FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: A18385B

Product name: SPANDIS

Chemical active substances:

Dicamba, 400 g/kg
Nicosulfuron, 100 g/kg
Prosulfuron, 40 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(new authorization)

Applicant: Syngenta

Submission date: xx/11/2020

MS Finalisation date: 11/07/2022

Version history

When	What
February 2021	Dossier sent for evaluation
November 2021	Updates following request of Poland (zRMS)
April 2022	zRMS evaluation of dRR
July 2022	Final version prepared by zRMS after Commenting period

Table of Contents

zRMS comments:
The text highlighted in grey was provided by the evaluator.

5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are ~~not~~ available for the active substance(s) and relevant impurities in the plant protection product.

Noticed data gaps are: none

- ~~data gap 1~~
- ~~data gap 2~~
- ~~data gap 3~~

In the context of the authorisation request sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.

The text of the applicant was not rewritten. The evaluator text is on grey background. The submitted data are sufficient for the evaluation.

The intended GAP is a combination of the representative GAPs of prosulfuron, nicosulfuron and dicamba. All analytical pre- and post-authorisation methods for prosulfuron, nicosulfuron and dicamba were evaluated during the EU review and considered sufficient and acceptable. However, the applicant submitted also some acceptable supplementary validation data.

Noticed data gaps are: in the context of the authoristation request – none.

Commodity/crop	Supported/ Not supported
Maize	Supported

5.2 Methods used for the generation of pre-authorization data (KCP 5.1)

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

5.2.1.1 Determination of active substance and/or variant in the plant protection product (KCP 5.1.1)

An overview on the acceptable methods and possible data gaps for analysis of prosulfuron, nicosulfuron and dicamba in plant protection product A18385B is provided as follows:

Comments of zRMS:	The method is accepted and can be applied for analysing prosulfuron, nicosulfuron and dicamba in the PPP.

Reference:	KCP 5.1.1/01
Report	Analytical method SF-570/1, Dos Santos Alves A.M., 2012, Report No. 10494732, Syngenta File No. A18385B_10044; VV-128246
Guideline(s):	No (method technical procedure)
Deviations:	N/A
GLP:	No (method technical procedure)
Acceptability:	Yes

Reference:	KCP 5.1.1/02

Report	Prosulfuron / Dicamba / Nicosulfuron A18385B – Validation of Analytical Method SF-570/1, De Benedictis S., 2014, Report No. 126609, Syngenta File No. A18385B_10045; VV-406938
Guideline(s):	SANCO 3030
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

An analytical method has been developed for the determination of the active substances prosulfuron, dicamba and nicosulfuron in A18385B.

Prosulfuron, dicamba and nicosulfuron are determined simultaneously by HPLC on a Nucleosil C18 column (column length 75 mm, column internal diameter 4.6 mm). Elution was by a 0.1% aqueous phosphoric acid and acetonitrile gradient. Detection was spectrophotometrically by an UV detector operating at 240 nm. Quantification was obtained by comparing peak areas of test samples with the areas from calibrated analytical standard solutions (external standard).

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances prosulfuron, nicosulfuron and dicamba in plant protection product A18385B

	Prosulfuron	**Nicosulfuron**	**Dicamba**
Author(s), year	De Benedictis S., 2014		
Principle of method	HPLC-UV		
Linearity (linear between 50-150% of the declared content) (correlation coefficient, expressed as r)	The linearity was tested using six samples (two determinations of each) of spiked formulation blank.		
	0.99996	0.99995	0.99993
Precision – Repeatability Mean n = ~~10~~6 (% RSD)	0.45%	0.61%	0.43%
Horrat value (H_r)*	0.2	0.3	0.3
Accuracy n = 4, spiked with 70-130% of the nominal amounts of the active substances (% Recovery)	98.3%	98.9%	100.4%
Interference/ Specificity	An examination of the chromatograms for A18385B, prosulfuron technical, dicamba technical, nicosulfuron technical and formulation blank showed no significant co-elution between the active ingredients and the formulation components. The analytical method is able to separate the active substances prosulfuron, dicamba and nicosulfuron from the formulation blank and solvent with no significant co-elution.		
Comment	-	-	-

* Not given in report; calculated with % RSD, the mean % AI found values in Tables 1-3 of the report and the equations given in SANCO/3030/99 rev. 5, Appendix 2

Conclusion

The method is suitable for the specific, accurate and precise determination of prosulfuron, dicamba and nicosulfuron in A18385B.

5.2.1.2 Description of analytical methods for the determination of relevant impurities (KCP 5.1.1)

Nicosulfuron: There are no relevant impurities and therefore no methods are required.

Dicamba: There are no relevant impurities and therefore no methods are required.

Prosulfuron: Impurity CGA 159902 (2-(3,3,3-trifluoro-propyl)-benzenesulfonamide) is considered as a relevant impurity as explained in Volume 4 of the RAR (France, 2014). Nevertheless, as this is a starting material of the manufacturing process of the technical active substance, this impurity is not formed during manufacture or storage of the formulations hence no analytical method is required for its determination in formulations. EFSA agreed to this assessment in their conclusion on the peer review (EFSA, 2014).
However, an analytical method for the determination of CGA159902 has been developed and validated and is described in the following.

Comments of zRMS:	This method meet all the requirements and can be used for analysing the impurity in this PPP.

Reference:	KCP 5.1.1/03
Report	Determination of Prosulfuron Relevant Impurity CGA159902 in Formulation by HPLC, De Benedictis S., 2013, Report No. SD-1693/1, Syngenta File No.: A18385B_10031; VV-128236
Guideline(s):	No (method technical procedure)
Deviations:	N/A
GLP:	No (method technical procedure)
Acceptability:	Yes

Reference:	KCP 5.1.1/04
Report	A18385B – Validation of Analytical Method SD-1693/1 for the Determination of CGA159902 in Formulation Containing Prosulfuron, De Benedictis S., 2013a, Report No. 126361, Syngenta File No.: A18385B_10030; VV-406129
Guideline(s):	SANCO 3030/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The relevant impurity CGA159902 is determined in prosulfuron formulations by HPLC using a Phenomenex Kinetex PFP column (2.6 µm particle size, length: 150 mm; column Internal diameter 4.6 mm) using a methanol/acetonitrile/1% phosphoric acid gradient with UV detection at 216 nm.

Quantification is by comparison of peak areas ratios to those of a reference solution.

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) A18385B

	CGA159902
Author(s), year	De Benedictis S., 2013
Principle of method	HPLC-UV
Linearity (correlation coefficient, expressed as r)	The linearity was tested with spiked formulation blank also containing dicamba and nicosulfuron technical material using pure reference substance of CGA159902 at six levels (two injections each) over the range of 0.1% to 1.36% for CGA159902 relative to the amount of prosulfuron. The coefficient of variation was calculated: 0.99841
Precision – Repeatability Mean (% RSD) **Horrat value (H_r)***	The repeatability was tested with twelve determinations (six weights, double injection each) of CGA159902 in formulation A18385B. mean [0.296% w/w]: 1.69% 0.5
Accuracy (% Recovery)	The recovery was tested with spiked formulation blank also containing dicamba and nicosulfuron technical materials, using pure reference substance of CGA159902 at six levels (two injections each) over the range of 0.1 % to 1.36 % relative to the amount of prosulfuron. The mean recovery for CGA159902was determined: 95%
Interference/ Specificity	No significant interference was observed. The analytical method is able to separate the impurity CGA159902 from the active ingredients, the formulation blank and solvent with no significant co-elution.
LOQ	The Limit of Quantification (LOQ) is considered to be 0.05% relative to prosulfuron.

* Not given in report; calculated with % RSD, the mean % w/w value and the equations given in SANCO/3030/99 rev. 5, Appendix 2

Conclusion

The method is suitable for the specific, accurate and precise determination of the relevant impurity CGA159902 in A18385B.

5.2.1.3 Description of analytical methods for the determination of formulants (KCP 5.1.1)

There are no formulants or constituents of formulants within the preparation or formed during storage, that are of toxicological, ecotoxicological or environmental relevance. Therefore, this point is not relevant.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

No CIPAC method is available for the determination of prosulfuron, dicamba and nicosulfuron in mixed WG formulations such as A18385B.

No CIPAC method is available for the determination of prosulfuron in WG formulations.

A CIPAC method is available for the determination of nicosulfuron in WG formulations. Nicosulfuron is determined by high performance liquid chromatography using a Zorbax® SB column, UV detection at 245 nm and internal standardisation (3-methyl-1,1-diphenylurea). The active substance content is quantified using a calibration curve. (CIPAC Handbook M, page 21)

A CIPAC method is available for the determination of dicamba in WG formulations. Dicamba is dis-

solved in methanol and determined by high performance liquid chromatography on a reversed phase column (RP18) using UV detection and external standardisation. (CIPAC Handbook K, page 32)

5.2.2 Methods for the determination of residues of prosulfuron (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of prosulfuron for the generation of pre-authorization data is given in the following table. Note that only analytical methods used in new residue trials reported in the framework of this application are listed.

Table 5.2-3: Validated methods for the generation of pre-authorization data for prosulfuron (KCP 5.1.2.1 in support of environmental fate studies)

Table not included;

No specific analytical methods were used to support the environmental fate data generated on this product.

Table 5.2-4: Validated methods for the generation of pre-authorization data for prosulfuron (KCP 5.1.2.2 in support of efficacy studies)

Table not included;

No specific analytical methods were used to support the efficacy data generated on this product.

Table 5.2-5: Validated methods for the generation of pre-authorization data for prosulfuron (KCP 5.1.2.3 in support of toxicological studies)

Table not included;

No analytical methods were used to support the toxicology data generated on this product.

Table 5.2-6: Validated methods for the generation of pre-authorization data for prosulfuron (KCP 5.1.2.4 in support of operator, worker, resident and bystander exposure studies)

Table not included;

No specific operator, worker, resident or bystander exposure studies were conducted to support this product. Consequently no analytical methods were required.

Table 5.2-7: Validated methods for the generation of pre-authorization data for prosulfuron in plant and animal products (KCP 5.1.2.5 in support of residue studies)

Component of residue definition: prosulfuron				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
No new residue trials are reported in the framework of this application, thus all relevant methods for data generation of pre-authorization data have already been evaluated on EU level.				

Table 5.2-8: Validated methods for the generation of pre-authorization data for prosulfuron in test media (KCP 5.1.2.6 in support of ecotoxicological studies)

Component of residue definition: prosulfuron				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
-	Test media	1.99 µg	LC-MS/MS	Method & Validation:

Component of residue definition: prosulfuron				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	(algae)	prosulfuron/L		Liedtke, 2013 Report D75032 EU agreed (zonal level for authorization of A18385B)
-	Test media (*Lemna*)	0.0489 µg prosulfuron/L	LC-MS/MS	Method & Validation: Liedtke, 2013a Report D75010 EU agreed (zonal level for authorization of A18385B)

Table 5.2-9: Validated methods for the generation of pre-authorization data for prosulfuron (KCP 5.1.2.7 in support of physical and chemical properties tests)

Table not included;

No specific analytical methods were used to support the physical and chemical properties generated on this product.

5.2.3 Methods for the determination of residues of nicosulfuron (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of nicosulfuron for the generation of pre-authorization data is given in the following table. Note that only analytical methods used in new residue trials reported in the framework of this application are listed.

Table 5.2-10: Validated methods for the generation of pre-authorization data for nicosulfuron (KCP 5.1.2.1 in support of environmental fate studies)

Table not included;

No specific analytical methods were used to support the e environmental fate studies generated on this product.

Table 5.2-11: Validated methods for the generation of pre-authorization data for nicosulfuron (KCP 5.1.2.2 in support of efficacy studies)

Table not included;

No specific analytical methods were used to support the efficacy data generated on this product.

Table 5.2-12: Validated methods for the generation of pre-authorization data for nicosulfuron (KCP 5.1.2.3 in support of toxicological studies)

Table not included;

No analytical methods were used to support the toxicology data generated on this product.

Table 5.2-13: Validated methods for the generation of pre-authorization data for nicosulfuron (KCP 5.1.2.4 in support of operator, worker, resident and bystander exposure studies)

Table not included;

No specific operator, worker, resident or bystander exposure studies were conducted to support this product. Consequently no analytical methods were required.

Table 5.2-14: Validated methods for the generation of pre-authorization data for nicosulfuron in plant and animal products (KCP 5.1.2.5 in support of residue studies)

Component of residue definition: nicosulfuron				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
No new residue trials are reported in the framework of this application, thus all relevant methods for data generation of pre-authorization data have already been evaluated on EU level.				

Table 5.2-15: Validated methods for the generation of pre-authorization data for nicosulfuron (KCP 5.1.2.6 in support of ecotoxicological studies)

Table not included;

No specific analytical methods were used to support the ecotoxicology data generated on this product.

Table 5.2-16: Validated methods for the generation of pre-authorization data for nicosulfuron (KCP 5.1.2.7 in support of physical and chemical properties tests)

Table not included;

No specific analytical methods were used to support the physical and chemical properties generated on this product.

5.2.4 Methods for the determination of residues of dicamba (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of dicamba for the generation of pre-authorization data is given in the following table. Note that only analytical methods used in new residue trials reported in the framework of this application are listed.

Table 5.2-17: Validated methods for the generation of pre-authorization data for dicamba (KCP 5.1.2.1 in support of environmental fate studies)

Table not included;

No specific analytical methods were used to support the environmental fate studies generated on this product.

Table 5.2-18: Validated methods for the generation of pre-authorization data for dicamba (KCP 5.1.2.2 in support of efficacy studies)

Table not included;

No specific analytical methods were used to support the efficacy data generated on this product.

Table 5.2-19: Validated methods for the generation of pre-authorization data for dicamba (KCP 5.1.2.3 in support of toxicological studies)

Table not included;

No analytical methods were used to support the toxicology data generated on this product.

Table 5.2-20: Validated methods for the generation of pre-authorization data for dicamba (KCP 5.1.2.4 in support of operator, worker, resident and bystander exposure studies)

Table not included;

No specific operator, worker, resident or bystander exposure studies were conducted to support this product. Consequently no analytical methods were required.

Table 5.2-21: Validated methods for the generation of pre-authorization data for dicamba in plant and animal products (KCP 5.1.2.5 in support of residue studies)

Component of residue definition: dicamba				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
REM 193.01 (post-authorization method, see 5.3)	High water content High acid content High oil content High protein/high starch content (dry)	0.01 mg/kg	GC-MS	Method: Gasser A., 1998 / Report No. REM 193.01 Validation: Maffezzoni M., 2004 / Report No. SYN/DIC/03041 EU agreed (Denmark, 2010)[(a)]
P-14.063.02	High protein/high starch content (dry)	0.01 mg/kg	GC-MS	Method: Schmidt F., 1994 / Report No. P-14.063.02 EU agreed (Denmark, 2010)
AM-0691B	High water content High protein/high starch content (dry)	0.01 mg/kg	GC-ECD GC-MS (confirmatory)	Method & Validation: Jimenez N., 1993 / AM-0691B-0593-3 EU agreed (Denmark, 2010)

(a): EFSA, 2011 identified a data gap as the hydrolysis step is not validated

For the additional residue trials reported in the framework of this application, residue analytical methods were used that have already been evaluated on EU level, i.e. REM 193.01 (post-registration method), P-14.063.02 or AM-0691B.

Table 5.2-22: Validated methods for the generation of pre-authorization data for dicamba (KCP 5.1.2.6 in support of ecotoxicological studies)

Table not included;

No specific analytical methods were used to support the ecotoxicology data generated on this product.

Table 5.2-23: Validated methods for the generation of pre-authorization data for dicamba (KCP 5.1.2.7 in support of physical and chemical properties tests)

Table not included;

No specific analytical methods were used to support the physical and chemical properties generated on this product.

5.3 Methods for post-authorization control and monitoring purposes (KCP 5.2)

5.3.1 Analysis of the plant protection product (KCP 5.2)

The methods already submitted in accordance with the requirements set out in point 5.2.1 can be applied.

5.3.2 Description of analytical methods for the determination of residues of prosulfuron (KCP 5.2)

5.3.2.1 Overview of residue definitions and levels for which compliance is required

Compared to the residue definition proposed in the Draft and Renewal Assessment Report (incl. its addenda) the current legal residue definition is identical.

Table 5.3-1: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Prosulfuron[(a)]	0.01 mg/kg	MRL (Reg. (EU) No 617/2014)
Plant, high acid content		0.01 mg/kg	MRL (Reg. (EU) No 617/2014)
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	MRL (Reg. (EU) No 617/2014)
Plant, high oil content		0.02 mg/kg	MRL (Reg. (EU) No 617/2014)
Muscle	Prosulfuron	0.02 mg/kg	MRL (Reg. (EU) No 617/2014)
Milk		0.02 mg/kg	MRL (Reg. (EU) No 617/2014)
Eggs		0.02 mg/kg	MRL (Reg. (EU) No 617/2014)
Fat		0.02 mg/kg	MRL (Reg. (EU) No 617/2014)
Liver		0.05 mg/kg	MRL (Reg. (EU) No 617/2014)
Kidney		0.02 mg/kg	MRL (Reg. (EU) No 617/2014)
Soil (Ecotoxicology)	Prosulfuron	0.131 mg/kg	NOEC for soil microoranisms (nitrogen mineralisation) (EFSA, 2014)
Drinking water (Human toxicology)	Prosulfuron	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Prosulfuron	0.016 mg/L	Biomass $E_bC_{50}$for *Pseudo-kirchneriella subcapitata* (72 h-static) (EFSA, 2014)
Air	Prosulfuron	1 µg/m^3	AOEL: 0.06 mg/kg bw/d (EFSA, 2014)
Tissue (meat or liver)	Prosulfuron	Not required	Not classified as T / T+
Body fluids		Not required	Not classified as T / T+

(a): Draft residue definition subject to the data gap on the genotoxicity of CGA150829 (EFSA, 2014)

5.3.2.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of prosulfuron in plant matrices is given in the following table.

Table 5.3-2: Validated methods for food and feed of plant origin

Component of residue definition: prosulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
High water content	Primary	0.01 mg/kg	LC-MS/MS	Method: McDonald T.J., 2011 / Report: GRM034.02A Validation: McDonald T.J., 2011 / Report: TK0039700 EU agreed (France, 2014)
	ILV	0.01 mg/kg	LC-MS/MS	Daneva E., Zetsch A., 2012 / Report: S11-03699 EU agreed (France, 2014)
	Confirmatory (if required)	-	-	Not required, primary method was confirmed by validation of two different mass transitions
High acid content	Primary	0.01 mg/kg	LC-MS/MS	Method: McDonald T.J., 2011 / Report: GRM034.02A Validation: McDonald T.J., 2011 / Report: TK0039700 EU agreed (France, 2014)
	ILV	0.01 mg/kg	LC-MS/MS	Daneva E., Zetsch A., 2012 / Report: S11-03699 EU agreed (France, 2014)
	Confirmatory (if required)	-	-	Not required, primary method was confirmed by validation of two different mass transitions
High oil content	Primary	0.01 mg/kg	LC-MS/MS	Method: McDonald T.J., 2011 / Report: GRM034.02A Validation: McDonald T.J., 2011 / Report: TK0039700 EU agreed (France, 2013)
	ILV	0.01 mg/kg	LC-MS/MS	Daneva E., Zetsch A., 2012 / Report: S11-03699 EU agreed (France, 2014)
	Confirmatory (if required)	-	-	Not required, primary method was confirmed by validation of two different mass transitions

Component of residue definition: prosulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
High protein/high starch content (dry)	Primary	0.01 mg/kg	LC-MS/MS	Method: McDonald T.J., 2011 / Report: GRM034.02A Validation: McDonald T.J., 2011 / Report: TK0039700 EU agreed (France, 2014)
	ILV	0.01 mg/kg	LC-MS/MS	Daneva E., Zetsch A., 2012 / Report: S11-03699 EU agreed (France, 2014)
	Confirmatory (if required)	-	-	Not required, primary method was confirmed by validation of two different mass transitions

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Not required, because:	Residues >LOQ are not expected in matrix types relevant for the target crops. Furthermore, recovery rates both in the primary method and in the ILV were in an acceptable range between 70 and 120%, and the method was considered acceptable in the peer review of renewal of approval.

5.3.2.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of prosulfuron in animal matrices is given in the following table.

Table 5.3-4: Validated methods for food and feed of animal origin

Component of residue definition: prosulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
Milk	Primary	0.01 mg/kg	LC-MS/MS	Method: Mayer L., 2011 / Report: GRM034.03A Validation: Mayer L., 2011 / Report: TK0039703 EU agreed (France, 2014)
	ILV	0.01 mg/kg	LC-MS/MS	Amic S., 2012 / Report: S11-03979 EU agreed (France, 2014)
	Confirmatory (if required)	-	-	Not required, primary method was confirmed by validation of two different mass transitions

Component of residue definition: prosulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
Eggs	Primary	0.01 mg/kg	LC-MS/MS	Method: Mayer L., 2011 / Report: GRM034.03A Validation: Mayer L., 2011 / Report: TK0039703 EU agreed (France, 2014)
	ILV	0.01 mg/kg	LC-MS/MS	Amic S., 2012 / Report: S11-03979 EU agreed (France, 2014)
	Confirmatory (if required)	-	-	Not required, primary method was confirmed by validation of two different mass transitions
Muscle	Primary	0.01 mg/kg	LC-MS/MS	Method: Mayer L., 2011 / Report: GRM034.03A Validation: Mayer L., 2011 / Report: TK0039703 EU agreed (France, 2014)
	Confirmatory (if required)	-	-	Not required, primary method was confirmed by validation of two different mass transitions
Fat	Primary	0.01 mg/kg	LC-MS/MS	Method: Mayer L., 2011 / Report: GRM034.03A Validation: Mayer L., 2011 / Report: TK0039703 EU agreed (France, 2014)
	Confirmatory (if required)	-	-	Not required, primary method was confirmed by validation of two different mass transitions
Kidney, liver	Primary	0.01 mg/kg	LC-MS/MS	Method: Mayer L., 2011 / Report: GRM034.03A Validation: Mayer L., 2011 / Report: TK0039703 EU agreed (France, 2014)
	ILV	0.01 mg/kg	LC-MS/MS	Amic S., 2012 / Report: S11-03979 EU agreed (France, 2014)
	Confirmatory (if required)	-	-	Not required, primary method was confirmed by validation of two different mass transitions

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Not required, because:	Residues >LOQ are not expected in animal matrices after feeding of prosulfuron-treated plantstuffs originating from the target crops. Furthermore, recovery rates both in the primary method and in the ILV were in an acceptable range between 70 and 120%, and the method was considered acceptable in the peer review of renewal of approval.

5.3.2.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of prosulfuron in soil is given in the following table.

Table 5.3-6: Validated methods for soil

Component of residue definition: prosulfuron			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
Primary	0.5 µg/kg	LC-MS/MS	Wiepke T., 1994 / Report: ABR-94055 EU agreed (France, 2014)
Confirmatory	0.5 µg/kg	LC-MS	Vargo. J.D., 1992 / Report: AG-600 EU agreed (France, 2014)

5.3.2.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of prosulfuron in ground, surface and drinking water is given in the following table.

Table 5.3-7: Validated methods for water

Component of residue definition: prosulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
Drinking water	Primary	0.05 µg/L	LC-MS/MS	Method: Richardson M., 2007 / Report: GRM034.01A Validation: Wiesner F., Gizler A., 2007 / Report: SYN-0729-V EU agreed (France, 2014)
	ILV	-	-	Not required, old data requirements apply
	Confirmatory	-	-	Not required, primary method was confirmed by validation of two different mass transitions
Surface water	Primary	0.05 µg/L	LC-MS/MS	Method: Richardson M., 2007 / Report: GRM034.01A Validation: Wiesner F., Gizler A., 2007 / Report: SYN-0729-

Component of residue definition: prosulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
				V EU agreed (France, 2014)
	Confirmatory	-	-	Not required, primary method was confirmed by validation of two different mass transitions
Ground water	Primary	0.05 µg/L	LC-MS/MS	Method: Richardson M., 2007 / Report: GRM034.01A Validation: Wiesner F., Gizler A., 2007 / Report: SYN-0729-V EU agreed (France, 2014)
	Confirmatory	-	-	Not required, primary method was confirmed by validation of two different mass transitions

5.3.2.6 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of prosulfuron in air is given in the following table.

Table 5.3-8: Validated methods for air

Component of residue definition: prosulfuron			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
Primary	1 µg/m^3	LC-MS/MS	Tummon O.J., 2004 / Report: RJ3551B EU agreed (France, 2014)
Confirmatory	-	-	Not available; however, a method in air is not required (France, 2013)

5.3.2.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

Prosulfuron is not classified as toxic or very toxic, therefore analytical methods for the determination of residues in body fluids and tissues are not required.

5.3.2.8 Other studies/ information

No additional studies required.

5.3.3 Description of analytical methods for the determination of residues of nicosulfuron (KCP 5.2)

5.3.3.1 Overview of residue definitions and levels for which compliance is required

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical.

Table 5.3-9: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Nicosulfuron	0.01 mg/kg	MRL (Reg. (EU) No 617/2014)
Plant, high acid content		0.01 mg/kg	MRL (Reg. (EU) No 617/2014)
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	MRL (Reg. (EU) No 617/2014)
Plant, high oil content		0.02 mg/kg	MRL (Reg. (EU) No 617/2014)
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	MRL (Reg. (EU) No 617/2014)
Muscle	Nicosulfuron	0.02 mg/kg	MRL (Reg. (EU) No 617/2014)
Milk		0.02 mg/kg	MRL (Reg. (EU) No 617/2014)
Eggs		0.02 mg/kg	MRL (Reg. (EU) No 617/2014)
Fat		0.02 mg/kg	MRL (Reg. (EU) No 617/2014)
Liver, kidney		0.02 mg/kg	MRL (Reg. (EU) No 617/2014)
Soil (Ecotoxicology)	Nicosulfuron	>1000 mg/kg (dry weight)	LC_{50} for *Eisenia fetida* (acute 14 days) (EFSA, 2007)
Drinking water (Human toxicology)	Nicosulfuron	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Nicosulfuron	1.7 µg/L	EC_{50} for *Lemna gibba* (7 day frond count) (EFSA, 2007)
Air	Nicosulfuron	1.2 µg/m^3	AOEL: 0.8 mg/kg bw/d (EFSA, 2007)
Tissue (meat or liver)	Nicosulfuron	Not required	Not classified as T / T+
Body fluids		Not required	Not classified as T / T+

5.3.3.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of nicosulfuron in plant matrices is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-10: Validated methods for food and feed of plant origin

Component of residue definition: nicosulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
High water content	Primary	0.01 mg/kg	LC-MS/MS	Steinhilper D., 2008 / Report No. 107 NIS New data
	ILV	0.01 mg/kg	LC-MS/MS	Schwarz T., 2008 / Report No.

Component of residue definition: nicosulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
				119 NIS New data
	Confirmatory (if required)	-	-	Not required, primary method was confirmed by validation of two different mass transitions
High acid content	Primary	-	-	Not validated for this matrix type
	ILV	-	-	
	Confirmatory (if required)	-	-	
High oil content	Primary	-	-	Not validated for this matrix type
	ILV	-	-	
	Confirmatory (if required)	-	-	
High protein/high starch content (dry)	Primary	0.01 mg/kg	LC-MS/MS	Steinhilper D., 2008 / Report No. 107 NIS New data
	ILV	0.01 mg/kg	LC-MS/MS	Schwarz T., 2008 / Report No. 119 NIS New data
	Confirmatory (if required)	-	-	Not required, primary method was confirmed by validation of two different mass transitions
Other (maize stover/straw)	Primary	0.01 mg/kg	LC-MS/MS	Steinhilper D., 2008 / Report No. 107 NIS New data
	ILV	0.01 mg/kg	LC-MS/MS	Schwarz T., 2008 / Report No. 119 NIS New data
	Confirmatory (if required)	-	-	Not required, primary method was confirmed by validation of two different mass transitions

For any special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices, please refer to Appendix 2.

Table 5.3-11: Statement on extraction efficiency

	Method for products of plant origin
Not required, because:	Residues >LOQ are generally not expected in matrix types relevant for the target crops. Furthermore, recovery rates both in the primary method and in the ILV were in an acceptable range between 70 and 120%.

5.3.3.3 Description of analytical methods for the determination of residues in animal

matrices (KCP 5.2)

An analytical method suitable for the determination of residues in animal tissues, milk and eggs is not required as the old data requirements apply; no residue definition was proposed by EFSA.

Nevertheless, a method of analysis was reviewed by the RMS during the last peer review process (United Kingdom, 2005). An overview on the methods for analysis of nicosulfuron in animal matrices is given in the following table.

Table 5.3-12: Validated methods for food and feed of animal origin

Component of residue definition: nicosulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
Foodstuff of animal origin	Primary	No details provided	HPLC-UV	Schulz M., Ullrich-Mitzel A., 1996 / Report No. 606543 EU agreed (United Kingdom, 2005)

5.3.3.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of nicosulfuron in soil is given in the following tables.

Table 5.3-13: Validated methods for soil (if appropriate)

Component of residue definition: nicosulfuron			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
Primary	0.05 µg/kg	LC-MS	Wais A., 2000a / Report No. 770117 EU agreed (United Kingdom, 2005)
Confirmatory	No details provided		Mirbach M.J., 1998 / Report No. 699873 EU agreed (United Kingdom, 2005)

5.3.3.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of nicosulfuron in surface and drinking water is given in the following tables.

Table 5.3-14: Validated methods for water

Component of residue definition: nicosulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
Drinking water	Primary	0.05 µg/L	HPLC-UV	Schulz M., Ullrich-Mitzel A., 1995a / Report No. 604383 EU agreed (United Kingdom, 2005)

Component of residue definition: nicosulfuron				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
	ILV	-	-	Not required, old data requirements apply
	Confirmatory	0.05 µg/L	LC-DAD	Wais A., 2000b / Report No. 770128 EU agreed (United Kingdom, 2005)
Surface water	Primary	0.05 µg/L	LC-DAD	Wais A., 2000b / Report No. 770128 EU agreed (United Kingdom, 2005)
	Confirmatory	No details provided		Mirbach M.J., 1998 / Report No. 699873 EU agreed (United Kingdom, 2005)

5.3.3.6 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of nicosulfuron in air is given in the following tables.

Table 5.3-15: Validated methods for air

Component of residue definition: nicosulfuron			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
Primary	1.2 µg/m^3	HPLC-UV	Method: Schulz M., Ullrich-Mitzel A., 1995b / Report No. 385470 Validation: Wais A., 2000c / Report No. 765358 EU agreed (United Kingdom, 2005)
Confirmatory	No details provided		Mirbach M.J., 1998 / Report: 699873 EU agreed (United Kingdom, 2005)

5.3.3.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

Nicosulfuron is not classified as toxic or very toxic, therefore analytical methods for the determination of residues in body fluids and tissues are not required.

5.3.3.8 Other studies/ information

No additional studies required.

5.3.4 Description of analytical methods for the determination of residues of dicamba (KCP 5.2)

5.3.4.1 Overview of residue definitions and levels for which compliance is required

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical.

In the Draft Assessment Report (Denmark, 2007), parent dicamba was proposed as residue definition for both plant and animal matrices. In their Conclusion (EFSA, 2011), EFSA proposed to define the residue for monitoring as dicamba and its salts (free and conjugated). However, MRLs are currently set for dicamba.

Table 5.3-16: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL level/ Remarks
Plant, high water content	Dicamba	0.05 mg/kg	MRL (Reg. (EU) No 2015/845)
Plant, high acid content		0.05 mg/kg	MRL (Reg. (EU) No 2015/845)
Plant, high protein/high starch content (dry commodities)		0.05 mg/kg	MRL (Reg. (EU) No 2015/845)
Plant, high oil content		0.05 mg/kg	MRL (Reg. (EU) No 2015/845)
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	MRL (Reg. (EU) No 2015/845)
Muscle	Dicamba	0.05 mg/kg (poultry 0.02 mg/kg)	MRL (Reg. (EU) No 2015/845)
Milk		0.2 mg/kg	MRL (Reg. (EU) No 2015/845)
Eggs		0.05 mg/kg	MRL (Reg. (EU) No 2015/845)
Fat		0.07 mg/kg (poultry 0.04 mg/kg)	MRL (Reg. (EU) No 2015/845)
Liver, kidney		0.7 mg/kg (poultry 0.07 mg/kg)	MRL (Reg. (EU) No 2015/845)
Soil (Ecotoxicology)	Dicamba (Banvel 480 SL)	> 480 mg/kg (dry weight)	LC_{50} for *Eisenia fetida* (acute 14 days) (EFSA, 2011)
Drinking water (Human toxicology)	Dicamba	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Dicamba	1.8 mg/L	Biomass E_bC_{50} for *Skeletonema costatum* (72 h-static) (EFSA, 2011)
Air	Dicamba	Open (21 µg/m^3 not accepted)	AOEL: 0.3 mg/kg bw/d (EFSA, 2011)
Tissue (meat or liver)	Dicamba	Not required	Not classified as T / T+

Matrix	Residue definition	MRL / limit	Reference for MRL level/ Remarks
Body fluids		Not required	Not classified as T / T+

5.3.4.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of dicamba in plant matrices is given in the following tables.

Table 5.3-17: Validated methods for food and feed of plant origin

Component of residue definition: dicamba and its salts (free and conjugated)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
High water content	Primary	0.01 mg/kg	GC-MS	Method: Gasser A., 1998 / Report No. REM 193.01 Validation: Maffezzoni M., 2004 / Report No. SYN/DIC/03041 EU agreed (Denmark, 2010)[(a)]
	ILV	0.01 mg/kg	GC-MS	Steinhauer S., 2004 / Report No. ADE-0402V EU agreed (Denmark, 2010)[(a)]
	Confirmatory (if required)	-	-	Not required, a second fragment ion (*m/z* >100) was used for confirmation
High acid content	Primary	0.01 mg/kg	GC-MS	Method: Gasser A., 1998 / Report No. REM 193.01 Validation: Maffezzoni M., 2004 / Report No. SYN/DIC/03041 EU agreed (Denmark, 2010)[(a)]
	ILV	0.01 mg/kg	GC-MS	Steinhauer S., 2004 / Report No. ADE-0402V EU agreed (Denmark, 2010)[(a)]
	Confirmatory (if required)	-	-	Not required, a second fragment ion (*m/z* >100) was used for confirmation
High oil content	Primary	0.01 mg/kg	GC-MS	Method: Gasser A., 1998 / Report No. REM 193.01 Validation: Maffezzoni M., 2004 / Report No. SYN/DIC/03041 EU agreed (Denmark, 2010)[(a)]
	ILV	0.01 mg/kg	GC-MS	Steinhauer S., 2004 / Report No. ADE-0402V EU agreed (Denmark, 2010)[(a)]
	Confirmatory (if required)	-	-	Not required, a second fragment ion (*m/z* >100) was used for confirmation

Component of residue definition: dicamba and its salts (free and conjugated)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
High protein/high starch content (dry)	Primary	0.01 mg/kg	GC-MS	Method: Gasser A., 1998 / Report No. REM 193.01 Validation: Maffezzoni M., 2004 / Report No. SYN/DIC/03041 EU agreed (Denmark, 2010)[(a)]
	ILV	0.01 mg/kg	GC-MS	Steinhauer S., 2004 / Report No. ADE-0402V EU agreed (Denmark, 2010)[(a)]
	Confirmatory (if required)	-	-	Not required, a second fragment ion (*m/z* >100) was used for confirmation

(a): EFSA, 2011 identified a data gap as the hydrolysis step is not validated

Table 5.3-18: Statement on extraction efficiency

	Method for products of plant origin
Not required, because:	Recovery rates both in the primary method and in the ILV were in an acceptable range between 70 and 120%.

5.3.4.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of dicamba in animal matrices is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-19: Validated methods for food and feed of animal origin

Component of residue definition: dicamba and its salts (free and conjugated)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
Milk	Primary	0.01 mg/kg	GC-MS	Method: Richardson M., 2008 / Report No. GRM022.03A Validation: Heillaut C., 2008 / Report No. T010322-04-REG New data
	ILV	0.01 mg/kg	GC-MS	Morriss A, 2009 / Report No. CEMR-3620; Class T., Kuhn T., 2010 / Report No. B 1836 G New data
	Confirmatory (if required)	-	-	Not required, primary method was confirmed by validation of three ions
Eggs	Primary	0.01 mg/kg	GC-MS	Method: Richardson M., 2008 / Report No. GRM022.03A

Component of residue definition: dicamba and its salts (free and conjugated)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
				Validation: Heillaut C., 2008 / Report No. T010322-04-REG New data
	ILV	0.01 mg/kg	GC-MS	Morriss A, 2009 / Report No. CEMR-3620; Class T., Kuhn T., 2010 / Report No. B 1836 G New data
	Confirmatory (if required)	-	-	Not required, primary method was confirmed by validation of three ions
Muscle	Primary	0.01 mg/kg	GC-MS	Method: Richardson M., 2008 / Report No. GRM022.03A Validation: Heillaut C., 2008 / Report No. T010322-04-REG New data
	ILV	0.01 mg/kg	GC-MS	Morriss A, 2009 / Report No. CEMR-3620 New data
	Confirmatory (if required)	-	-	Not required, primary method was confirmed by validation of three ions
Fat	Primary	0.01 mg/kg	GC-MS	Method: Richardson M., 2008 / Report No. GRM022.03A Validation: Heillaut C., 2008 / Report No. T010322-04-REG New data
	ILV	0.01 mg/kg	GC-MS	Morriss A, 2009 / Report No. CEMR-3620 New data
	Confirmatory (if required)	-	-	Not required, primary method was confirmed by validation of three ions
Kidney, liver	Primary	0.01 mg/kg	GC-MS	Method: Richardson M., 2008 / Report No. GRM022.03A Validation: Heillaut C., 2008 / Report No. T010322-04-REG New data
	ILV	0.01 mg/kg	GC-MS	Morriss A, 2009 / Report No. CEMR-3620; Class T., Kuhn T., 2010 / Report No. B 1836 G New data
	Confirmatory (if required)	-	-	Not required, primary method was confirmed by validation of three ions

For any special comments or remarkable points concerning the analytical methods for the determination of residues in animal matrices, please refer to Appendix 2.

Table 5.3-20: Statement on extraction efficiency

	Method for products of animal origin
Not required, because:	Recovery rates both in the primary method and in the ILV were in an acceptable range between 70 and 120%.

5.3.4.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of dicamba in soil is given in the following tables.

Table 5.3-21: Validated methods for soil

Component of residue definition: dicamba			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
Primary	0.01 mg/kg	GC-MS	Method: Gasser A., 2000a / Report No. REM 193.02 Validation: Gasser A., 2000b / Report No. 301/00 EU agreed (Denmark, 2010)
Confirmatory	-	-	Not required, a second fragment ion (*m/z* >100) was used for confirmation; further ions are specified in the methods for identification

Component of residue definition: DCSA			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
Primary	0.01 mg/kg	GC-MS	Method: Gasser A., 2000a / Report No. REM 193.02 Validation: Gasser A., 2000b / Report No. 301/00 EU agreed (Denmark, 2010)
Confirmatory	-	-	Not required, a second fragment ion (*m/z* >100) was used for confirmation; further ions are specified in the methods for identification

5.3.4.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of dicamba in surface and drinking water is given in the following tables.

Table 5.3-22: Validated methods for water

Component of residue definition: dicamba				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
Drinking water	Primary	0.05 µg/L	GC-MS	Method: Gasser A., 2000c / Report No. REM 193.03 Validation: Gasser A., 2000d / Report No. 302/00 EU agreed (Denmark, 2010)
	ILV	-	-	Not required, old data requirements apply
	Confirmatory	-	-	Not required, a second fragment ion (*m/z* >100) was used for confirmation; further ions are specified in the methods for identification
Surface water	Primary	0.1 µg/L	GC-MS	Method: Gasser A., 2000c / Report No. REM 193.03 Validation: Gasser A., 2000d / Report No. 302/00 EU agreed (Denmark, 2010)
	Confirmatory	-	-	Not required, a second fragment ion (*m/z* >100) was used for confirmation; further ions are specified in the methods for identification

Component of residue definition: DCSA				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
Drinking water	Primary	0.05 µg/L	GC-MS	Method: Gasser A., 2000c / Report No. REM 193.03 Validation: Gasser A., 2000d / Report No. 302/00 EU agreed (Denmark, 2010)
	ILV	-	-	Not required, old data requirements apply
	Confirmatory	-	-	Not required, a second fragment ion (*m/z* >100) was used for confirmation; further ions are specified in the methods for identification
Surface water	Primary	0.1 µg/L	GC-MS	Method: Gasser A., 2000c / Report No. REM 193.03 Validation: Gasser A., 2000d / Report No. 302/00 EU agreed (Denmark, 2010)

Component of residue definition: DCSA				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
	Confirmatory	-	-	Not required, a second fragment ion (*m/z* >100) was used for confirmation; further ions are specified in the methods for identification

5.3.4.6 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of dicamba in air is given in the following tables.

Table 5.3-23: Validated methods for air

Component of residue definition: dicamba			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / Report No. / EU agreed**
Primary	21 µg/m^3	HPLC-UV	Kettner R., Karapally YJ., 1993 / Report No. 21401 EU agreed (Denmark, 2010)[(a)]
Primary	2 µg/m^3 (or 0.002 µg/L)	GC-MS	Method: Hargreaves S. L., 2007 / Report No. GRM022.01A Validation: Emburey S. N., 2007 / Report No. T010135-04-REG New data
Confirmatory	-	-	Not required, two additional fragment ions (*m/z* >100) were used for confirmation

(a) EFSA, 2011 identified a data gap as the method is not fully validated

5.3.4.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

Dicamba is not classified as toxic or very toxic, therefore analytical methods for the determination of residues in body fluids and tissues are not required.

5.3.4.8 Other studies/ information

No additional studies required.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner SYN = Syngenta
KCP 5.1.1/01	Dos Santos A.	2012	A18385B - Analytical Method SF-570/1 Syngenta Syngenta Crop Protection, Munchwilen, Switzerland, 10494732 Not GLP not published Syngenta File No A18385B_10044 ; VV-128246	N	SYN
KCP 5.1.1/02	De Benedictis S.	2014	A18385B - Validation of Analytical Method SF-570/1 Syngenta Syngenta Crop Protection, Munchwilen, Switzerland, 126609 GLP not published Syngenta File No A18385B_10045; VV-406938	N	SYN
KCP 5.1.1/03	De Benedictis S.	2013	A18385B - Analytical Method SD-1693/1 Syngenta Syngenta Crop Protection, Munchwilen, Switzerland, 10543871 Not GLP not published Syngenta File No A18385B_10031; VV-128236	N	SYN
KCP 5.1.1/04	De Benedictis S.	2013a	A18385B - Validation of Analytical Method SD-1693/1 for Determination of CGA159902 in Formulations Containing Prosulfuron Syngenta Syngenta Crop Protection, Munchwilen, Switzerland, 126361 GLP not published Syngenta File No A18385B_10030; VV-406129	N	SYN
KCP 5.1.2./01	Liedtke A.	2013	Prosulfuron/dicamba/nicosulfuron WG (A18385B) plus adigor (A12127R) - Toxicity to Pseudokirchneriella subcapitata in a 96-hour algal growth inhibition test	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner SYN = Syngenta
(used in KCP 10.2.1 / 01)			Syngenta Harlan Laboratories Ltd., Itingen, Switzerland, D75032 GLP not published Syngenta File No A18385B_10020; VV-405587		
KCP 5.1.2./02 (used in KCP 10.2.1 / 02)	Liedtke A.	2013a	Prosulfuron/dicamba/nicosulfuron WG (A18385B) plus adigor (A12127R) - Toxicity to the aquatic higher plant Lemna gibba in a 7-day growth inhibition test Syngenta Harlan Laboratories Ltd., Itingen, Switzerland, D75010 GLP not published Syngenta File No A18385B_10021; VV-405419	N	Syngenta
KCP 5.2/01	Richardson M.	2008	Dicamba - Analytical Method for the Determination of Residues of Dicamba (SAN837) and its metabolite NOA414746 in Animal Matrices. Final Determination by GC-MSD Syngenta - Jealott's Hill, Bracknell, United Kingdom Syngenta - Jealott's Hill, Bracknell, United Kingdom, GRM022.03A Not GLP not published Syngenta File No SAN837_11276; VV-127750	N	Cheminova A/S
KCP 5.2/02	Heillaut C	2008	Dicamba - Validation of Residue Method GRM022.03A for Dicamba (SAN837) and NOA414746 Metabolite in Animal Matrices (milk, eggs, muscle, fat, liver and kidney) SynTech Research France SAS, La Chapelle de Guinchay, France ADME - Bioanalyses, Vergeze, France, T010322-04-REG GLP not published Syngenta File No SAN837_10997; VV-382384	N	Cheminova A/S
KCP 5.2/03	Morriss A	2009	Independent Laboratory Validation of a Method (GRM022.03A) for the Determination of Residues of Dicamba and its metabolite NOA414746 in Animal Matrices Syngenta - Jealott's Hill, Bracknell, United Kingdom CEMAS, North Ascot, United Kingdom, CEMR-3620	N	SYN

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner SYN = Syngenta
			GLP not published Syngenta File No SAN837_11279; VV-383427		
KCP 5.2/04	Class T, Kuhn T	2010	Dicamba - Independent Laboratory Validation of Analytical Method GRM022.03A for the Determination of Residues of Dicamba and its Metabolite NOA414746 in Animal Materials by GC/MS (NCI) Syngenta - Jealott's Hill, Bracknell, United Kingdom PTRL Europe, Ulm, Germany, B 1836 G GLP not published Syngenta File No SAN837_11330; VV-386364	N	SYN
KCP 5.2/05	Hargreaves S. L.	2007	Dicamba - Residue Method for the Determination of Residues in Air Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott's Hill, Bracknell, United Kingdom, GRM022.01A GLP not published Syngenta File No SAN837/6677; VV-124517	N	SYN
KCP 5.2/06	Emburey S.	2007	Dicamba - Validation of an Analytical Method for the Determination of Residues of Dicamba Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott's Hill, Bracknell, United Kingdom, T010135-04-REG GLP not published Syngenta File No SAN837/6678; VV-334321	N	SYN

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for prosulfuron

A 2.1.1 Methods used for the generation of pre-authorization data (KCP 5.1)

A 2.1.1.1 Description of analytical methods for the determination of residues in support of environmental fate studies (KCP 5.1.2.1)

No new or additional studies have been submitted.

A 2.1.1.2 Description of analytical methods for the determination of residues in support of efficacy studies (KCP 5.1.2.2)

No new or additional studies have been submitted.

A 2.1.1.3 Description of analytical methods for the determination of residues in support of toxicological studies (KCP 5.1.2.3)

No new or additional studies have been submitted.

A 2.1.1.4 Description of analytical methods for the determination of residues in support of operator, worker, resident and bystander exposure studies (KCP 5.1.2.4)

No new or additional studies have been submitted.

A 2.1.1.5 Description of analytical methods for the determination of residues in support of residues studies (KCP 5.1.2.5)

No new or additional studies have been submitted.

A 2.1.1.6 Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2.6)

A 2.1.1.6.1 Study 1

Comments of zRMS:	The method was verified and confirmed as acceptable based on the original report provided by the applicant.

The following study on algae has not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but was evaluated in the Central zone for previous authorization of A18385B and considered acceptable. Therefore no method summary is provided.

Reference:	KCP 5.1.2/01
Report	Liedtke A, 2013, Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - Toxicity to *Pseudokirchneriella subcapitata* in a 96-hour algal growth inhibition test, Report Number D75032, Harlan Laboratories Ltd., Zelgiwelg 1, 4452 Itingen, Switzerland. Syngenta File No. A18385B_10020; VV-405587
Guideline(s):	OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006) Commission Regulation (EC) No. 761/2009 C.3: Algal Inhibition Test, 2009 US EPA Ecological Effects Test Guidelines, OPPTS 850.5400: Algal Toxicity, Tiers I and II, (1996)

Deviations: No.

GLP: Yes.

Acceptability: Yes

Conclusion

The analytical method included in the ecotoxicology study is considered adequate.

A 2.1.1.6.2 Study 2

Comments of zRMS:	The method was verified and confirmed as acceptable based on the original report provided by the applicant.

The following study on Lemna has not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but was evaluated in the Central zone for authorization of A18385B and considered acceptable. Therefore no method summary is provided.

Reference: KCP 5.1.2/02

Report: Liedtke A, 2013a, Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) – Toxicity to the Aquatic Higher Plant *Lemna gibba* in a 7-Day Growth Inhibition Test. Report Number D75010. Harlan Laboratories Ltd., Zelgliweg 1, 4452 Itingen, Switzerland.

Syngenta file no A18385B_10021; VV-405419

Guideline(s): OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 221: *Lemna* sp. Growth Inhibition Test (2006)

Commission Regulation (EC) No. 761/2009 laying down test methods pursuant to Regulation (EC) No. 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), 2009, C.26: Lemna sp. Growth Inhibition Test.

US EPA Ecological Effects Test Guidelines, OPPTS 850.4400: Aquatic Plant Toxicity using *Lemna* spp., Tiers I and II, (1996).

Deviations: No.

GLP: Yes.

Acceptability: Yes

Conclusion

The analytical method included in the ecotoxicology study is considered adequate.

A 2.1.1.7 Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1.2.7)

No new or additional studies have been submitted.

A 2.1.2 Methods for post-authorization control and monitoring purposes (KCP

5.2)

A 2.1.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted.

A 2.2 Analytical methods for nicosulfuron

A 2.2.1 Methods used for the generation of pre-authorization data (KCP 5.1)

No new or additional studies have been submitted.

A 2.2.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.2.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

A 2.2.2.1.1 Multiresidue method (LC-MS/MS)

A 2.2.2.1.1.1 Method validation

The following residue analytical method for crop parts and associated validation study have not previously been submitted for review/reviewed under Council Directive 91/414/EEC and are provided in support of this assessment. Please refer to Cheminova for report, letter of access provided.

Comments of zRMS:	The method is considered acceptable (since the method reported and well described below by the applicant provides only additional support in the context of the authorisation request, obtaining a report from Cheminova was deemed not necessary). The method validation meets the requirements.

Reference:	KCP 5.2
Report:	Validation of a Multiresidue method for the determination of Nicosulfuron in maize, Steinhilper D., 2008, Report No. 107 NIS
Guideline(s):	Yes (SANCO/825/00 rev.7)

Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

As part of this study the multi-residue method DFG S19 extraction procedure was attempted however recovery was very poor (11-12%). Therefore the following method was developed.

Control samples of plant, grain and stover were homogenised in the presence of dry-ice by chopping. Nicosulfuron was extracted from fortified samples by macerating with methanol/water (1/1, v/v) for approx. 5 minutes. The extract is then centrifuged for 5 minutes at 5000 rpm and an aliquot filtered through a 0.45 µm filter for determination by liquid chromatography with tandem mass spectrometry (LC-MS-MS). For stover, sweet corns and dried kernels an aliquot was taken from clean-up with an Enu+SPE cartridge. The elution mixture was evaporated under nitrogen and reconstituted in methanol/5 mM ammonium acetate (1/9, v/v) ready for determination of nicosulfuron by LC-MS/MS with ion transitions for quantification and confirmation.

Results and discussions

The accuracy was assessed from the recovery nicosulfuron obtained from plant, grain and stover from fortified control samples. The overall mean recovery from samples fortified at 0.01 mg/kg and 0.1 mg/kg ranged from 86 to 94%. Mean recoveries at each fortification level and overall were within the acceptable range of 70 to 110% for all matrices. Similar results were shown using the confirmatory mass transitions.

The precision was assessed from the variation obtained from the analysis of 5 fortified replicates at 2 concentration levels for all corn matrices. The overall RSD from samples fortified at 0.01 mg/kg and 0.1 mg/kg ranged from 5 to 7%. The RSD values at individual fortification levels were all less than the acceptable value of 20%. No outliers were removed before statistical analysis. Similar results were shown using the confirmatory mass transitions.

Table A 1: Recovery results from method validation of nicosulfuron using the analytical method

Sample matrix	Fortification level (mg/kg)	Range Recovery (%)	Mean Recovery (%)	RSD (%)	N
Quantification (411 → 182 m/z)					
Plant	0.01	80 - 91	87	5	6
	0.1	81 - 91	87	5	5
	Overall	80 – 91	87	5	11
Grain	0.01	76 - 89	84	6	5
	0.1	82 - 95	88	6	5
	Overall	76 – 95	86	6	10
Stover	0.01	81 - 96	89	7	5
	0.1	94 – 101	98	3	5
	Overall	81 - 101	94	7	10
Confirmation (411 → 213 m/z)					
Plant	0.01	82 - 99	91	7	6
	0.1	83 - 94	90	5	5
	Overall	82 – 99	90	6	11
Grain	0.01	77 – 95	82	9	5
	0.1	84 – 96	89	5	5
	Overall	77 – 96	86	8	10
Stover	0.01	83 - 103	92	8	5
	0.1	86 – 98	95	5	5
	Overall	83 - 103	93	7	10

Table A 2: Characteristics for the analytical method used for validation of nicosulfuron residues in maize matrices

	Nicosulfuron
Specificity	Control extracts of all maize matrices were free from components that interfered with the analysis of Nicosulfuron. Any components observed in control chromatograms were therefore below a concentration equivalent to 30% of the LOQ. The analytical procedure was considered specific for nicosulfuron. As the LC-MS-MS method used is considered self-confirmatory, re-analysis of final extracts, using a suitable selective and sensitive alternative chromatographic technique, was not required.

	Nicosulfuron
Calibration (type, number of data points)	The calibration was demonstrated using 10 standards over the range 0.2 to 100 ng/mL for all matrices. No significant matrix affects were noted and therefore samples were analysed using calibration standards prepared in methanol/water (1:1 v/v). The calibration response was linear (y = mx + c) with a coefficient of determination (r2) of 0.9997 for the primary and confirmatory mass transitions. Representative calibration lines are presented in the report.
Calibration range	0.2 to 100 ng/mL
Assessment of matrix effects is presented	No
Limit of determination/quantification	Acceptable accuracy and precision was obtained at 0.01 mg/kg for all maize matrices.

Conclusion

The analytical method has been successfully validated for the determination of nicosulfuron residues in maize matrices with a LOQ of 0.01 mg/kg.

A 2.2.2.1.1.2 Independent laboratory validation

Comments of zRMS:	The method is considered acceptable. The method validation meets the requirements.

Reference:	KCP 5.2
Report:	Independent Laboratory Validation (ILV) of a residue analytical method for the determination of residue of nicosulfuron in maize plant, straw and grain, using LC/MS/MS, Schwarz T., 2008, Report No. 119NIS
Guideline(s):	Yes (SANCO/825/00 rev.7; SANCO/3029/99 rev. 4; ENV/JM/MONO(2007)17)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The method as described in the report by Steinhilper (2008) was validated by an independent laboratory. Minor modifications were made to the method due to different LC-MS/MS instrumentation. None of the modifications were considered to change the integrity of the original methodology.

Results and discussions

This study is an independent laboratory validation conducted to satisfy reproducibility requirements for the analytical method.

The accuracy was assessed from the recovery nicosulfuron obtained from plant, grain and straw from fortified control samples. The overall mean recovery from samples fortified at 0.01 mg/kg and 0.1 mg/kg ranged from 79 to 104%. Mean recoveries at each fortification level and overall were within the acceptable range of 70 to 110% for all matrices. Similar results were shown using the confirmatory mass transitions.

The precision was assessed from the variation obtained from the analysis of 5 fortified replicates at two concentration levels for all corn matrices. The overall RSD from samples fortified at 0.01 mg/kg and

0.1 mg/kg ranged from 3 to 7%. The RSD values at individual fortification levels were all less than the acceptable value of 20%. No outliers were removed before statistical analysis. Similar results were shown using the confirmatory mass transitions.

Table A 3: Recovery results from independent laboratory validation of nicosulfuron using the analytical method

Sample matrix	Fortification level (mg/kg)	Range Recovery (%)	Mean Recovery (%)	RSD (%)	N
Quantification (411 → 182 m/z)					
Plant	0.01	75 - 81	79	3	5
	0.1	77 - 82	80	3	5
	Overall	75 – 82	79	3	10
Grain	0.01	83 - 95	92	5	5
	0.1	99 - 107	103	3	5
	Overall	83 – 107	98	7	10
Straw	0.01	102 – 110	104	3	5
	0.1	101 – 107	104	2	5
	Overall	101 - 110	104	3	10
Confirmation (411 → 213 m/z)					
Plant	0.01	73 - 79	74	3	5
	0.1	80 - 83	82	1	5
	Overall	73 – 83	78	6	10
Grain	0.01	87 – 93	91	3	5
	0.1	97 – 106	103	4	5
	Overall	87 – 106	97	8	10
Stover	0.01	89 - 108	100	7	5
	0.1	101 – 106	103	2	5
	Overall	89 - 108	101	5	10

Table A 4: Characteristics for the analytical method used for independent laboratory validation of nicosulfuron residues in maize

	Nicosulfuron
Specificity	Control extracts of all maize matrices were free from components that interfered with the analysis of nicosulfuron. Any components observed in control chromatograms were therefore below a concentration equivalent to 30% of the LOQ. The analytical procedure was considered specific for nicosulfuron. As the LC-MS-MS method used is considered self-confirmatory, re-analysis of final extracts, using a suitable selective and sensitive alternative chromatographic technique, was not required.

	Nicosulfuron
Calibration (type, number of data points)	The calibration was demonstrated using 8 standards over the range 0.025 to 25 ng/mL for all matrices. No significant matrix affects were noted following suitable dilution and therefore samples were analysed using calibration standards prepared in methanol/water (1:1 v/v). The calibration response was linear (y = mx + c) with correlation coefficients (r) of >0.995 for the primary and confirmatory mass transitions. Representative calibration lines are presented in the report.
Calibration range	0.025 to 25 ng/mL
Assessment of matrix effects is presented	No
Limit of determination/quantification	Acceptable accuracy and precision was obtained at 0.01 mg/kg for all maize matrices.

Conclusion

The analytical method has been successfully validated by an independent laboratory for post registration monitoring for the determination of nicosulfuron residues in maize matrices with a LOQ of 0.01 mg/kg.

A 2.2.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted.

A 2.2.2.7 Other Studies/ Information

No new or additional studies have been submitted.

A 2.3 Analytical methods for dicamba

A 2.3.1 Methods used for the generation of pre-authorization data (KCP 5.1)

No new or additional studies have been submitted.

A 2.3.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.3.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.3.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

A 2.3.2.2.1 GRM022.03A

A 2.3.2.2.1.1 Method validation

Comments of zRMS:	The method is considered acceptable. No GLP, however is not obligatory for the method. The method GRM022.03A was developed for the determination of residues of dicamba (SAN837) and its metabolite NOA414746 in animal matrices at the LOQ of 0.01 mg/kg for each analyte. After derivatization final determination was done by GC-MSD. The validation parameters meet the requirements.

Reference:	KCP 5.2/01
Report:	Dicamba – Analytical Method for the Determination of Residues of Dicamba (SAN837) and its metabolite NOA414746 in Animal Matrices. Final Determination by GC-MSD., Richardson M., 2008, Report No. GRM022.03A, Syngenta File No. SAN837_11276; VV-127750
Guideline(s):	Not mentioned in A18385B dRR, 2013
Deviations:	Not mentioned in A18385B dRR, 2013
GLP:	Yes (validation) / No (method; not necessary)
Acceptability:	Yes

Reference:	KCP 5.2/02
Report	Dicamba – Validation of Residue Method GRM022.03A for Dicamba (SAN837) and NOA414746 Metabolite in Animal Matrices (Milk, Eggs, Muscle, Fat, Liver and Kidney), Heillaut C., 2008, Report No. T010322-04-REG, Syngenta File No. SAN837_10997; VV-382384
Guideline(s):	Not mentioned in A18385B dRR, 2013
Deviations:	Not mentioned in A18385B dRR, 2013
GLP:	No
Acceptability:	Yes

Materials and methods

Milk and eggs

Samples are extracted with acetonitrile and centrifuged. The supernatant is added to 1M HCl in high purity water. Samples are heated at 95ºC for 1.5 h. Aliquots are extracted with DCM after the addition of sodium chloride. The extracts are combined and evaporated to dryness and then reconstituted in 1M HCl solution. Samples are subjected to SPE and the analytes eluted with 0.1% v/v acetic acid in acetonitrile. Samples are evaporated to dryness and residues reconstituted in acetone. Dicamba and NOA414756 are derivatised with N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide (MTBSTFA) to form the tertiary butyl dimethyl silyl esters. Final determination is by negative ion chemical ionisation (NICI) gas chromatography with mass selective detection (GC-MSD).

Liver, muscle, fat and kidney

Samples are extracted with 1M HCl in high purity water by heating at 95ºC for 1.5 h. Aliquots are extracted with DCM after the addition of sodium chloride. The DCM extracts are combined and evaporated to dryness and are then reconstituted in 1M HCl solution. Samples are subjected to a solid phase extrac-

tion procedure and the analytes are eluted in 0.1% v/v acetic acid in acetonitrile. Samples are evaporated to dryness and residues reconstituted in acetone. Dicamba and NOA414756 are derivatised with N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide (MTBSTFA) to form the tertiary butyl dimethyl silyl esters. Final determination is by GC-MSD.

Results and discussions

Method GRM022.03A was validated in dairy cattle tissues (fat, kidney, liver, muscle). Validation samples were fortified at levels between 0.01 and 0.1 mg/kg with dicamba and DCSA (NOA414746).

The overall mean recovery values for all animal matrices in the validation study were between 70% and 110% for both dicamba and NOA414746 and therefore meet the current requirements according to the EU guidance SANCO825/00 rev.7 (March 2004) demonstrating the method has satisfactory accuracy.

Overall relative standard deviations (RSDs) for all matrices were below 20% for both dicamba and NOA414746 and therefore meet the current guidelines according to the EU guidance SANCO825/00 rev.7 (March 2004).

Table A 5: Recovery results from method validation of dicamba using the analytical method

Matrix	Fortification level (mg/kg)	Recovery	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Dicamba (*m/z* 184)					
Milk	0.01	75, 84, 86, 87, 86	84	6	75-87
	0.10	83, 86, 86, 94, 86	87	5	83-94
	Overall		**85**	**5**	**75-94**
Eggs	0.01	94, 97, 100, 96, 94	96	3	94-100
	0.10	89, 83, 86, 88, 79	85	5	79-89
	Overall		**91**	**8**	**79-100**
Liver	0.01	86, 91, 84, 86, 84	86	3	84-91
	0.10	104, 97, 99, 95, 93	98	4	93-104
	Overall		**92**	**8**	**84-104**
Kidney	0.01	89, 93, 96, 99, 96	95	4	89-99
	0.10	89, 96, 98, 98, 95	95	4	89-98
	Overall		**95**	**4**	**89-99**
Muscle tissue	0.01	80, 85, 89, 88, 92	87	5	80-92
	0.10	92, 93, 95, 95, 91	93	2	91-95
	Overall		**90**	**5**	**80-95**
Fat	0.01	99, 96, 96, 94, 96	96	2	94-99
	0.10	80, 79, 83, 81, 87	82	4	79-87
	Overall		**89**	**9**	**79-99**
Dicamba (*m/z* 185)					
Milk	0.01	64, 72, 76, 76, 75	73	7	64-76
	0.10	84, 86, 88, 94, 86	88	4	84-94

Matrix	Fortification level (mg/kg)	Recovery	Mean Recovery (%)	RSD (%)	Recovery Range (%)
	Overall		**80**	**11**	**64-94**
Eggs	0.01	91, 94, 97, 92, 91	93	3	91-97
	0.10	90, 83, 86, 89, 79	85	5	79-90
	Overall		**89**	**6**	**79-97**
Liver	0.01	86, 93, 85, 87, 82	87	4	82-93
	0.10	102, 96, 99, 94, 94	97	4	94-102
	Overall		**92**	**7**	**82-102**
Kidney	0.01	90, 92, 96, 97, 94	94	3	90-97
	0.10	88, 94, 97, 98, 93	94	4	88-98
	Overall		**94**	**3**	**88-98**
Muscle tissue	0.01	78, 82, 88, 87, 88	85	5	78-88
	0.10	92, 92, 95, 95, 90	93	2	90-95
	Overall		**89**	**6**	**78-95**
Fat	0.01	97, 95, 96, 94, 95	95	1	94-97
	0.10	81, 79, 82, 81, 87	82	4	79-87
	Overall		**89**	**8**	**79-97**
Dicamba (*m/z* 186)					
Milk	0.01	73, 81, 85, 85, 85	82	6	73-85
	0.10	84, 86, 88, 94, 87	88	4	84-94
	Overall		**85**	**6**	**73-94**
Eggs	0.01	92, 95, 100, 93, 94	95	3	92-100
	0.10	91, 84, 87, 89, 80	86	5	80-91
	Overall		**91**	**6**	**80-100**
Liver	0.01	85, 90, 84, 87, 83	86	3	83-90
	0.10	106, 99, 101, 97, 96	100	4	96-106
	Overall		**93**	**9**	**83-106**
Kidney	0.01	91, 93, 97, 98, 96	95	3	91-98
	0.10	91, 94, 97, 98, 94	95	3	91-98
	Overall		**95**	**3**	**91-98**
Muscle tissue	0.01	78, 81, 87, 87, 87	84	5	78-87
	0.10	92, 92, 96, 95, 91	93	3	91-96
	Overall		**89**	**7**	**78-96**
Fat	0.01	96, 93, 96, 93, 95	95	1	93-96
	0.10	80, 80, 83, 82, 88	83	4	80-88
	Overall		**89**	**8**	**80-96**

Table A 6: Recovery results from method validation of DCSA (NOA414746) using the analytical method

Matrix	Fortification level (mg/kg)	Recovery	Mean Recovery (%)	RSD (%)	Recovery Range (%)
NOA414746 (*m/z* 227)					
Milk	0.01	71, 76, 81, 83, 86	79	8	71-86
	0.10	89, 88, 87, 94, 88	89	3	87-94
	Overall		**84**	**8**	**71-94**
Eggs	0.01	92, 95, 101, 95, 93	95	4	92-101
	0.10	93, 85, 88, 91, 84	88	5	84-93
	Overall		**92**	**6**	**84-101**
Liver	0.01	85, 90, 68, 78, 66	77	13	66-90
	0.10	72, 76, 78, 77, 75	75	3	72-78
	Overall		**76**	**9**	**66-90**
Kidney	0.01	81, 91, 91, 92, 90	89	5	81-92
	0.10	86, 96, 96, 93, 87	92	5	86-96
	Overall		**90**	**5**	**81-96**
Muscle tissue	0.01	79, 88, 94, 93, 92	89	7	79-94
	0.10	86, 94, 92, 90, 86	90	4	86-94
	Overall		**89**	**5**	**79-94**
Fat	0.01	83, 76, 68, 79, 75	76	8	68-83
	0.10	85, 82, 85, 84, 90	85	4	82-90
	Overall		**81**	**8**	**68-90**
NOA414746 (*m/z* 284)					
Milk	0.01	72, 77, 83, 85, 96	83	11	72-96
	0.10	88, 87, 87, 95, 87	89	4	87-95
	Overall		**86**	**8**	**72-96**
Eggs	0.01	66, 86, 95, 83, 87	84	13	66-95
	0.10	90, 84, 87, 89, 84	87	3	84-90
	Overall		**85**	**9**	**66-95**
Liver	0.01	92, 90, 64, 71, 64	76	18	64-92
	0.10	75, 79, 80, 80, 78	78	3	75-80
	Overall		**77**	**12**	**64-92**
Kidney	0.01	86, 104, 105, 111, 115	104	11	86-115
	0.10	86, 98, 97, 92, 89	92	5	86-98
	Overall		**98**	**11**	**86-115**
Muscle tissue	0.01	77, 105, 96, 95, 104	95	12	77-105

Matrix	Fortification level (mg/kg)	Recovery	Mean Recovery (%)	RSD (%)	Recovery Range (%)
	0.10	86, 93, 93, 89, 88	90	4	86-93
	Overall		**93**	**9**	**77-105**
Fat	0.01	83, 66, 70, 71, 74	73	9	66-83
	0.10	84, 81, 85, 82, 87	84	3	81-87
	Overall		**78**	**10**	**66-87**
NOA414746 (*m/z* 285)					
Milk	0.01	74, 75, 82, 83, 86	80	7	74-86
	0.10	91, 90, 88, 95, 90	91	3	88-95
	Overall		**85**	**8**	**74-95**
Eggs	0.01	95, 99, 105, 97, 97	99	4	95-105
	0.10	92, 84, 86, 89, 83	87	4	83-92
	Overall		**93**	**8**	**83-105**
Liver	0.01	89, 92, 70, 78, 67	79	14	67-92
	0.10	73, 75, 78, 77, 74	75	3	73-78
	Overall		**77**	**10**	**67-92**
Kidney	0.01	81, 93, 93, 94, 92	90	6	81-94
	0.10	86, 95, 94, 92, 86	91	5	86-95
	Overall		**91**	**5**	**81-95**
Muscle tissue	0.01	78, 86, 90, 90, 86	86	6	78-90
	0.10	86, 94, 91, 89, 87	89	3	86-94
	Overall		**88**	**5**	**78-94**
Fat	0.01	82, 75, 99, 78, 74	82	12	74-99
	0.10	84, 83, 85, 84, 91	85	4	83-91
	Overall		**83**	**9**	**74-99**

Table A 7: Characteristics for the analytical method used for validation of dicamba residues in animal matrices

	Dicamba	DCSA (NOA414746)
Specificity	NICI GC-MSD monitoring three fragment ions is a highly specific detection technique and a confirmatory method is not required. Interference arising from the matrices tested was not observed. Using high purity solvents and reagents, interference has not been observed. The method uses mainly disposable labware and provided all re-usable glassware is detergent washed and rinsed with HPLC-grade methanol, acetone or acetonitrile before use, interference from labware should not be observed.	
Calibration (type, number of data points)	The linearity of the NICI-MSD detector responses for	

	Dicamba	DCSA (NOA414746)
Calibration range	dicamba and NOA414746 were tested over the range from 5 pg to 200 pg injected on column (equivalent to 0.005 ug/mL to 0.2 ug/mL standards when using a 1 uL injection volume) and was found to be linear. If a residue beyond the tested concentration range is expected, the extract can be diluted to bring it within the linear range prior to quantification.	
Assessment of matrix effects is presented	Yes	
Limit of determination/quantification	The limit of quantification (LOQ) of a method is defined as the lowest analyte concentration in a sample at which the methodology has been validated and for which a mean recovery of 70-110% with a relative standard deviation (RSD) of <20% has been obtained. The limit of quantification (LOQ) for dicamba and NOA414746 has been set at 0.01 mg/kg.	

Conclusion

The repeatability and specificity of the method were demonstrated and GRM022.03A was successfully validated for the determination of residues of dicamba and NOA414746 in animal matrices at the LOQ of 0.01 mg/kg for each analyte.

Stability of extracts

The stability of dicamba and NOA414746 in final extracts stored at 4ºC (between 0 and 9ºC) was assessed in eggs. Samples were re-analysed after a 12 day interval. Results determined from this matrix at the 12 day interval were similar to those from the original analysis (the mean recovery rate was in the range 70-110%). The results indicate the stability of dicamba and NOA414746 in final extracts when stored at 4ºC.

Table A 8: Storage stability of dicamba in eggs final extract

Storage interval (days)	Fortification level (mg/kg)	Recovery (%)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Dicamba (Quantifyer ion *m/z* 184)					
1	0.01	94, 97, 100, 96, 94	96	3	94-100
12	0.01	77, 82, 81, 76, 77	79	4	76-82
NOA414746 (Quantifyer ion *m/z* 227)					
1	0.01	92, 95, 101, 95, 93	95	4	92-101
12	0.01	82, 86, 90, 84, 84	85	4	82-90

A 2.3.2.2.1.2 Independent laboratory validation

Comments of zRMS:	The method GRM022.03A ILV is considered acceptable. The validation parameters meet the requirements.

Reference: KCP 5.2/03

Report: Independent Laboratory Validation of a Method (GRM022.03A) for the Determination of Residues of Dicamba and its metabolite NOA414746 in Animal Matrices, Morriss A., 2009, Report No. CEMR-3620, Syngenta File

	No. SAN837_11279; VV-383427
Guideline(s):	Not mentioned in A18385B dRR, 2013
Deviations:	Not mentioned in A18385B dRR, 2013
GLP:	Yes
Acceptability:	Yes

Reference:	KCP 5.2/04
Report	Dicamba - Independent Laboratory Validation of Analytical Method (GRM022.03A) for the Determination of Residues of Dicamba and its metabolite NOA414746 in Animal Materials, Class T. and Kuhn T., 2010, Report No. B 1836 G, Syngenta File No. SAN837_11330; VV-386364
Guideline(s):	Not mentioned in A18385B dRR, 2013
Deviations:	Not mentioned in A18385B dRR, 2013
GLP:	Yes
Acceptability:	Yes

Materials and methods

See method GRM022.03A above.

The ILV was repeated due to the failure of the data to meet the requirement of <20% for the %RSD. Due to the problems with the method procedures observed the method was modified to use GC-MS with negative chemical ionization (NCI) after silylation to analyse the samples. As before, control specimens were analysed in duplicate and fortified specimens were analysed in quintuplet for both fortification levels. The validation was conducted on milk, eggs, and dairy cattle tissues (liver). Fortification levels were set at the LOQ and ten times that level. Samples were analysed using primary GC-MS with negative chemical ionization (NCI) after silylation. Results of the repeated ILV are presented in the second table below.

Results and discussions

For the ILV, overall mean recovery values for dicamba for liver, muscle, milk and eggs were between 70 – 110% using both non-matrix and matrix matched bracketing standards, and for kidney with non-matrix matched bracketing standards, and therefore meet the current requirements according to the EU guidance SANCO/825/00 Rev.7 (Mar 2004).

Poor recovery values were seen for both bracketing standards for dicamba for fat and for the matrix-matched bracketing standard for kidney. Due to poor recovery values, the study monitor was contacted and some method check work was conducted to improve the observed results. The SPE and derivatisation phases were further investigated.

The overall mean recovery values for DCSA (NOA414746) for the non-matrix-matched bracketing standard for muscle and liver were between 70 -110% and therefore meet the current requirements according to the EU guidance SANCO/825/00 Rev.7 (Mar 2004).

The independent validation was deemed acceptable for dicamba for liver, milk, eggs and kidney (as described above). In muscle, dicamba mean recoveries were within acceptable limits but the variance (%RSD) failed to meet current requirements (<20%). Validation for fat failed to meet current guidance requirements.

In the repeated ILV, the overall mean recovery values for dicamba for liver, milk and eggs were between 70 – 110% for the primary GC-MS (NCI) ions 183 m/z (liver) or 184 m/z (milk, eggs), and for the GC-MS (NCI) confirmatory ions 185 m/z and 186 m/z (with the exception of liver at 186 m/z, with an overall

mean recovery of 65% but with excellent overall RSD of 8%). Therefore, the results meet the current requirements according to the EU guidance SANCO/825/00 Rev.7 (Mar 2004).

The overall mean recovery values for DCSA (NOA414746) in milk and liver were acceptable (68 - 98% with RSD ≤18%) for the primary GC-MS (NCI) ions 229 m/z (liver) and 227 m/z (milk), and also for the GC-MS (NCI) confirmatory ions 284 m/z and 285 m/z.

For egg, mean recoveries for NOA414746 obtained in the 3rd ILV set were in the range of 52% to 74%; however, relative standard deviations were ≤ 11 % for the two fortification levels.

The independent validation was deemed acceptable for dicamba and its metabolite NOA414746 in liver, milk, eggs and kidney at the LOQ of 0.01 mg/kg and over concentration ranges typical of those for which the method will be used.

Overall relative standard deviations (RSDs) for most animal matrices for dicamba in the ILV study were below 20% with the exception of muscle (both standards) and fat (using non matrix-matched standard).

In the repeated ILV, overall RSDs for all matrices for dicamba and its metabolite NOA414746 in the ILV study were below 20% and therefore meet the current guidelines according to the EU guidance SAN-CO/825/00 Rev.7 (Mar 2004).

Table A 9: Recovery results from independent laboratory validation of dicamba and DCSA (NOA414746) using the analytical method

Matrix	Fortification Level (mg/kg)	Number of Analyses	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Dicamba					
Muscle (MMS)	0.01	5	75	25.9	49 – 93
	0.10	5	72	18	60 – 89
	Overall	**10**	**73**	**21.3**	**49 – 93**
Muscle (NMMS)	0.01	5	78	27.1	51 – 104
	0.10	5	67	18.3	55 – 81
	Overall	**10**	**72**	**24.1**	**51 – 104**
Fat (MMS)	0.01	5	24	13.6	21 – 29
	0.10	5	24	20.5	17 – 30
	Overall	**10**	**24**	**16.6**	**17 – 30**
Fat (NMMS)	0.01	5	33	21.7	24 – 44
	0.10	5	27	21.3	19 – 34
	Overall	**10**	**30**	**23.3**	**19 – 44**
Liver (MMS)	0.01	5	73	5.3	68 – 77
	0.10	5	78	10.2	67 – 87
	Overall	**10**	**75**	**8.7**	**67 – 87**
Liver (NMMS)	0.01	5	89	7.5	78 – 95
	0.10	5	89	10.1	76 – 99
	Overall	**10**	**89**	**8.4**	**76 – 99**
Kidney (MMS)	0.01	5	61	10.4	54 – 68
	0.10	5	69	5.8	64 – 72
	Overall	**10**	**65**	**9.9**	**54 – 72**
Kidney (NMMS)	0.01	5	70	9.3	62 – 78
	0.10	5	83	4.8	78 – 86
	Overall	**10**	**77**	**10.8**	**62 – 86**
Milk (MMS)	0.01	5	74	10.8	64 – 84
	0.10	5	89	16.4	68 – 109
	Overall	**10**	**81**	**16.9**	**64 – 109**
Milk (NMMS)	0.01	5	94	12.2	80 – 109
	0.10	5	115	16.8	88 – 142
	Overall	**10**	**105**	**18.1**	**80 – 142**
Eggs (MMS)	0.01	5	79	7.7	73 – 87

Matrix	Fortification Level (mg/kg)	Number of Analyses	Mean Recovery (%)	RSD (%)	Recovery Range (%)
	0.10	5	87	21.6	57 – 101
	Overall	**10**	**83**	**16.9**	**57 – 101**
Eggs (NMMS)	0.01	5	95	7.9	88 – 106
	0.10	5	102	21.2	66 - 118
	Overall	**10**	**99**	**16.0**	**66 - 118**
DCSA					
Muscle (MMS)	0.01	5	63	26.3	44 – 85
	0.10	5	54	14.8	46 – 65
	Overall	**10**	**58**	**22.4**	**44 – 85**
Muscle (NMMS)	0.01	5	85	25.3	59 – 108
	0.10	5	69	14.8	59 – 82
	Overall	**10**	**77**	**23.4**	**59 – 108**
Fat (MMS)	0.01	5	12	31.4	7 – 17
	0.10	5	13	21.5	9 – 16
	Overall	**10**	**13**	**25.7**	**7 – 17**
Fat (NMMS)	0.01	5	19	31.3	13 – 29
	0.10	5	18	20.0	13 – 22
	Overall	**10**	**19**	**25.2**	**13 – 29**
Liver (MMS)	0.01	5	41	11.6	36 – 49
	0.10	5	45	15.4	36 – 53
	Overall	**10**	**43**	**13.9**	**36 – 53**
Liver (NMMS)	0.01	5	75	6	70 – 82
	0.10	5	76	15.1	61 – 89
	Overall	**10**	**75**	**11.0**	**61 – 89**
Kidney (MMS)	0.01	5	50	8.6	45 – 55
	0.10	5	47	7.7	43 – 53
	Overall	**10**	**49**	**8.2**	**43 – 55**
Kidney (NMMS)	0.01	5	66	6.7	59 – 71
	0.10	5	67	8.9	60 – 76
	Overall	**10**	**67**	**7.5**	**59 – 76**
Milk (MMS)	0.01	5	54	7.0	48 – 58
	0.10	5	55	15.8	41 – 63
	Overall	**10**	**55**	**11.6**	**41 – 63**
Milk (NMMS)	0.01	5	62	6.8	55 – 66
	0.10	5	64	15.9	48 – 74
	Overall	**10**	**63**	**11.7**	**48 – 74**
Eggs (MMS)	0.01	5	48	10.7	40 – 54
	0.10	4	58	11	49 – 64
	Overall	**9**	**52**	**14.1**	**40 – 64**
Eggs (NMMS)	0.01	5	42	10.9	34 – 45
	0.10	4	51	11	43 – 56
	Overall	**9**	**46**	**14.4**	**34 – 56**

Table A 10: Recovery results from repeated independent laboratory validation of dicamba and DCSA (NOA414746) using the analytical method

Matrix	GC-MS Transition	Fortification Level (mg/kg)	Number of Analyses	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Dicamba						
Milk	184 m/z	0.01	5	76	8	67-83
		0.10	5	90	8	83-97

Matrix	GC-MS Transition	Fortification Level (mg/kg)	Number of Analyses	Mean Recovery (%)	RSD (%)	Recovery Range (%)
		Overall	**10**	**83**	**12**	**67-97**
	185 m/z	0.01	5	78	6	72-84
		0.10	5	90	9	81-101
		Overall	**10**	**84**	**10**	**72-101**
	186 m/z	0.01	5	76	7	68-82
		0.10	5	89	8	81-98
		Overall	**10**	**83**	**11**	**68-98**
Eggs	184 m/z	0.01	5	71	6	64-75
		0.10	4	73	11	67-85
		Overall	**9**	**72**	**8**	**64-85**
	185 m/z	0.01	5	79	7	69-84
		0.10	4	77	11	71-89
		Overall	**9**	**78**	**8**	**69-89**
	186 m/z	0.01	5	75	7	67-80
		0.10	4	75	10	70-86
		Overall	**9**	**75**	**8**	**67-86**
Liver	183 m/z*	0.01	5	73	3	71-77
		0.10	5	73	7	69-81
		Overall	**10**	**73**	**5**	**69-81**
	185 m/z	0.01	5	71	9	66-79
		0.10	5	75	8	68-82
		Overall	**10**	**73**	**8**	**66-82**
	186 m/z	0.01	5	62	9	56-70
		0.10	5	68	4	65-71
		Overall	**10**	**65**	**8**	**56-71**
DCSA						
Milk	227 m/z	0.01	5	93	5	87-97
		0.10	5	103	10	89-115
		Overall	**10**	**98**	**10**	**87-115**
	284 m/z	0.01	5	77	5	72-81
		0.10	5	102	10	91-114
		Overall	**10**	**90**	**17**	**72-114**
	285 m/z	0.01	5	75	3	72-77
		0.10	5	100	12	89-114
		Overall	**10**	**87**	**18**	**72-114**
Egg	227 m/z	0.01	4	74	7	67-79
		0.10	5	59	10	52-68
		Overall	**9**	**66**	**14**	**52-79**
	284 m/z	0.01	4	67	5	64-72
		0.10	5	63	11	53-72
		Overall	**9**	**65**	**9**	**53-72**
	285 m/z	0.01	4	52	10	47-58
		0.10	5	54	8	47-58
		Overall	**9**	**53**	**9**	**47-58**
Liver	227 m/z	0.01	5	79	6	74-86
		0.10	5	67	6	61-71
		Overall	**10**	**73**	**10**	**61-86**
	284 m/z	0.01	5	74	5	71-80
		0.10	5	81	13	69-93
		Overall	**10**	**77**	**10**	**69-93**
	285 m/z	0.01	5	71	10	62-79
		0.10	5	65	6	58-69
		Overall	**10**	**68**	**10**	**58-79**

Table A 11: Characteristics for the analytical method used for independent laboratory validation of dicamba residues in animal matrices

	Dicamba	DCSA (NOA414746)
Specificity	All samples were analysed using matrix-matched and non-matrix matched bracketing standards. An assessment of the matrix effects was conducted for both analytes using liver, fat and egg matrices. The results showed that significant matrix effects were likely to be observed for both analytes. In the repeated ILV, fortified sample extracts were evaluated with a multi-point calibration obtained from matrix-matched standards. For all animal matrices, recovery calculations were carried out using matrix matched standards to compensate any significant effects.	
Calibration (type, number of data points)	For the ILV, linearity of the method for dicamba and DCSA (NOA414746) was in the range 0.005 – 0.20 µg/mL with correlation coefficients > 0.9965. In the repeated ILV, linearity of the method for dicamba and DCSA (NOA414746) was in the range 2.5 – 160 ng/mL with correlation coefficients > 0.98.	
Calibration range		
Assessment of matrix effects is presented	Yes (see under specificity above)	
Limit of determination/quantification	0.01 mg/kg	0.01 mg/kg

Conclusion

It is concluded that method GRM022.03A was independently validated. Accordingly, the analytical method GRM022.03A is a specific method suitable for routine analysis and enforcement for dicamba in animal matrices.

A 2.3.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted.

A 2.3.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted.

A 2.3.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

A 2.3.2.5.1 GRM022.01A

A 2.3.2.5.1.1 Method validation

Comments of zRMS:	The method is considered acceptable for the residues determination in air. The validation parameters meet the requirements. The LOQ of the method has been set at 2 µg m^{-3}.

Reference: KCP 5.2/05

Report: Dicamba: Residue Method for the Determination of Residues in Air, Hargreaves S. L., 2007, Report No. GRM022.01A
Syngenta File No SAN837/6677; VV-124517

Guideline(s): SANCO/825/00 rev.7; SANCO/3029/99 rev.4

Deviations:	Not mentioned in Registration Report
GLP:	No (method; not necessary)
Acceptability:	Yes

Reference:	KCP 5.2/06
Report	Dicamba: Validation of an Analytical Method for the Determination of Residues of Dicamba in Air, Emburey S. N., 2007, Syngenta File No. T010135-04-REG Syngenta File No SAN837/6678; VV-334321
Guideline(s):	SANCO/825/00 rev.7; SANCO/3029/99 rev.4
Deviations:	Not mentioned in Registration Report
GLP:	Yes
Acceptability:	Yes

Materials and methods

Air was drawn through an OVS (Occupational Safety and Health Administration (OSHA) Versatile Sampler) tube containing two layers of Tenax adsorbent at a rate of 0.25 L min^{-1} for a period of up to six hours, using a pre-calibrated motorised pump. After this time period the Tenax absorbent, both layers, were removed from the tube and residue of dicamba, separately for each layer, was desorbed by ultrasonication in acidified acetonitrile. An aliquot of the acidified acetonitrile solution was then evaporated to dryness before being redissolved in acetone. This acetone sample was derivatised to form the tert-butyl dimethylsilyl ester using N-(tert-Butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA). Final determination was by negative-ion chemical ionisation gas liquid chromatography with mass selective detection (NICI GC-MSD).

Acquisition parameters

Compound name	Low mass resolution	SIM	MODE
Dicamba	Yes	Target ion	184 *m/z*
		Qualifier 1	185 *m/z*
		Qualifier 2	186 *m/z*
		Retention time	10.2 min

Chromatography conditions

Column	Varian CPSIL-8 (30.0 m x 0.25 mm i.d., df = 0.25 µm)
Injection port	Splitless glass wool plug
Carrier gas and head pressure	Helium at 1.0 mL/min constant flow
Injection mode	Pulsed (pulse pressure 30.0 psi)
Purge time	2 min
Purge flow	50 mL/min
Injection volume	1 µL

Injector temperature	275°C
Detector temperature	300°C
Transfer line temperature	280°C
Ion source temperature	150°C
Quadrupole temperature	106°C
Temperature programme	60°C (hold for 1 minute), 20°C/min to 300°C (hold for 1 minute)
MSD conditions	
Mode	Negative CI
Reagent gas	Methane
Electron energy	Maximum 230 eV (set by autotune)
System calibration	Autotune

Results and discussions

The percentage recovery obtained for each sample (separately for the upper and lower adsorbent layers) was calculated and these results were used to assess the relative standard deviation and limit of quantification of the analytical method. The mean recovery was in the range of 70 -120%.

Repeatability of this method was demonstrated by the standard deviation of the recovery values given in Table A 12. The relative standard deviation of recovery data obtained is within the guideline of ≤20%. This method is adequate for determining dicamba residues in air.

Table A 12: Recovery results from method validation of dicamba using the analytical method

Conditions	Fortification level (ng/L)	Recovery (%)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Dicamba (*m/z* 184)					
33-34°C and 77-85% humidity 6 hour monitoring	Control	<LOQ, <LOQ*			
	2.0** (upper layer)	90, 93, 87, 84, 81	87	6	81-93
	20 (upper layer)	81, 91, 93, 92, 87	89	6	81-93
		Overall	**88**	**5**	**81-93**
	2.0** (lower layer)	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ	ND	ND	ND
	20 (lower layer)	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ	ND	ND	ND
Dicamba (*m/z* 185)					
33-34°C and 77-85% humidity 6 hour monitoring	Control	<LOQ, <LOQ*			
	2.0** (upper layer)	90, 93, 88, 85, 81	87	5	81-93

Conditions	Fortification level (ng/L)	Recovery (%)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
	20 (upper layer)	79, 93, 91, 92, 82	87	7	79-93
		Overall	**87**	**6**	**79-93**
	2.0** (lower layer)	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ	ND	ND	ND
	20 (lower layer)	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ	ND	ND	ND
Dicamba (*m/z* 186)					
33-34°C and 77-85% humidity 6 hour monitoring	Control	<LOQ, <LOQ*			
	2.0** (upper layer)	87, 87, 84, 83, 83	85	3	83-87
	20 (upper layer)	81, 91, 94, 92, 86	89	6	81-94
		Overall	**87**	**5**	**81-94**
	2.0** (lower layer)	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ	ND	ND	ND
	20 (lower layer)	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ	ND	ND	ND

* Two control samples were analysed with each analytical batch. No residues were measured above the LOD in any of the samples. All recovery data were generated using non-matrix matched standards.
** Limit of quantification, defined by the lowest validated fortification level.
ND Not determined (insufficient data points)

Table A 13: Characteristics for the analytical method used for validation of dicamba residues in animal matrices

	Dicamba	DCSA (NOA414746)
Specificity	GC-MS as a detection technique with two additional fragment ions (m/z > 100) is considered to be highly specific and therefore according to the guidance (see guidance section of this summary) further confirmation is not required. No significant interferences arising from the matrice, the lab ware, reagents or solvents tested have been observed at the retention time of interest analyte. A mass spectrum was provided to justify the selection of the additional ions.	
Calibration (type, number of data points)	The detector showed linear response for dicamba in the range from 0.625 ng/mL to 50 ng/mL (equivalent to 0.625 pg to 50 pg injected on column when using a 1 µL injection volume) with a correlation coeficient 1 (for target ion m/z = 184 and both qualifier ions m/z = 185 and 186). Standards at 5 different concentration levels (n = 5) were injected in triplicate and the mean response plotted against amount injected.	
Calibration range		
Assessment of matrix effects is presented	-	

	Dicamba	DCSA (NOA414746)
Limit of determination/quantification	The limit of quantification (LOQ) is 2 µg/m^3 (or 0.002 µg/L), equivalent to 0.18 µg dicamba adsorbed on the Tenax adsorbent. LOQ comply with the concentration calculated from the $AOEL_{systemic}$. The limit of detection (LOD) was estimated to be 0.037 ng/L in air (or 0.037 µg/m^3 air) based on 0.25 L/min air flow and 6 hour sampling for the dicamba target ion. The LOD for the dicamba confirmatory ions was 0.067 ng/L and 0.052 ng/L in air. No residues were detected in the control samples above the LOD i.e. residues were less than 30 % of the LOQ. Residues of dicamba were detected above the LOD but below the LOQ (i.e less than 10% of applied compound) in the lower sorbent layer in 3 of the 5 replicates for the 20 ng/L fortifications; it means no signifiant breakthrough occurs.	

Conclusion

This method complies with EU guidelines SANCO/3029/99 rev. 4, SANCO/825/00 rev. 7 and US EPA guideline OPPTS 850.7100.

A 2.3.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted.

A 2.3.2.7 Other Studies/ Information

No new or additional studies have been submitted.