395) M-037237-02-1
wheat	EU-N	EC	2 x 250, BBCH 71, PHI 34 d	grain straw	0.02 0.85	<0.02 0.43	gr35196 M-037252-02-1
barley	EU-N	EC	2 x 187.5, BBCH 73, PHI 35 d (42 d)	grain straw	0.07 0.32	0.02 0.36 (0.38)	gr33696 M-035384-01-1
barley	EU-N	EC	2 x 187.5, BBCH 75, PHI 35 d (42 d)	grain straw	0.03 0.17 (0.23)	<0.02 0.05 (0.07)	gr3295 M-035410-02-1
barley	EU-N	EC	2 x 250, BBCH 83, PHI 35 d (42 d)	grain straw	<0.02 (0.02) 0.68	<0.02 0.12	gr37296 M-035500-01-1

EU-N: northern Europe FL = formulation TFS = trifloxystrobin

Additional trials are available conducted in northern Europe at the respective GAP. These trials are summarised below, but were also already submitted in October 2008 to CRD to support the assessment of all existing MRLs in accordance with "Article 12(2) of regulation (EC) No 396/2005".

For wheat and barley grain the supplementary trials show higher trifloxystrobin residues (wheat grain up to 0.14 mg/kg, barley grain up to 0.40 mg/kg) than the trials evaluated for Annex I inclusion under Directive 91/414/EEC. For barley grain nevertheless the PHI 35 result of trial gr37399 seems questionable, since residues at day 42 after last application are below LOQ and residues in ear at day 13 and 20 are lower than the grain results at day 35. In addition it has to be noted that the last application was quite late (BBCH 83). The results of the other barley trials are in line with the already evaluated trials (northern and southern Europe).

Further residue trials with trifloxystrobin in cereals that were conducted in 2013 and will include isomer analysis will be submitted when available.
The additional cereal trials including isomer analysis are summarised further down (highlighted in yellow) in this addendum document.

Supplementary wheat trials (northern Europe)

Report:	**KCA 6.10/08, [redacted]; 1998**; M-069205-01-1
Title:	Residues of CGA 279202 + CGA 321113 in winter wheat (test product: NAD 21180 F - A9604A, EC 125)
Document No & Report No:	M-069205-01-1 GR49197
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.10/09, [redacted]; 2000**; M-054730-02-1
Title:	Determination of CGA 279202 and the metabolite CGA 321113 in spring wheat
Document No & Report No:	M-054730-02-1 GR38499
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.10/10, [redacted]; 2001**; M-030968-01-1
Title:	Determination of residues of CGA 279202 and the metabolite CGA 321113 in winter wheat
Document No & Report No:	M-030968-01-1 gr 57100
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.10/11, [redacted]; 2001** ; M-030971-01-1
Title:	Determination of residues of CGA 279202 and the metabolite CGA 321113 in winter wheat
Document No & Report No:	M-030971-01-1 gr 58200
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Test system

In 1997 to 2000 four trials were performed in northern Europe on wheat with trifloxystrobin EC or SC formulations. The products were applied two times to wheat at application rates of 0.25 kg trifloxystrobin/ha.
Grain samples were taken on day 34 to 36 after the last application in all trials. In two trials, additional samples of grain were taken at later time points.

Residues of trifloxystrobin and CGA 321113 were determined according to method REM 177.03. The limit of quantitation was 0.01, 0.02 or 0.05 mg/kg for grain, plant or straw.

Findings

- Storage stability: The maximum storage period of deep-frozen samples was up to 211 days for trifloxystrobin and CGA 321113 and is covered by the storage stability studies.

- Residue results:
Residues of trifloxystrobin in grain at day 34-36 after last application ranged between <0.02 and 0.14 mg/kg. Residues of CGA 321113 were between <0.01 and 0.061 in grain at day 34-36 after last application.

Residues of trifloxystrobin in straw at day 34-36 after last application ranged between 0.31 and 1.81 mg/kg. Residues of CGA 321113 were between 0.07 and 0.82 (0.88) mg/kg in straw at day 34-36 (42) after last application.

Table 6.10-21: Application data and residues of trifloxystrobin and CGA 321113 in/ on wheat in northern Europe treated with Trifloxystrobin EC or SC formulations

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Residues Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
gr49197 BRD-gr49197 GLP: yes 1997	Wheat, winter Ritmo	Germany [redacted] Europe, North	125 EC	2	0.250	0.0625	75	whole plant without roots	0* 0	<0.02 4.30	0.02 0.06
								ear	14 21	0.79 0.24	0.06 0.05
								rest of plant	14 21	1.86 0.29	0.59 0.10
								grain	**35** **42**	0.02 0.05	<0.02 0.02
								straw	**35** 42	1.81 1.43	0.82 0.88
gr38499 BRD-2144-99 GLP: yes 1999	Wheat, spring Hanno	Germany [redacted] Europe, North	125 EC	2	0.250	0.0625	71	whole plant without roots	0	3.32	0.09
								ear	13 22	0.04 0.02	<0.02 <0.02
								rest of plant	13 22	0.51 0.31	0.05 0.05
								grain	**34** 41	<0.02 <0.02	<0.02 <0.02
								straw	**34** 41	0.31 0.18	0.07 <0.05
gr57100 BRD-gr57100 GLP: yes 2000	Wheat, winter Dekan	Germany [redacted] Europe, North	500 SC	2	0.250	0.0625	75	whole plant without roots	0	3.6/ 0.017**	0.048
								grain	**35**	0.14	0.061
								straw	**35**	1.3/ 0.1**	0.31
gr58200 BRD-gr58200 GLP: yes 2000	Wheat, winter Ritmo	Germany [redacted] Europe, North	500 SC	2	0.250	0.0625	75	whole plant without roots	0	3.0	<0.01
								grain	**36**	0.024	<0.01
								straw	**36**	1.6	0.14

FL: Formulation No: number of applications GS = growth stage (BBCH code) at application
DALT = days after last treatment * prior to last treatment ** residues in control

Supplementary barley trials (northern Europe)

Report:	**KCA 6.10/12, [redacted]; 2001** ; M-022006-01-1
Title:	Residue study with CGA 279202 in or on spring barley in France (North)
Document No & Report No:	M-022006-01-1 2022/99
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.10/13, [redacted]; 1999** ; M-057584-01-1
Title:	Residue study with CGA 279202 + cyproconazole in or on barley in North of France
Document No & Report No:	M-057584-01-1 9813201
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.10/14, [redacted]; 2000** ; M-055021-02-1
Title:	Determination of residues of CGA 279202 and the metabolite CGA 321113 in spring barley
Document No & Report No:	M-055021-02-1 GR35199
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.10/15, [redacted]; 2000** ; M-054967-02-1
Title:	Determination of residues of CGA 279202 and the metabolite CGA 321113 in winter barley
Document No & Report No:	M-054967-02-1 GR37399
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Report:	**KCA 6.10/16, [redacted]; 2001** ; M-030958-01-1
Title:	Determination of residues of CGA 279202 and the metabolite CGA 321113 in winter barley
Document No & Report No:	M-030958-01-1 gr 99100
Guidelines:	EU Council Directive 91/414/EEC Annex II, part A section 6 and Annex III, part A, section 8 residues in or on treated products, food and feed
GLP	yes

Test system

In 1998 to 2000 five residue trials were performed in northern Europe on barley with trifloxystrobin EC or SC formulations. The products were applied two times to barley at application rates of 0.19 to 0.26 kg trifloxystrobin/ha.
Grain samples were taken on day 34 to 39 after the last application in all trials. In three trials, additional samples of fruit were taken at later time points.

Residues of trifloxystrobin and CGA 321113 were determined according to method REM 177.03. The limit of quantitation was 0.01, 0.02 or 0.05 mg/kg for grain, plant or straw.

Findings

- Storage stability: The maximum storage period of deep-frozen samples was up to 386 days for trifloxystrobin and CGA 321113 and is covered by the storage stability studies.

- Residue results:
Residues of trifloxystrobin in grain at day 34-39 after last application ranged between <0.02 and 0.40 mg/kg. Residues of CGA 321113 were between <0.02 and 0.06 in grain at day 34-39 after last application.

Residues of trifloxystrobin in straw at day 34-39 after last application ranged between <0.05 and 1.58 mg/kg. Residues of CGA 321113 were between <0.05 and 0.42 in straw at day 34-39 after last application.

Table 6.10-22: Application data and residues of trifloxystrobin and CGA 321113 in/ on barley in northern Europe treated with Trifloxystrobin EC or SC formulations

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
2022/99 FRA-2022-99 GLP: yes 1999	Barley, spring Prisma	France [redacted] Europe, North	125 EC	2	0.248-0.261	0.0624-0.0625	59	grain	**39** **39**	0.044 0.055	0.010 0.014
9813201 FRA-9813201 GLP: yes 1998	Barley, winter Esterel	France [redacted] Europe, North	267.5 EC	2	0.1903 - 0.1927	0.04679 - 0.04681	51	grain straw	**35** **35** **47** **47** **35** **35** **47** **47**	<0.02 <0.02 <0.02 <0.02 <0.05 <0.05 0.09 0.09	<0.02 <0.02 <0.02 <0.02 <0.05 <0.05 <0.05 <0.05
gr35199 BRD-2141-99 GLP: yes 1999	Barley, spring Henni	Germany [redacted] Europe, North	125 EC	2	0.250	0.063	69	whole plant without roots ear rest of plant grain straw	0 13 20 13 20 **34** 41 **34** **41**	3.12 0.25 0.12 0.72 0.55 0.05 0.04 0.35 0.49	0.05 0.03 0.03 0.06 0.09 0.02 0.03 0.18 0.18
gr37399 BRD-2143-99 GLP: yes 1999	Barley, winter Theresa	Germany [redacted] Europe, North	125 EC	2	0.250	0.063	83	whole plant without roots ear rest of plant grain straw	0 13 20 13 20 **35** 42 **35** 42	5.40 0.32 0.28 0.63 0.51 0.40 <0.02 1.58 <0.05	0.07 0.05 0.05 0.15 0.16 0.06 <0.02 0.29 <0.05

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
gr59100 BRD-gr59100 GLP: yes 2000	Barley, winter[redacted] Theresa	Germany [redacted] Europe, North	500 SC	2	0.250	0.0625	73	whole plant without roots grain straw	0 34 34	5.5* 0.027** 0.12 1.1	<0.01 <0.01 0.42

FL: Formulation No: number of applications
GS = growth stage (BBCH code) at application DALT = days after last treatment
* prior to last treatment ** residues in control

Supplementary wheat trials (northern and southern Europe) – including isomer analysis

Report:	**KCA 6.10/17, [redacted]; [redacted]; 2014** ; M-485198-01-1
Title:	Determination of the residues of cyproconazole and trifloxystrobin in/on wheat after spray application of cyproconazole & trifloxystrobin SC 535 in the field in United Kingdom, Germany, Italy and Spain
Document No & Report No:	M-485198-01-1 13-2085
Guidelines:	Regulation (EC) No 1107/2009 EC guidance working document 7035/VI/95 rev.5 OECD 509, OECD guideline for the testing of chemicals, Crop Field Trial
GLP	yes

Test system

In 2013 two trials were performed in northern Europe and 2 trials in southern Europe on wheat with trifloxystrobin SC formulation (mixture with cyproconazole). The product was applied two times to wheat at application rates of 0.1875 kg trifloxystrobin/ha.
Grain samples were taken on day 35 to 36 after the last application in all trials. In three trials, additional samples of grain were taken when the grain was ripe for harvest (day 51 to 62 after last application).

Residues of trifloxystrobin, its isomers CGA 357261, CGA 357262, CGA 373466, its metabolite CGA 321113 and isomer CGA 373466 were determined according to method 01313/M001. The analytical method was validated by recovery experiments prior to and during the analysis of the samples by spiking control samples with all analytes. The limit of quantitation was 0.01 in all cases.

Findings

- Method performance: Overall mean recoveries at fortification levels between 0.01 and 0.10 (10.0) mg/kg were within the acceptable range of 70-110 %, RSD <20%.

- Storage stability: The maximum storage period of deep-frozen samples was up to 273 days for trifloxystrobin, CGA 321113, CGA 357261, CGA 357262, CGA 331409 and CGA 373466 and is covered by the storage stability studies.

- Residue results:
Residues of trifloxystrobin in grain at day 35 after last application or at harvest were <0.01 mg/kg, except in one trial where residues at 0.020 mg/kg were found. Residues of CGA 321113, CGA 357261, CGA 357262, CGA 331409 and CGA 373466 were below LOQ in grain at day 35/36 after last application or at harvest.
In straw residues of trifloxystrobin ranged between 0.20 and 7.6 mg/kg at day 35 after last application or at harvest. Residues of CGA 321113 were between 0.010 and 0.10 mg/kg in straw. Residues of CGA 357261 were between <0.01 and 0.27 mg/kg in straw. Residues of CGA 357262 were between <0.01 and 0.13 mg/kg in straw. Residue of CGA 331409 were between 0.018 and 0.13 mg/kg in straw. Residues of CGA 373466 were between <0.01 and 0.041 mg/kg in straw.

Table 6.10-23: Application data and residues of trifloxystrobin and CGA 321113 in/ on wheat treated with a Trifloxystrobin SC formulation in the field in northern and southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application						Residues			
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
13-2085 13-2085-01 13-2085-01-T GLP: yes 2013	Wheat Alderon; Spring wheat	United Kingdom [redacted] Europe, North	535 SC	2	0.1875	0.094	69	green material grain straw	0 14 35 35	4.4 2.9 0.020 7.6	<0.01 <0.01 <0.01 0.042
13-2085 13-2085-02 13-2085-02-T GLP: yes 2013	Wheat Winne-tou; Winter wheat	Germany [redacted] Europe, North	535 SC	2	0.1875	0.062	69	green material grain straw	0 9 35 61 35 61	4.6 0.69 <0.01 <0.01 0.42 0.26	0.015 <0.01 <0.01 <0.01 0.093 0.10
13-2085 13-2085-03 13-2085-03-T GLP: yes 2013	Wheat Forblanc ; white variety	Italy [redacted] (Ferrara) Europe, South	535 SC	2	0.1875	0.047	65	green material grain straw	0 17 36 51 36 51	5.5 0.17 <0.01 <0.01 0.20 0.23	0.017 <0.01 <0.01 <0.01 0.034 0.036

13-2085 13-2085-04 13-2085-04-T GLP: yes 2013	Wheat Avispa; Durum wheat	Spain [redacted] Europe, South	535 SC	2	0.1875	0.062	65	green material grain straw	0 14 35 62 35 62	6.9 2.3 <0.01 <0.01 2.9 2.2	<0.01 <0.01 <0.01 <0.01 0.012 0.010

FL: Formulation No: number of applications GS = growth stage (BBCH code) at application
DALT = days after last treatment

Table 6.10-24: Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in/ on wheat treated with a Trifloxystrobin SC formulation in the field in northern and southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Portion analysed	DALT (days)	Residues CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
13-2085 13-2085-01 13-2085-01-T GLP: yes 2013	Wheat Alderon; Spring wheat	United Kingdom [redacted] Europe, North	green material grain straw	0 14 35 35	0.027 0.061 <0.01 0.27	<0.01 0.032 <0.01 0.13	<0.01 <0.01 <0.01 0.13	<0.01 <0.01 <0.01 0.017
13-2085 13-2085-02 13-2085-02-T GLP: yes 2013	Wheat Winnetou; Winter wheat	Germany [redacted] Europe, North	green material grain straw	0 9 35 61 35 61	<0.01 0.023 <0.01 <0.01 0.019 0.019	<0.01 0.015 <0.01 <0.01 0.015 0.016	<0.01 0.024 <0.01 <0.01 0.030 0.023	<0.01 <0.01 <0.01 <0.01 0.032 0.041
13-2085 13-2085-03 13-2085-03-T GLP: yes 2013	Wheat Forblanc; white variety	Italy [redacted] (Ferrara) Europe, South	green material grain straw	0 17 36 51 36 51	<0.01 0.014 <0.01 <0.01 <0.01 0.020	<0.01 0.012 <0.01 <0.01 <0.01 0.011	<0.01 0.016 <0.01 <0.01 0.018 0.018	<0.01 <0.01 <0.01 <0.01 <0.01 0.012
13-2085 13-2085-04 13-2085-04-T GLP: yes 2013	Wheat Avispa; Durum wheat	Spain [redacted] Europe, South	green material grain	0 14 35 62	0.035 0.096 <0.01 <0.01	0.025 0.060 <0.01 <0.01	<0.01 0.065 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01

			straw	35 62	0.22 0.26	0.12 0.13	0.10 0.11	<0.01 <0.01

DALT = days after last treatment

Supplementary barley trials (northern and southern Europe) – including isomer analysis

Report:	**KCA 6.10/18, [redacted]; [redacted]; 2014** ; M-485010-02-1
Title:	Determination of the residues of cyproconazole and trifloxystrobin in/on barley after spraying application of Cyproconazole & Trifloxystrobin SC 535 in the United Kingdom, Germany and southern France
Document No & Report No:	M-485010-02-1 13-2084
Guidelines:	Regulation (EC) No 1107/2009 EC guidance working document 7035/VI/95 rev.5 OECD 509, OECD guideline for the testing of chemicals, Crop Field Trial
GLP	yes

Test system

In 2013 two trials were performed in northern Europe and 1 trial in southern Europe on barley with trifloxystrobin SC formulation (mixture with cyproconazole). One additional trial conducted in southern Europe had to be cancelled (application not according GAP) and was repeated in 2014, but results are not yet available.
The product was applied two times to barley at application rates of 0.1875 kg trifloxystrobin/ha.
Grain or ear samples were taken on day 35 to 36 after the last application in all trials. In two trials, additional samples of grain were taken when the grain was ripe for harvest (day 48 or to 59 after last application).

Residues of trifloxystrobin, its isomers CGA 357261, CGA 357262, CGA 373466, its metabolite CGA 321113 and isomer CGA 373466 were determined according to method 01313/M001. The analytical method was validated by recovery experiments prior to and during the analysis of the samples by spiking control samples with all analytes. The limit of quantitation was 0.01 in all cases.

Findings

- Method performance: Overall mean recoveries at fortification levels between 0.01 and 0.10 (10.0) mg/kg were within the acceptable range of 70-110 %, RSD ≤20%..

- Storage stability: The maximum storage period of deep-frozen samples was up to 255 days for trifloxystrobin, CGA 321113, CGA 357261, CGA 357262, CGA 331409 and CGA 373466 and is covered by the storage stability studies.

- Residue results:
Residues of trifloxystrobin in grain or ear at day 35 (36) after last application were between 0.025 and 0.051 mg/kg.. Residues of CGA 321113, CGA 357262, CGA 331409 and CGA 373466 were below LOQ in grain and ear at day 35/36 after last application or at harvest. Residues of CGA 357261were between <0.01 mg/kg and 0.014 mg/kg in grain.
In straw residues of trifloxystrobin ranged between 0.12 and 7.0 mg/kg at day 35 (36) after last application or at harvest. Residues of CGA 321113 were between <0.01 and 0.12 mg/kg in straw. Residues of CGA 357261 were between 0.013 and 0.49 mg/kg in straw. Residues of CGA 357262 were between <0.01 and 0.18 mg/kg in straw. Residue of CGA 331409 were between <0.01 and 0.28 mg/kg in straw.
Residues of CGA 373466 were between <0.01 and 0.061 mg/kg in straw.

Table 6.10-25: Application data and residues of trifloxystrobin and CGA 321113 in/ on barley treated with a Trifloxystrobin SC formulation in the field in northern and southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Trifloxy-strobin (mg/kg)	CGA 321113 (mg/kg)
13-2084 13-2084-01 13-2084-01-T GLP: yes 2013	Barley Irina; Spring barley	United Kingdom [redacted] Europe, North	535 SC	2	0.1875	0.094	61	green material	0 18	12 2.7	<0.01 0.016
								grain	36	0.051	<0.01
								straw	36	7.0	0.12
13-2084 13-2084-02 13-2084-02-T GLP: yes 2013	Barley Duett	Germany [redacted] Europe, North	535 SC	2	0.1875	0.062	61	green material	0 13 27	4.5 4.3	<0.01 <0.01
								grain	35 48	0.027 0.020	<0.01 <0.01
								straw	35 48	0.31 0.12	0.021 <0.01
13-2084 13-2084-04 13-2084-04-T GLP: yes 2013	Barley Cervoise Winter barley	France [redacted] Europe, South	535 SC	2	0.1875	0.062	61	green material	0 27	3.8 0.29	<0.01 <0.01
								ear	35	0.025	<0.01
								rest of plant	35	0.53	<0.01
								grain	59	<0.01	<0.01
								straw	59	0.39	0.014

FL: Formulation No: number of applications GS = growth stage (BBCH code) at application
DALT = days after last treatment

Table 6.10-26: Residues of CGA 357261, CGA 357262, CGA 331409 and CGA 373466 in/ on barley treated with a Trifloxystrobin SC formulation in the field in northern and southern Europe

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Portion analysed	DALT (days)	Residues CGA 357261 (mg/kg)	CGA 357262 (mg/kg)	CGA 331409 (mg/kg)	CGA 373466 (mg/kg)
13-2084 13-2084-01 13-2084-01-T GLP: yes 2013	Barley Irina; Spring barley	United Kingdom [redacted] Europe, North	green material	0	0.062	<0.01	<0.01	<0.01
				18	0.082	0.025	0.061	<0.01
			grain	36	0.014	<0.01	<0.01	<0.01
			straw	36	0.49	0.18	0.28	0.061
13-2084 13-2084-02 13-2084-02-T GLP: yes 2013	Barley Duett	Germany [redacted] Europe, North	green material	0	0.018	<0.01	<0.01	<0.01
				13	0.057	0.023	0.044	<0.01
			grain	35	<0.01	<0.01	<0.01	<0.01
				48	<0.01	<0.01	<0.01	<0.01
			straw	35	0.036	0.020	0.026	<0.01
				48	0.013	<0.01	<0.01	<0.01
13-2084 13-2084-04 13-2084-04-T GLP: yes 2013	Barley Cervoise Winter barley	France [redacted] Europe, South	green material	0	0.020	0.011	<0.01	<0.01
				27	0.025	0.010	0.016	<0.01
			ear	35	<0.01	<0.01	<0.01	<0.01
			rest of plant	35	0.027	0.016	0.025	<0.01
			grain	59	<0.01	<0.01	<0.01	<0.01
			straw	59	0.045	0.019	0.038	<0.01

DALT = days after last treatment

CA 6.10.1 Effect on the residue level in pollen and bee products

The objective of such studies would be to determine the residues in pollen and bee products for human consumption resulting from residues taken up by honeybees from crops at blossom.

Since an official published guideline for such studies is not yet available, no relevant study was conducted for trifloxystrobin so far.