REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: ADM.4651.H.1.A

(former A18032E)

Product name: NIKITA

Chemical active substances:

Dicamba, 312.5 g/kg
Mesotrione, 150 g/kg
Nicosulfuron, 100 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Sponsor: ADAMA

Applicant: ADAMA

Submission date: June 2020

MS Finalisation date: March 2022 (initial Core Assessment)

June 2022 (final Core Assessment)

Version history

When	What
June 2020	Applicant initial dRR
March 2022	Initial assessment by the zRMS The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information is ~~struck through and shaded for transparency~~.
June 2022	Final report (Core Assessment updated following the commenting period). No additional information or assessments after the commenting period.

ADAMA use the code ADM.4651.H.1.A for the formulation but for consistency the former Syngenta code A18032E is used throughout the dRR.

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5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substances and relevant impurities in the plant protection product.

Noticed data gaps are: none

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.

Noticed data gaps are:

- A method for determination of dicamba in body fluids and tissues is required and should be provided at the renewal of dicamba.

Commodity/crop	Supported/ Not supported
Maize	Supported

zRMS comments:

Dicamba
The analytical methods were evaluated by RMS-Denmark (2007).
According to the EFSA Journal 2011;9(1):1965:
"A GC-MS method is available for plants that claims to analyse both free and conjugated dicamba, however the hydrolysis step is not validated, therefore a data gap has been identified. A method of analysis for animal products is not available and a data gap is identified as MRLs are proposed. For soil a GC-MS method is available to analyse for dicamba and DCSA. In water GC-MS methods are available for dicamba, DCSA and 5-OH-dicamba. It should be noted however, that as the methods for plants and soil contain a hydrolysis step they are not specific for dicamba and its salts as they will also hydrolyse esters if dicamba had been applied in an ester form. The air method is not fully validated, therefore a data gap has been identified. A method of analysis for body fluids and tissues is not required as the active substance is not classified as toxic or very toxic."

Analytical methods for residues (Regulation (Annex IIA, point 4.2)

Residue definitions for monitoring purposes

Food of plant origin	Dicamba and its salts and conjugated dicamba expressed as dicamba
Food of animal origin	Dicamba and its salts and conjugated dicamba expressed as dicamba
Soil	Dicamba, DCSA and their salts
Water surface	Dicamba, DCSA and their salts
drinking/ground	Dicamba, DCSA and their salts
Air	Dicamba

Monitoring/Enforcement methods

Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)	REM 193.01: Extraction was performed with 1 N hydrochloric acid and the extract was brought to > pH 8 by addition of 4 N potassium hydroxide. After centrifugation an aliquot is acidified and partitioned with diethyl ether. Dicamba is converted to dicamba methyl derivative by methylation with iodomethane. Extracts were cleaned-up using a silica gel column. Determination was performed by GC-MS using SIM. LOQ was 0.01 mg/kg for maize. Open for validation of the hydrolysis step.
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)	Open
Soil (analytical technique and LOQ)	Dicamba: GC-MS (LOQ: 0.01 mg/kg) DCSA: GC-MS (LOQ: 0.01 mg/kg)

Water (analytical technique and LOQ)	Drinking water: Dicamba: GC-MS (LOQ: 0.05 g/L) DCSA: GC-MS (LOQ: 0.05 g/L) 5-OH-dicamba: GC-MS (LOQ: 0.05 g/L)* Surface water: Dicamba: GC-MS (LOQ: 0.1 g/L) DCSA: GC-MS (LOQ: 0.1 g/L) 5-OH-dicamba: GC-MS (LOQ: 0.1 g/L)* * Not a part of the residue definition for monitoring
Air (analytical technique and LOQ)	Open
Body fluids and tissues (analytical technique and LOQ)	Not required as the active substance is not classified as toxic or very toxic.

Analytical methods for the determination of residues of dicamba and its metabolites in plant matrices are available and have been presented in the draft Assessment Report for dicamba (Vol.3, Section B.5.2, February 2007) and in the addendum to DAR (Vol.3, Section B.5.2, November 2010). During the peer review under Directive 91/414/EEC, the residue method REM 193.01 was demonstrated to be suitable for the determination of dicamba and its conjugates and was validated in high water- (pasture, maize plant), high starch- (maize grain), high oil- (rape seed), high acid-content matrices (orange) and dry matrices (maize straw), achieving a LOQ of 0.01 mg/kg. Suitable ILV data were provided for high water- (pasture) and high starch-content matrices (maize grain).
New analytical methods for the determination of

- dicamba residues in animal matrices,
- dicamba and NOA414746 residues in soil,
- dicamba and dicamba metabolite NOA414746 (DCSA) in water samples
- dicamba in air

have been provided by Applicant. These data are currently under evaluation for the renewal of approval of the active substance, dicamba. For the detailed evaluation of new studies it is referred to Appendix 2.
The studies are acceptable.

According to the EFSA Journal 2011;9(1):1965 a method of analysis for body fluids and tissues is not required as the active substance is not classified as toxic or very toxic.
However, in Commission Regulation (EU) No 283/2013 it is stated that "…*methods, with a full description, shall be submitted for the analysis in body fluids and tissues for active substance and relevant metabolites*".
In our opinion the analytical method for the determination of residues in body fluids and tissues is required and should be provided at the renewal of the active substance.

Mesotrione
The analytical methods were evaluated and validated in the RAR (2015).
The residue definition for enforcement and risk assessment is proposed as mesotrione only in cereal grains and pulses and oilseeds.
The residue definitions for animal commodities are provisionally not required for the representative use on maize.
In the EFSA Journal 2016;14(3):4419 - "*Peer review of the pesticide risk assessment of the active substance mesotrione*" it was concluded that mesotrione residues can be monitored in food and feed of plant origin by the QuEChERS method (LC-MS/MS) with LOQs of 0.01 mg/kg in each commodity group. Residue monitoring method for food of animal origin is not required as no MRLs were set, however mesotrione can be determined in food and feed of animal origin by the QuEChERS method (LC-MS/MS) with LOQs of 0.01 mg/kg in all animal matrices.
Residues of mesotrione and its metabolites AMBA and MNBA in soil can be monitored by LC-MS/MS with LOQs of 0.002 mg/kg for all three compounds. Appropriate LC-MS/MS method exists for monitoring residues of mesotrione and its metabolites AMBA and MNBA in ground water and surface water with a LOQ of 0.05 µg/L for all compounds. It should be mentioned however, that pending on the final residue definition for monitoring for the environmental compartment, additional data might be required. Residues of mesotrione in air can be monitored by LC-MS/MS with a LOQ of 0.45 µg/m^3.
The QuEChERS method (LC-MS/MS) can be used for monitoring mesotrione residues in blood with a LOQ of 0.01 mg/kg.

Analytical methods for residues (Regulation (EU) N° 283/2013, Annex Part A, point 4.2 & point 7.4.2)

Residue definitions for monitoring purposes

Food of plant origin	Mesotrione
Food of animal origin	Not required (provisional)
Soil	Mesotrione and metabolite A (open)
Sediment	Mesotrione and metabolite A (open)
Water surface	Mesotrione and metabolite A (open)
drinking/ground	Mesotrione and metabolite A (open)
Air	Mesotrione
Body fluids and tissues	Mesotrione

Monitoring/Enforcement methods

Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)	QuEChERS LC-MS/MS (LOQ 0.01 mg/kg) Maize forage (high water), maize kernel (dry), oilseed rape (high oil) and orange (high acid) ILV in maize forage (high water) and maize kernel (dry) LC-MS/MS
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)	QuEChERS LC-MS/MS (LOQ 0.01 mg/kg) in all animal matrices
Soil (analytical technique and LOQ)	Single method LC-MS/MS: Mesotrione: LOQ 0.002 mg/kg MNBA: LOQ 0.002 mg/kg AMBA: LOQ 0.002 mg/kg
Water (analytical technique and LOQ)	Single method LC-MS/MS (surface and ground water, ILV available for drinking water) Mesotrione: LOQ 0.05 µg/L MNBA: LOQ 0.05 µg/L AMBA: LOQ 0.05 µg/L
Air (analytical technique and LOQ)	LC-MS/MS Mesotrione: LOQ 0.45 µg/m^3
Body fluids and tissues (analytical technique and LOQ)	QuEChERS LC-MS/MS (LOQ 0.01 mg/kg in blood)

Additional analytical methods for mesotrione have been evaluated in Appendix 2.

Conclusions:
Sufficiently validated analytical methods are available to control mesotrione and metabolites in plant matrices, animal matrices, in soil, in water, in air and in human tissues and body fluids according to the definitions.

Nicosulfuron
The analytical methods were validated and evaluated by United Kingdom, 2007.
The residue definition for enforcement and risk assessment is proposed as nicosulfuron.
The residue definitions for animal commodities are unable to propose, however not required for representative use.

According to the EFSA Journal 2011;9(1):1965: *"Adequate analytical methods are available for the determination of nicosulfuron residues in food of plant origin (in grain and fodder maize), soil, water and air. As the residue definition for all matrices is nicosulfuron, further methods of analysis and validation data for impurities and metabolites are not*
required.
For the determination of residues of nicosulfuron in maize shoots (sprouts), grain and whole plants, a series of multistage methods based on extraction, partition and clean-up were used. Either HPLC or GC determination methods were used, with either LC/MS or GC/MS being used for confirmatory determination. Acceptable validation data were submitted for analysis of active substance, the validation data submitted for metabolite analysis were less satisfactory, however the residue definition for plant and products is 'parent nicosulfuron', therefore the lack of validation data in these cases is not a critical issue. Recovery data were obtained for nicosulfuron at levels

between 0.01 and 0.10 mg/kg with acceptable mean recoveries and RSD values. Only single methods for the determination of residues are available."
In EFSA Journal 2012;10(12):3048 it is stated that "*During the peer review under Directive 91/414/EEC, an analytical method using HPLC-MS/MS, and its ILV were evaluated and validated for the determination of parent nicosulfuron in plant matrices with an LOQ of 0.01 mg/kg in dry commodities (maize corn) and in maize straw (United Kingdom, 2007). Although this analytical method was previously considered acceptable by EFSA (2007b), the method was now re-evaluated according to the current guidelines. As this analytical method was validated only for one mass transition, the analytical method was not confirmed and confirmatory data are required.*
The multi-residue QuEChERS using diatomaceous earth clean up in combination with LC-MS/MS method, as described by CEN (2008), is also available to analyse the parent nicosulfuron in dry commodities. Nevertheless, the validation data reported are too limited to conclude on the validity of this analytical method (EURL, 2012).
Hence it is concluded that nicosulfuron can be enforced in food of plant origin with an LOQ of 0.01 mg/kg in dry commodities but confirmatory data are still required. Additionally, an analytical method, an ILV and a confirmatory method fully validated in high water content commodities are required.
Methods for enforcement of residues in food of animal origin
During the peer review under Directive 91/414/EEC, an analytical method using HPLC-UV for the determination of parent nicosulfuron was evaluated in food of animal origin but no validation data were submitted (United Kingdom, 2005).
However, considering that there is no significant intake of residues by livestock, no residue definition and no MRLs are proposed for commodities of animal origin (section 3.2). Therefore, an analytical method for enforcement of residues in food of animal origin is not necessary."

Analytical methods for residues (Regulation (Annex IIA, point 4.2)

Residue definitions for monitoring purposes

Food of plant origin	Nicosulfuron
Food of animal origin	Not proposed
Soil	Nicosulfuron
Sediment	Nicosulfuron
Water surface	Nicosulfuron
drinking/ground	Nicosulfuron
Air	Nicosulfuron
Body fluids and tissues	Nicosulfuron

Monitoring/Enforcement methods

Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)	HPLC-MS/MS, LOQ = 0.01 mg/kg
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)	Not required
Soil (analytical technique and LOQ)	Nicosulfuron: LC/MS, LOQ = 0.05 µg/kg
Water (analytical technique and LOQ)	Nicosulfuron: HPLC/UV, LOQ = 0.05 µg/L Confirmatory method: LC-DAD. LOQ = 0.05 µg/L
Air (analytical technique and LOQ)	Nicosulfuron: HPLC/UV. LOQ = 1.2 µg/m^3
Body fluids and tissues (analytical technique and LOQ)	Not required

EFSA concluded (EFSA, 2012) that all tentative MRLs still need to be confirmed by the following data:
- Confirmatory data for the HPLC-MS/MS method with an LOQ of 0.01 mg/kg in dry commodities;
- An analytical method, its ILV and a confirmatory method fully validated for the determination of parent nicosulfuron in high water content commodities.

Applicant submitted new analytical methods to address these EFSA requirements. For the detailed evaluation of new studies it is referred to Appendix 2. These data are currently under evaluation for the renewal of approval of the active substance, nicosulfuron. The studies are acceptable.

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 substances and/or variant in the plant protection product (KCP 5.1.1)

Method SF-568/1 for the determination of mesotrione, dicamba and nicosulfuron in A18032E
The method for the analysis of mesotrione, dicamba and nicosulfuron in the plant protection product A18032E has not been reviewed at EU level as a consequence of the review of mesotrione or dicamba or nicosulfuron. An overview on the acceptable methods and possible data gaps for analysis of mesotrione, dicamba and nicosulfuron in the plant protection product A18032E is provided as follows:

Comments of zRMS:	Acceptable validation data have been provided for the method. The method has been validated in accordance with the SANCO 3030/99 rev 4 guidance and is considered to be sufficient to determine of mesotrione, dicamba and nicosulfuron in the plant protection product A18032E.

Reference:	KCP 5.1.1/01
Report	Adolph S. (2012) Analytical Method SF-568/1 - mesotrione/dicamba/nicosulfuron WG (15731.25/10) in formulation, by HPLC, Syngenta Crop Protection, Münchwilen AG, Switzerland. Unpublished Report No. 10493506. Issued date 29.03.2012, Syngenta File No. A18032E_10062
Guideline(s):	none
Deviations:	none
GLP:	no
Acceptability:	Yes

Reference:	KCP 5.1.1/02
Report	De Benedictis S. (2013) A18032E – Validation of Analytical Method SF-568/1, Syngenta Crop Protection Münchwilen AG Münchwilen, Switzerland. Unpublished Report No. 10528232. Issued date 24.04.2013, Syngenta File No. A18032E_10063
Guideline(s):	SANCO 3030/99 rev. 4
Deviations:	none
GLP:	yes
Acceptability:	Yes

Materials and methods
Mesotrione, dicamba and nicosulfuron are determined in A18032E using liquid chromatography using a reversed phase column, an acetonitrile : water (1% phosphoric acid) eluent and UV detection at 260 nm. Quantification is achieved by comparison of ratios of peak areas to those of a standard solution (internal standard method).

(Adolph S., 2012)

Validation - Results and discussions
Full validation of the method SF-568/1 has been conducted. The method has been shown to be specific for the determination of mesotrione, dicamba and nicosulfuron in product A18032E and no significant interference was observed. Based on the results for repeatability, recovery, linearity and specificity, precision and accuracy of the method are established. Therefore, the method is suitable for the specific, accurate and precise determination of mesotrione, dicamba and nicosulfuron in product A18032E.

(De Benedictis S., 2013)

Table 5.2-1: Validation of Method SF-568/1 for the determination of active substances mesotrione dicamba and nicosulfuron in A18032E

	Mesotrione	Dicamba	Nicosulfuron
Author(s), year	De Benedictis S., 2013	De Benedictis S., 2013	De Benedictis S., 2013
Principle of method	HPLC and UV detection	HPLC and UV detection	HPLC and UV detection
Linearity n = 6 Tested between 50% - 150% of the declared content	r = 0.99997 y = 1.009x + 0.370	r = 0.99997 y = 0.997x + 1.940	0.99997 y = 1.009x + 0.096
Precision as repeatability n = 12	S_{rel} (%RSD): 0.70 mean concentration = 15.05 % w/w	S_{rel} (%RSD): 0.43 mean concentration = 31.03 % w/w	S_{rel} (%RSD): 0.61 mean concentration = 9.93 % w/w
Accuracy as recovery n = 4 Tested between 70% and 130% of declared content	mean recovery: 101.4%	mean recovery: 100.9%	mean recovery: 101.1 %
Interference/ Specificity	no significant interference, SF-568/1 is specific for the determination of mesotrione	no significant interference, SF-568/1 is specific for the determination of mesotrione	no significant interference, SF-568/1 is specific for the determination of mesotrione
Comment	SF-568/1 is acceptably validated	SF-568/1 is acceptably validated	SF-568/1 is acceptably validated

Conclusion
The method is suitable for the specific, accurate and precise determination of mesotrione, dicamba and nicosulfuron in A18032E.

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

An overview of the acceptable methods and possible data gaps for analysis of relevant impurities in the plant protection product A18032E is provided. A18032E contains the active substance mesotrione which contain relevant impurities:
Mesotrione relevant impurities:

- R287431 (6-methanesulfonyl-7-nitro-9-oxo-9H-xanthene-1-carbonitrile)
- R287432 (6-methanesulfonyl-9-oxo-9H-xanthene-1-carbonitrile)
- 1,2-dichloroethane

The methods for the analysis of mesotrione relevant impurities in the plant protection product A18032E have not been reviewed at EU level as a consequence of the review of mesotrione. An overview of the methods and possible data gaps for analysis of mesotrione relevant impurities in the plant protection product A18032E is provided.

Method SD-977/2 for the determination of R287431 (6-methanesulfonyl-7-nitro-9-oxo-9H-xanthene-1-carbonitrile) in A18032E
The relevant impurity R287431 (6-methanesulfonyl-7-nitro-9-oxo-9H-xanthene-1-carbonitrile) may be formed in trace amounts during the chemical synthesis of mesotrione technical material however, it cannot be formed from mesotrione or from other formulation components of A18032E; storage stability data for R287431 in formulated product A18032E is therefore not required.
The analytical method SD-977/2 determines the relevant impurity R287431 in mesotrione containing formulations using multiple-point external standard calibration based on liquid chromatography with mass spectroscopy detection (LC/MS). The detection is by MS/MS (tandem mass spectrometry) for a specific multiple reaction monitoring (SRM) of an analyte, therefore, high specificity for R287431 is inherent in the method. For R287431, transition of m/z 344 (parent ion) → m/z 207 (product ion) was used for

quantification. The chromatographic conditions and sample extraction procedure of the method were designed to handle water dispersible granule (WG) formulation type as demonstrated in the sample chromatograms included in the method (Syngenta file no. R287431/10003). The method allows identification and quantification of R287341 ranging from 1µg/g to 3µg/g in formulations for samples prepared as described in the method.
Full method validation of SD-977/2 was performed under GLP (Syngenta file no. R287431/10001) using formulation A18032E. Validation has demonstrated accuracy (recovery, linearity), precision (repeatability), specificity and limit of quantification of the method.
The results described in the validation report demonstrate the applicability and validity of the method SD-977/2 for the determination of R287431 in formulation A18032E.

Comments of zRMS:	The analytical method SD-977/2 (LC-MS) for the determination of R287431 relevant impurity of Mesotrion in A14203B, A13789C, A12909Q, A12738A, A15189G, A18032E, A15901A, A18219B, and A14203B formulated products, has been successfully validated according to SANCO/3030/99/rev.4. The proposed LOQ at 1 µg/g in formulation, is not low enough to support the specification for the relevant impurity according to Regulation (EU) 2017/725 which should by ≤ 0.3 µg/g in formulation however we agree with Syngenta statement that due to the extremely low concentration of R287431 in technical mesotrione and even lower concentration in formulated materials and the noise generated from the formulation matrix, quantification at such low concentrations is extremely challenging, even based on the latest technology available in mass spectrometry. Current LC/MS inlet designs and ionisation techniques result in a limit of detection (LOD) of approximately 0.1 - 0.2 µg/g for R287431 and a limit of quantification (LOQ) of approximately 1 µg/g. Taking into account the above considerations, the proposed analytical method is considered sufficiently validated.

Reference:	KCP 5.1.1/03
Report	Hager M. (2011) Analytical method SD-977/2. Syngenta Crop Protection, Inc., Greensboro, USA. Unpublished Report No. 10427012. Issued date 04.05.2011. Syngenta File No. R287431/10003
Guideline(s):	No
Deviations:	No
GLP:	No
Acceptability:	Yes

Reference:	KCP 5.1.1/04
Report	Hager M. (2011a) Validation of analytical method SD-977/2 – R287431 in A14203B, A13789C, A14351BX, A12909A, A15189G, A12738A and A18219B. Syngenta Crop Protection, Inc., Greensboro, USA. Unpublished Report No. 10427878. Issued date 12.05. 2011. Syngenta File No. R287431/10001
Guideline(s):	SANCO 3030/99 rev. 4
Deviations:	none
GLP:	yes
Acceptability:	Yes

To show the validity of the method for A18032E, a specificity test was performed on A18032E to demonstrate that none of the formulation components, active substances or by-products interferes with R287431.

Reference:	KCP 5.1.1/05
Report	Hager M. (2013) Mesotrione – Relevant Impurity in A18032E. Syngenta Crop Protection, Inc., Greensboro, USA. Unpublished Report No. 10538107. Issued date 02.07.2013. Syngenta File No. A18032E_10061

Guideline(s):	SANCO 3030/99 rev. 4
Deviations:	none
GLP:	no
Acceptability:	Yes

Reference:	KCP 5.1.1/06
Report	Hager M. (2017) A18032E- Statement on Validation of Analytical Method SD-977 /2 for the Determination of R287431 (Xan-1) in Formulation A18032E. Syngenta Crop Protection, Inc., Greensboro, USA. Unpublished Report No. 300074792. Issued date 03.02.2017. Syngenta File No. A18032E_10366
Guideline(s):	SANCO 3030/99 rev. 4
Deviations:	none
GLP:	no
Acceptability:	Yes

Reference:	KCP 5.1.1/07
Report	A18032E - Response to the Greek Regulatory Authority Concerning Relevant Impurity R287431, Hager M. *et al*, 2018, Syngenta File N.o. A18032E_10452
Guideline(s):	SANCO 3030/99 rev. 4
Deviations:	None stated
GLP:	No
Acceptability:	Yes

Materials and methods

The relevant impurity R287431 in A18032E is separated by HPLC on a BEH C18 column (100 mm x 2.1 mm). For elution a gradient of 5 mM ammonium formate in water and 1% formic acid in acetonitrile was used, followed by spectrophotometric detection at 270 nm (DAD). Identification was by ion trap mass spectroscopy with a source temperature of 150°C. Parent ion was m/z 344 and daughter ion at 207 m/z. Quantification is by comparison of peak areas ratios to those of a reference solution.

(Hager M., 2011)

Validation of method SD-977/2 - Results and discussions

Full validation of the method SD-977/2 has been conducted. The method has been shown to be specific for the determination of R287431 in product A18032E and no significant interference was observed. Based on the results for repeatability, recovery, linearity and specificity, precision and accuracy of the method are established. Therefore, the method is suitable for the specific, accurate and precise determination of R287431in product A18032E. A summary of the validation data is given in Table 5.2 2.

(Hager M., 2011a, 2013 & 2017)

Table 5.2-2: Validation of Method SD-977/2 for the determination of R287431 in A18032E

	R287431: maximum content in A18032E 0.30mg/kg (2mg/kg in technical mesotrione)
Author(s), year	Hager M., 2011a, 2013, 2017
Principle of method	High Performance Liquid Chromatography/Mass Spectroscopy (HPLC/MS)
Linearity n = 3 linear between 70 and 130% of the target concentration (2µg/g) of R287431	Range r = 0.98 - 0.99 y = 9481x + 42.58 (e.g. A14203B) Correlation coefficient: r = 0.997161, r^2 = 0.994330 Calibration curve: 10377.3 * x + -3.78906 (A18032E)
Precision as repeatability n = 6	Range S_{rel} (%RSD): 4.0 - 8.9 % mean concentration: 0.018 - 0.020 µg/ml

	R287431: maximum content in A18032E 0.30mg/kg (2mg/kg in technical mesotrione)
Accuracy n = 3 determined between 70 and 130% of the target concentration (2µg/g) of R287431	Range recovery: 90 % (A18032E)
Interference/ Specificity	No significant interference was observed. The analytical method is able to separate the impurity R287431 from the formulation blank and solvent with no significant co-elution.
LOQ[1]	The validation data prove that the limit of quantification for R287431 is established at concentration of ± 2 µg/g.
Comment	The method is suitable for the specific, accurate and precise determination of the relevant impurity R287431 in A18032E.

[1] Due to the extremely low concentration of R287431 in technical mesotrione and even lower concentration in formulated materials, quantification at such low concentrations is extremely challenging, even based on the latest technology available in mass spectrometry. Current LC/MS inlet designs and ionisation techniques result in a limit of detection (LOD) of approximately 0.2ug/g for R287431. Therefore, attempting quantifiable levels below 2ug/g is unreliable. Fundamentally, this is a consequence of the chemical structure of R287431; the molecule is difficult to ionise due to its chemical structure (ionisation is required to perform mass spectrometry). So the LC/MS method effectively only has access to a very small fraction of the injected quantity of R287431 to detect and quantify. As a result the sensitivity of the method is significantly reduced and this is the key technical reasoning behind the LOQ being set at 1 µg/g.

Additional Validation Data and Justification of the 1 µg/g Supportable LOQ (Hager M. et al, 2018, Syngenta File N.o. A18032E_10452)

A validated analytical method for the determination of R287431 in the mesotrione formulation A18032E is available (SD-977/2). Method SD-977/2 may be used with all mesotrione formulations as demonstrated by validation studies for eight different formulations. Method SD-977/2 uses MRM (multiple reaction monitoring) detection which gives high specificity for R287431 determination with parent (m/z 344) and daughter (m/z 207) ions being monitored. SD-977/2 also utilises standard addition which means at least three calibration points are conducted for every analysis conducted and the method is therefore considered self-validating.

Method SD-977/2 has been successfully validated according to SANCO 3030 rev.5 2017 for eight different mesotrione formulations, including A14203B, a water dispersible granule, expected to behave similarly to A18032E. SD-977/2 is specific, accurate and precise for the determination of R287431 in A14203B. This has been confirmed in supplemental non-GLP validation as reported in Statements (Syngenta report numbers A18032E_10061 and A18032E_10366). The limit of quantification (LOQ) of R287431 is considered to be 1 µg/g or 1ppm which Syngenta considers to be appropriate for the analysis of R287431 in mesotrione formulations.

In theory, because of the scaling factor i.e. from mesotrione technical material to formulated product, then a lower LOQ could be hypothesized. For A18032E, containing 150g/l of mesotrione, the theoretical maximum concentration of R287431 is:

2ppm x 150 / 1000 = 0.3ppm

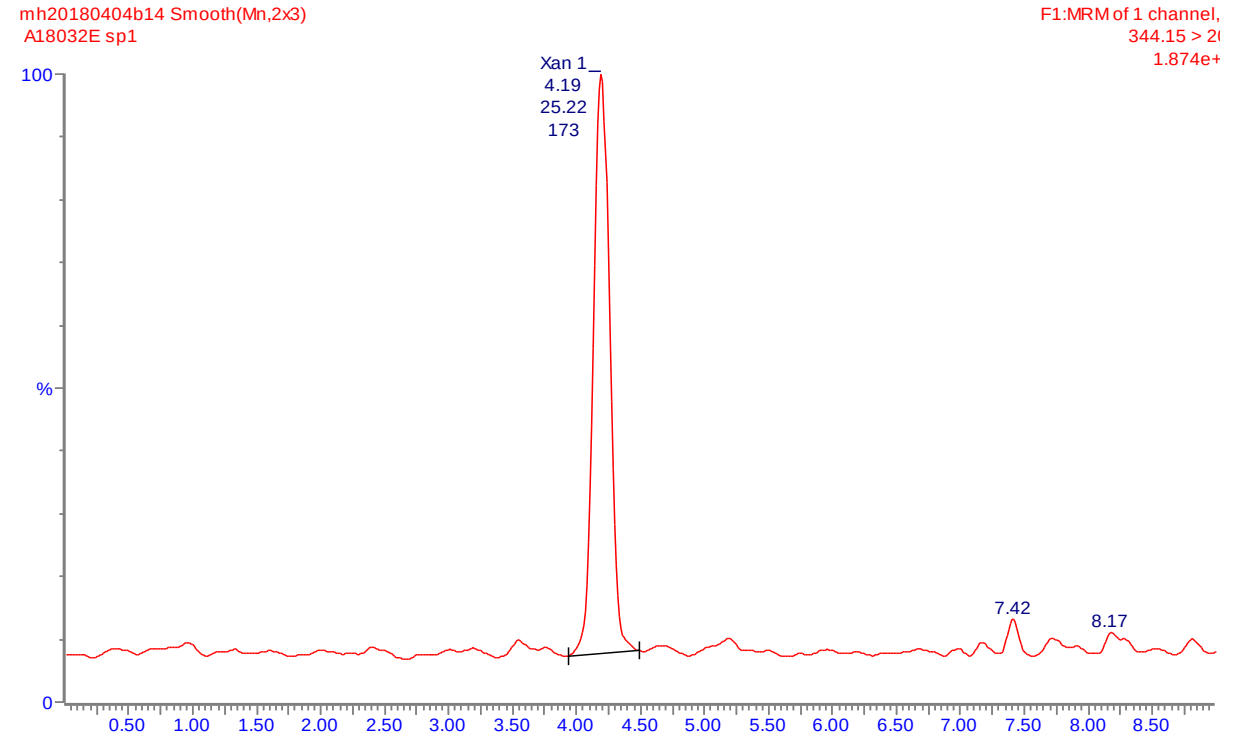
Therefore, an LOQ of 0.3ppm or less could be targeted, by simple proportion.

However, given that Syngenta guarantees that R287431 will always be below 2ppm in technical mesotrione and that this impurity cannot form in mesotrione formulations; R287431 will always be lower than 2ppm in formulated material and in fact very much lower by the appropriate scaling factor as demonstrated for A18032E. Therefore, Syngenta would argue that an LOQ lower than 1ug/g or 1ppm is not strictly necessary.

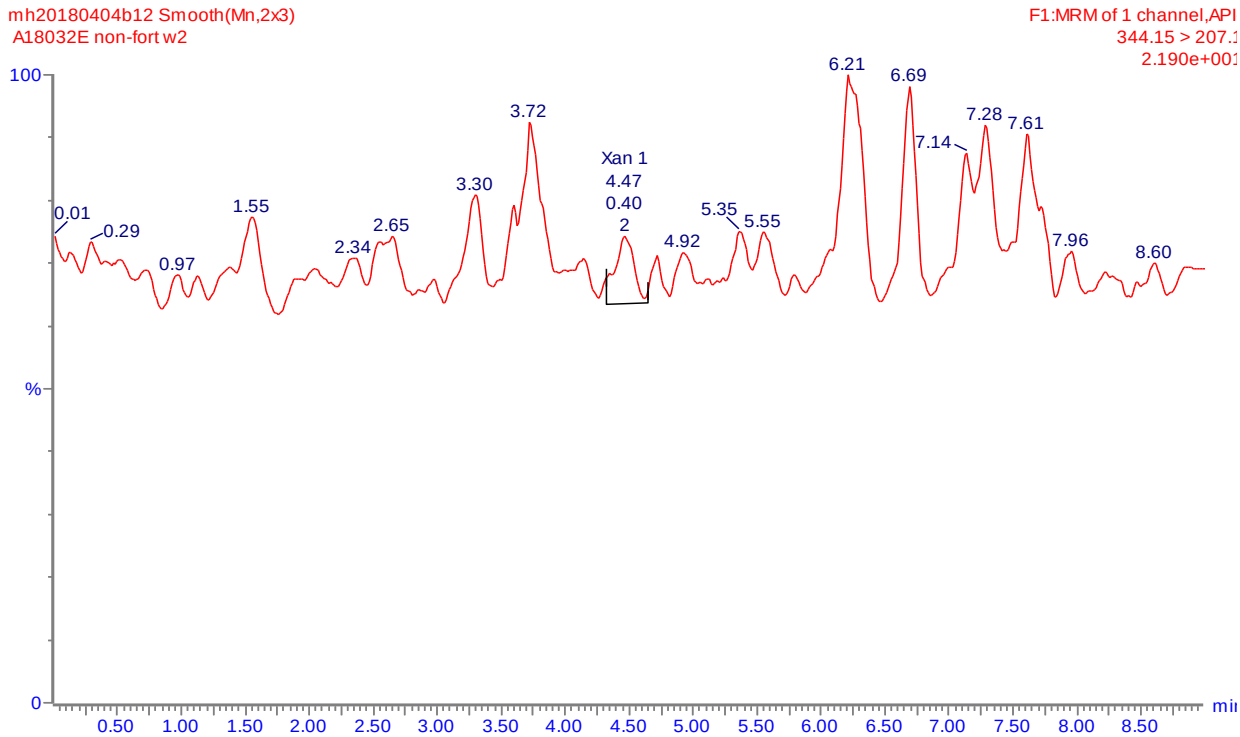
The following chromatograms demonstrate that the concentration of R287431 in an unfortified batch of A18032E is below the LOQ of the method (1µg/g or 1ppm).

Chromatograms to demonstrate that R287431 is below the LOQ of 1 µg/g (1 ppm) in A18032E

Test material = A18032E, LIMS no. 1013134, R287431, fortified at 1 µg/g



Test material = A18032E, LIMS no. 1013134, Non-fortified

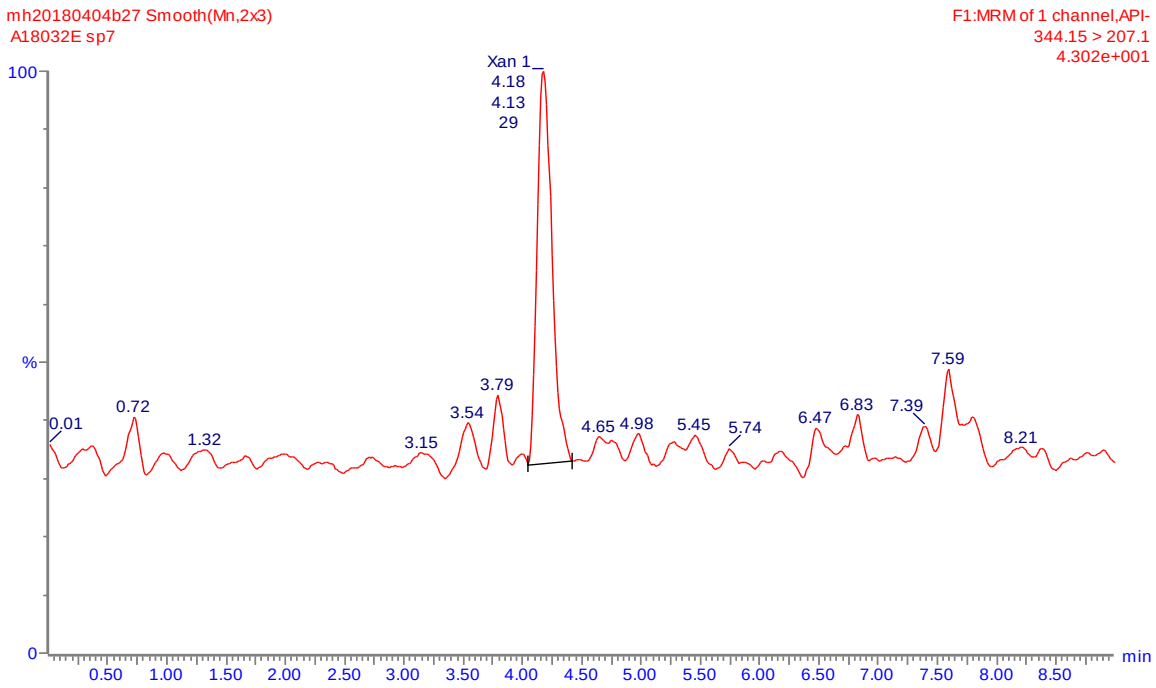


In addition, there are definite technical limitations associated with the analysis of R287431 in mesotrione formulations which mean that Syngenta believes that the method (SD-977/2) is not sufficiently robust to be able to define a sub-ppm LOQ. R287431 is not easily ionized by mass spectroscopy, giving little

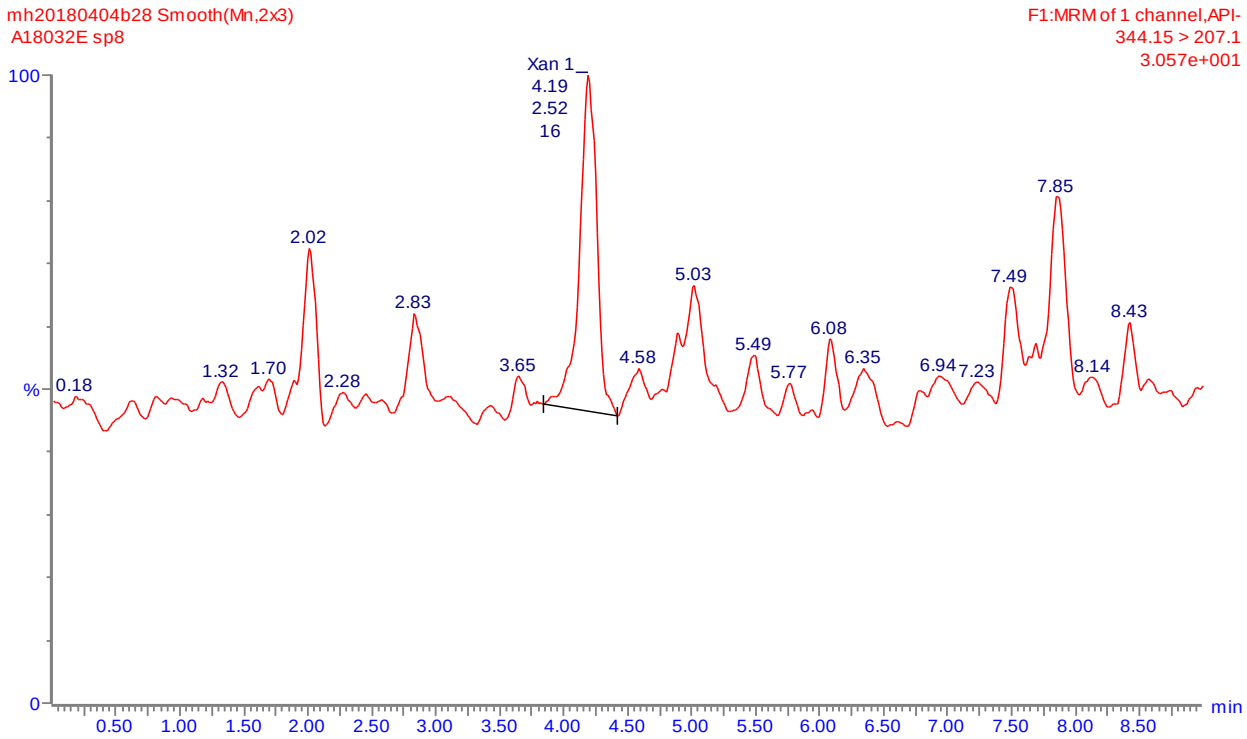
response by the more commonly used electrospray ionization. It must, instead, be analyzed by atmospheric pressure ionization mode. Thus, there are limits on mass spectrometry sensitivity for this compound. It has been found that 1 ppm is the practical LOQ with current state of the art, triple quad mass spectrometry detection.

Chromatograms to demonstrate that 0.2ppm response for R287431 is below the LOQ of the method

Test material = A18032E, LIMS no. 1013134, R287431, fortified at 0.2 µg/g (1st of 6 independently prepared solutions)



Test material = A18032E, LIMS no. 1013134, R287431, fortified at 0.2 µg/g (2nd of 6 independently prepared solutions)



The above chromatograms illustrate the 0.2ppm response for R287431 with respect to noise peaks that come and go with subsequent injections of test material. The average R287431 signal response from 13 injections of six independently prepared solutions was 3.65 area counts. By comparison, the noise response ranged 0.3 – 1.1 area counts and the typical noise estimated at 0.71 area counts. Therefore, applying the common practice of testing LOQ with criteria of 10x signal to noise, the 0.2 ppm test level fails to meet this LOQ test. Moreover, the 0.2ppm response for R287431 fails to meet SANCO/3030/99 rev.5 guidelines for LOQ. The 128% mean recovery on 0.2 µg/g fortifications (on 13 determinations) fails to meet the 75-125% requirement. Moreover, the 40.5% RSD obtained fails to meet the 20.4% RSD requirement, per Horwitz calculation, according to the SANCO guideline.

Moreover, according to the latest SANCO guidance, SANCO/3030/99 rev.5 2017 ('Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements….', the following text is noted:

> *"However, in certain cases the content of the active substance in the plant protection product can be too low in order to determine a relevant impurity at the level derived from the maximum content in the technical active substance. In this case, the validation must be performed at the lowest possible concentration. However, for relevant impurity it is necessary to demonstrate that it is not technically possible to reach the theoretically required LOQ (with chromatogram or some experiment data) and to provide a (eco) toxicological argumentation demonstrating that the reached LOQ is acceptable"*

Due to the extremely low concentration of R287431 in technical mesotrione and even lower concentration in formulated materials, plus the noise generated from the formulation matrix, quantification at such low concentrations is extremely challenging, even based on the latest technology available in mass spectrometry. Current LC/MS inlet designs and ionisation techniques result in a limit of detection (LOD) of approximately 0.1 - 0.2µg/g for R287431 and a limit of quantification (LOQ) of approximately 1 µg/g. Therefore, attempting quantifiable levels much below 1µg/g (1ppm) is unreliable as demonstrated in the data reported here. As mentioned above, solvent blanks gave noise response, 0.3 – 1.3 area counts (0.711 average measured). By comparison the 0.2ppm R287431 fortification (of A18032E), mean response, 3.645 area counts, is less than 10 fold signal to noise acceptance criteria.

Conclusions

In summary, Syngenta believes that the method (SD-977/2) is not sufficiently robust to be able to define a sub-ppm LOQ. Quantification at such low concentrations is extremely challenging, even based on the latest technology available in mass spectroscopy. Current LC/MS inlet designs and ionisation techniques result in a limit of detection (LOD) of approximately 0.2 µg/g for R287431. Therefore attempting measureable levels much below 1 µg/g would be unreliable.

Furthermore, Syngenta guarantees that R287431 will always be below 2ppm in technical mesotrione and that R287431 cannot form in mesotrione formulations. Therefore, R287431 will always be below 2ppm in technical material and even lower by the appropriate dilution factor in formulated mesotrione. This means that R287431 will always be lower than 0.002g/kg (2ppm) in A18032E which was established as the concentration of no (eco) toxicological concern during the EU review of mesotrione.

Therefore, Syngenta believes that an LOQ of 1ppm for the determination of R287431 in formulated mesotrione is appropriate to ensure compliance and safety with respect to A18032E and this position is validated by the current analytical method guidance SANCO/3030/99 rev.5 2015.

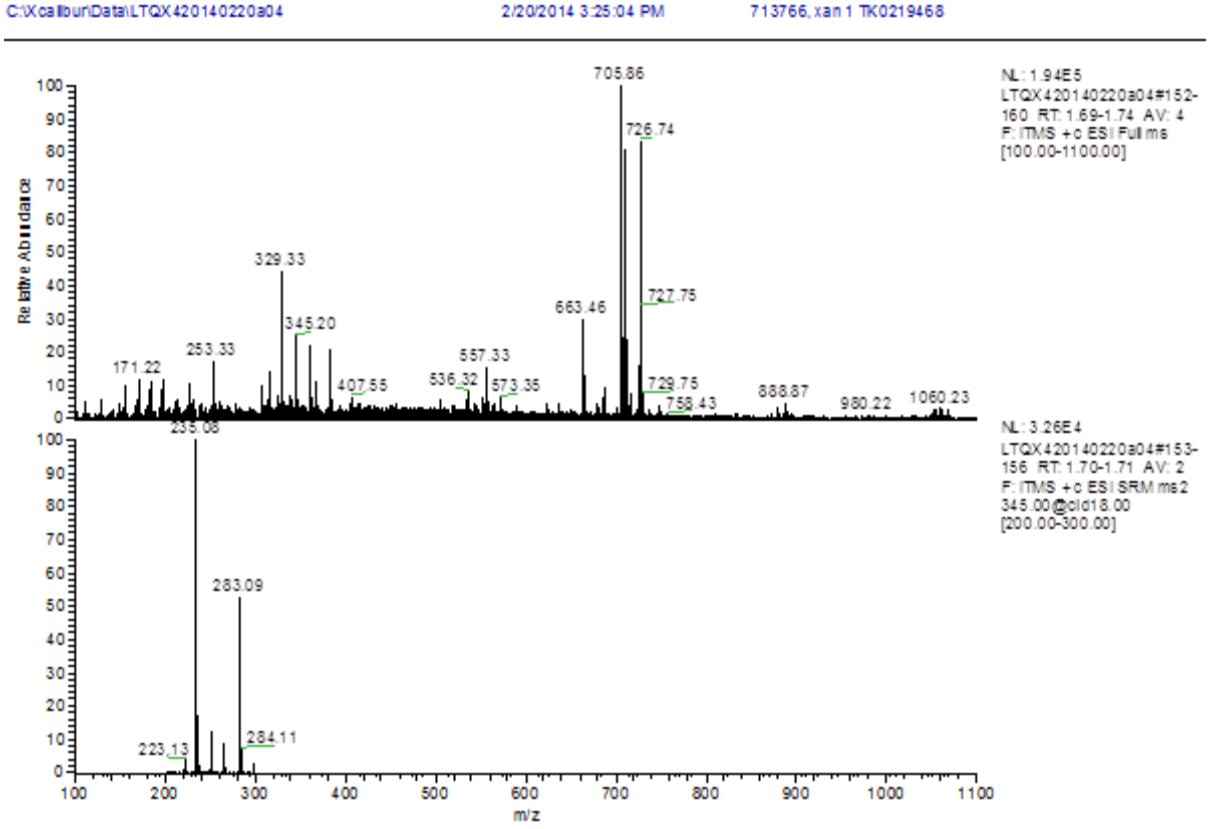
Confirmation of Identity of R287431

According to SANCO/3030/99: *Confirmatory techniques are required to support identification of the a.s. and significant and/or relevant impurities, when the primary method of determination is not GC-MS or another highly specific method such as HPLC-UV DAD.*

The analytical method SD-977/2 determines the relevant impurity R287431 in mesotrione containing formulations using multiple-point external standard calibration based on liquid chromatography with mass

spectroscopy detection (LC/MS). The detection is by MS/MS (tandem mass spectrometry) for a specific multiple reaction monitoring (SRM) of an analyte, therefore, high specificity for R287431 is inherent in the method and confirmation of identity of R287431 is inherent in the method.
As additional proof of identity i.e. Mass Spectra generated during the characterization of the reference material R2827431, Batch 713766 (Study TK0219468) are given below.

Figure 5.2-1: Mass Spectra for R287431 Analytical Standard, Batch713766 (Study TK0219468)



Conclusion

The method is suitable for the specific, accurate and precise determination of the relevant impurity R287431 in A18032E.

Method SD-1990/1 for the determination of R287432 (6-methanesulfonyl-9-oxo-9H-xanthene-1-carbonitrile) in A18032E

The relevant impurity R287432 (6-methanesulfonyl-9-oxo-9H-xanthene-1-carbonitrile) may be formed in trace amounts during the chemical synthesis of mesotrione technical material however, it cannot be formed from mesotrione or from other formulation components of A18032E; storage stability data for R287432 in formulated product A18032E is therefore not required.

The analytical method SD-1990/1 determines the relevant impurity R287432 in mesotrione containing formulations using multiple-point external standard calibration based on liquid chromatography with mass spectroscopy detection (LC/MS). The detection is by MS/MS (tandem mass spectrometry) for specific multiple reaction monitoring (MRM) of an analyte, therefore, high specificity for R287432 is inherent in the method. For R287432, a transition of m/z 300 (precursor ion) → m/z 221 (product ion) was used for quantification and a separate MRM transition of m/z 300→ m/z 209 was available as qualifier if needed. The chromatographic conditions and sample extraction procedure of the method were designed to handle a broad range of formulation types including suspo-emulsions (SE), suspension concentrates (SC), oil dispersions (OD) and water dispersible granules (WG) as demonstrated in the sample chromatograms included in the method (Syngenta file no. A13789C_50005). The method allows determination and quantification of R287342 ranging from 10 µg/g to 200 µg/g in formulations for samples prepared as described in the method.

Full method validation of SD-1990/1 was performed under GLP (Syngenta file no. A13789C_50004) using the representative formulation A13789C (mesotrione/terbuthylazine/S-metolachlor SE). Validation has demonstrated accuracy (recovery, linearity), precision (repeatability), specificity and limit of quantification of the method. It is proposed to rely on this full validation study to support the formulation A18032E, however supplementary validation data was generated for A18032E and is described below.

Method SD-1990/1 is also used for the determination of R287432 in the formulation A18032E (mesotrione/terbuthylazine SC). The different nominal concentrations of mesotrione in formulations A18032E and A13789C are not of relevance, as the sample weighing is adapted accordingly. Thus the concentration of mesotrione and its relevant impurity remains the same in the test solutions. Supplementary, non-GLP testing for specificity, recovery, precision and linearity using A18032E were also conducted and are described in a separate report (Syngenta file no. A18032E_10347).

The results described in both validation reports demonstrate the applicability and validity of the method SD-1990/1 for the determination of R287432 in the formulation A18032E.

Comments of zRMS:	The LC-MS method SD-1990/1, was fully validated for the determination of the relevant impurity R287432 in mesotrione in formulation A 13789C and has been re-validated for the determination of R287432 in product A18032E. The LC-MS method SD-1990/1 is considered fit for purpose according the guidance document SANCO/3030/99 rev. 4.

Reference:	KCP 5.1.1/0~~7~~8
Report	Huang S., (2016) ZA1296 - SD-1990/1 - Determination of R287432 in Mesotrione Related Formulations by Liquid Chromatography/Mass Spectrometry (LC/MS). Syngenta Crop Protection, Inc., Greensboro, USA. Unpublished Report No. 300068727. Issued date 10.11.2016 Syngenta File No. A13789C_50005)
Guideline(s):	No
Deviations:	No
GLP:	No
Acceptability:	Yes

Reference:	KCP 5.1.1/0~~8~~9
Report	Huang S., (2016a) Validation of analytical method SD-1990/1, Syngenta Crop Protection, Inc., Greensboro, USA. Unpublished Report No. USGR160250. Issued date 08.12.2016. Syngenta File No. A13789C_50004
Guideline(s):	SANCO 3030/99 rev. 4
Deviations:	none

GLP:	yes
Acceptability:	Yes

Reference:	KCP 5.1.1/0~~9~~10
Report	Huang S., (2016b) A18032E - Statement on Validation of Analytical Method SD-1990/1 for Determination of R287432 in Formulation A18032E (SAN837/ZA1296/nicosulfuron WG (31.25/15/10)). Syngenta Crop Protection, Inc., Greensboro, USA. Unpublished Report No. 300072567. Issued date 15.12.2016. Syngenta File No. A18032E_10347
Guideline(s):	SANCO 3030/99 rev. 4
Deviations:	none
GLP:	no
Acceptability:	Yes

Materials and methods

The relevant impurity R287432 in A18032E is separated by HPLC on a fused silica BEH-C18 column (100 mm x 2.1 mm) at 40°C. For elution a gradient of 0.1% formic acid in water and 0.1% formic acid in acetonitrile was used. Detection and identification was by ion trap mass spectroscopy after vaporizing the sample at 450°C under nitrogen. Parent ion was m/z 300 and MRM product ions are 209 m/z and 221 m/z with the latter being used for quantification.
Quantification is by comparison of peak areas ratios to those of a reference solution.

(Huang S., 2016)

Validation of method SD-1990/1 - Results and discussions

Full validation of the method SD-1990/1 has been conducted. The method has been shown to be specific for the determination of R287432 in product A18032E and no significant interference was observed. Based on the results for repeatability, recovery, linearity and specificity, precision and accuracy of the method are established. Therefore, the method is suitable for the specific, accurate and precise determination of R287432 in product A18032E. A summary of the validation data is given in Tables 5.2-3 and 5.2-4.

(Huang S., 2016a & 2016 b)

Table 5.2-3: Validation of Method SD-1990/1 for the determination of R287432 in A13789C

	R287432: maximum content in A13789C 0.075g/L, (2g/kg in technical mesotrione)
Author(s), year	Huang S., 2016a (Unpublished Report No. USGR160250)
Principle of method	Liquid Chromatography/Mass Spectroscopy (LC/MS)
Linearity n = 6 linear range 10 - 200 µg/g in formulation	r = 0.9998 y = 0.9991x + 0.0005
Precision as repeatability n = 6	S_{rel} (%RSD): 1.5% mean concentration: 40 µg/g in formulation
Accuracy n = 4	mean recovery: ~~98.4~~ 100% (determined between 10 and 200 µg/g of R287432 in formulation) The method accuracy was considered acceptable since the recovery for R287432 is between 70 and 130%
Interference/ Specificity	No significant interference was observed. The analytical method is able to separate the impurity R287432 from the formulation blank with no significant co-elution.
LOQ	The validation data prove that the limit of quantification for R287432 is established at concentration of 10 µg/g.
Comment	The method is suitable for the specific, accurate and precise determination of the relevant impurity R287432 in formulation.

Table 5.2-4: Validation of Method SD-1990/1 for the determination of R287432 in A18032E

	R287432: maximum content in A18032E 0.3 g/kg, (2g/kg in technical mesotrione)
Author(s), year	Huang S., 2016b (Unpublished Report No. 300072567)
Principle of method	Liquid Chromatography/Mass Spectroscopy (LC/MS)
Linearity n = 6 linear range 10 to 200 µg/g in A18032E	r = 0.9999 y = 0.9962x + 0.0034
Precision as repeatability n = 6	S_{rel} (%RSD): 4.7% mean concentration: 40 µg/g
Accuracy n = 6	mean recovery: 98.4 % (determined at 40 µg/g of R287432 in A18032E)
Interference/ Specificity	No significant interference was observed. The analytical method is able to separate the impurity R287432 from the formulation blank with no significant co-elution.
LOQ	The validation data prove that the limit of quantification for R287432 is established at concentration of 10 µg/g.
Comment	The method is suitable for the specific, accurate and precise determination of the relevant impurity R287432 in A18032E.

Special note in relation to LOQ:

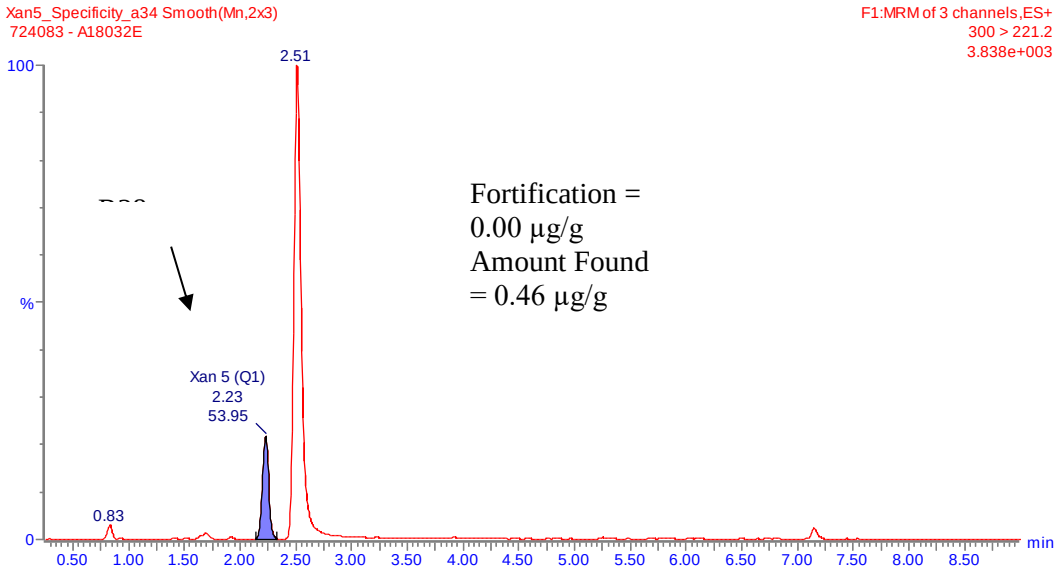
According to SANCO/3030/99 rev.5 2015:

However, in certain cases the content of the active substance in the plant protection product can be too low in order to determine a relevant impurity at the level derived from the maximum content in the technical active substance. In this case, the validation must be performed at the lowest possible concentration, and by means of chromatograms it must be demonstrated that the content of the analyte is below the LOQ.

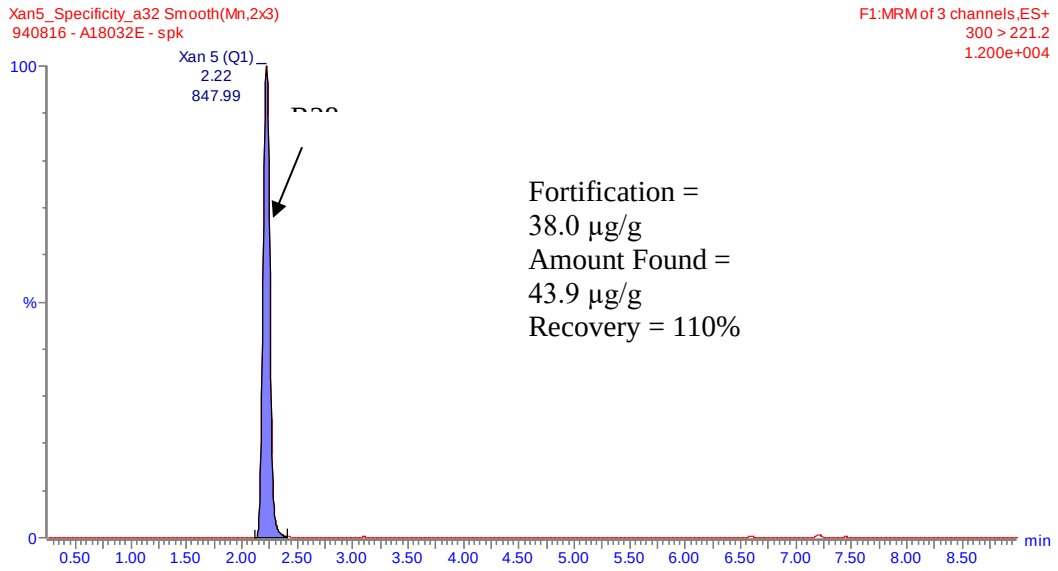
Figure 5.2-2: Chromatogram to demonstrate that R287432 is below the LOQ of 10 µg/g (10 ppm) in A18032E

The following chromatograms demonstrate that the concentration of R287432 in an unfortified batch of A18032E is below the LOQ (10 µg/g or 10 ppm).

A18032E (ID 940816) at ~5.0 mg/mL fortified with R287432 at ~ 0.00 µg/mL



A18032E (ID 940816) at ~5.0 mg/mL fortified with R287432 at ~ 0.20 µg/mL



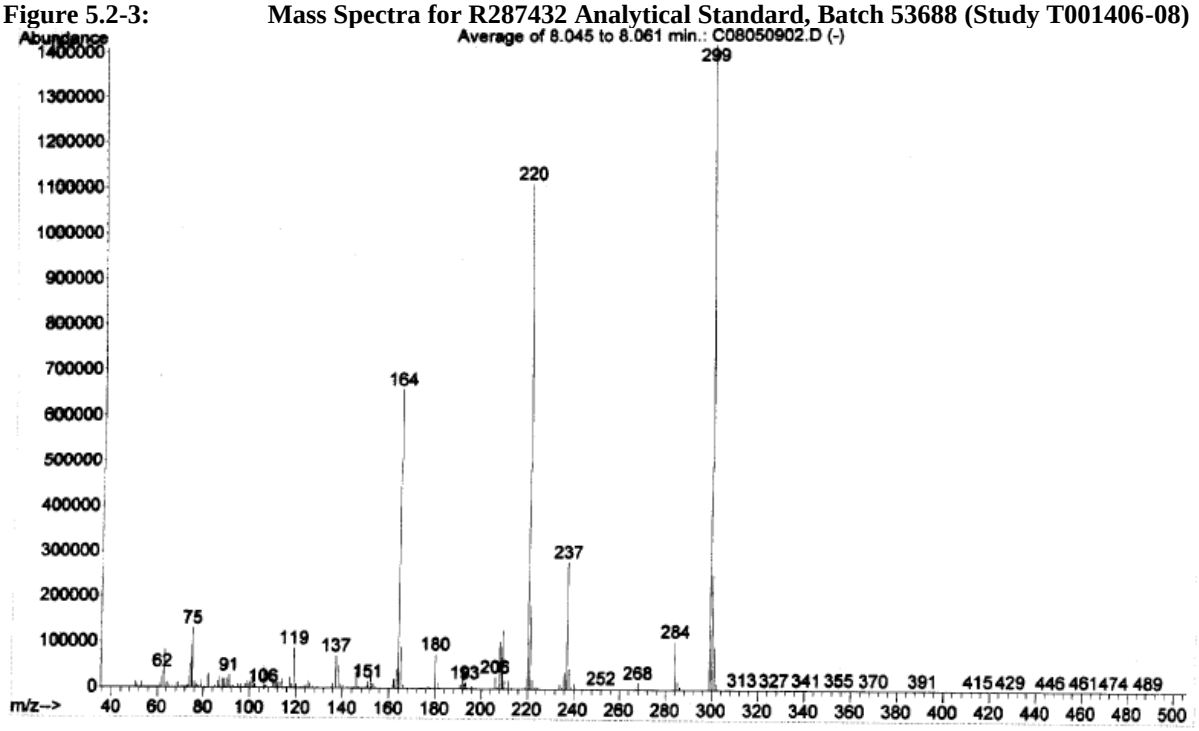
Confirmation of Identity of R287432

According to SANCO/3030/99: *Confirmatory techniques are required to support identification of the a.s. and significant and/or relevant impurities, when the primary method of determination is not GC-MS or another highly specific method such as HPLC-UV DAD.*

The analytical method SD-1990/1 determines the relevant impurity R287432 in mesotrione containing formulations using multiple-point external standard calibration based on liquid chromatography with mass spectroscopy detection (LC/MS). The detection is by MS/MS (tandem mass spectrometry) for specific multiple reaction monitoring (MRM) of an analyte, therefore, high specificity for R287432 is inherent in the method and confirmation of identity of R287432 is inherent in the method.

As additional proof of identity i.e. Mass Spectra generated during the characterization of the reference material R2827432, Batch 53688 (Study T001406-08) are given below.

Figure 5.2-3: Mass Spectra for R287432 Analytical Standard, Batch 53688 (Study T001406-08)



Conclusion

The method is suitable for the specific, accurate and precise determination of the relevant impurity R287432 in A18032E.

Method SD-1973/1 for the determination of 1,2-dichloroethane in A18032E

The relevant impurity 1,2-dichloroethane is a process solvent used during the chemical synthesis of mesotrione technical material, it therefore cannot be formed from mesotrione or from other formulation components of A18032E; storage stability data for 1,2-dichloroethane in A18032E is therefore not required.
The analytical method SD-1973/1 determines relevant impurity 1,2-dichloroethane (DCE) in mesotrione containing formulations using a multiple point, standard addition calibration based on headspace gas chromatography with flame ionization detection (HS/GC/FID). The chromatographic conditions and sample extraction procedure of the method were designed to handle a broad range of formulation types including suspo-emulsions (SE), suspension concentrates (SC), oil dispersions (OD) and water dispersible granules (WG) as demonstrated in the sample chromatograms included in the method (Syngenta file no. A13789C_50002).The method allows quantification of DCE levels ranging from 0.05% to 0.2% relative to mesotrione active ingredient in formulation for samples prepared as described in method SD-1973/1.
Full method validation of SD-1973/1 was performed under GLP (Syngenta file no. A13789C_50001) using the representative formulation A13789C (mesotrione/terbuthylazine/S-metolachlor SE). Validation has demonstrated accuracy (recovery, linearity), precision (repeatability), specificity and limit of quantification of the method. It is proposed to rely on this full validation study to support the formulation A18032E, however supplementary validation data was generated for A18032E and is described below.
Method SD-1973/1 is also used for the determination of 1,2-dichloroethane (DCE) in formulation A18032E (mesotrione, dicamba, nicosulfuron WG). The different nominal concentrations of mesotrione in formulations A18032E and A13789C are not of relevance, as the sample weighing is adapted accordingly. Thus the concentration of mesotrione and its relevant impurity remains the same in the test solutions. Supplementary, non-GLP testing for specificity, linearity, accuracy and precision using A18032E were also conducted and are described in a separate report (Syngenta file no. A18032E_10346).
The results described in both validation reports demonstrate the applicability and validity of the method SD-1973/1 for the determination of 1,2-dichloroethane (DCE) in the formulation A18032E.

Comments of zRMS:	The GC-FID method (SD-1973/1) was fully validated for the determination of relevant impurity 1,2-dichloroethane (DCE) in product A13789C and has been re-validated for the determination determination of 1,2 dichloroethane in product A18032E. The GC-FID

	method is considered fit for purpose according the guidance document SANCO/3030/99 rev. 4.

Reference:	KCP 5.1.1/~~10~~11
Report	Zhang Y. *et al* (2016) ZA1296 - SD-1973/1 - Determination of Impurity DCE (1,2-dichloroethane) in Mesotrione Related Formulations by Headspace Gas Chromatography. Syngenta Crop Protection, Inc., Greensboro, USA. Unpublished Report No. 300066025, Issue date 25.10.2016 (Syngenta File No. A13789C_50002)
Guideline(s):	No
Deviations:	No
GLP:	No
Acceptability:	Yes

Reference:	KCP 5.1.1/~~11~~12
Report	Meyerhoffer W. (2016). Validation of analytical method SD-1973/1. Syngenta Crop Protection, Inc., Greensboro, USA. Unpublished Report No. USGR160249, Issue date 14.12.2016. (Syngenta File No. A13789C_50001)
Guideline(s):	SANCO 3030/99 rev. 4
Deviations:	no
GLP:	yes
Acceptability:	Yes

Reference:	KCP 5.1.1/~~12~~13
Report	Meyerhoffer W. (2016b). Statement on validation of analytical method SD-1973/1 for Determination of 1,2-dichloroethane (DCE) in formulation A18032E, Syngenta Crop Protection, Inc., Greensboro, USA. Unpublished Report No. 300072403, Issue date 16.12.2016. (Syngenta File No. A18032E_10346)
Guideline(s):	SANCO 3030/99 rev. 4
Deviations:	no
GLP:	no
Acceptability:	Yes

Materials and methods

The relevant impurity 1,2-dichloroethane (DCE) is determined in mesotrione containing formulations by gas chromatography on a fused silica DB-1 column (30m, 0.25 mm i.d., temperature program 40°C up to 230°C), using helium as a carrier gas and flame ionisation detection (FID). Quantification is by comparison of peak areas ratios to those of a reference solution.

(Zhang Y. *et al*., 2016)

Validation of method SD-1973/1 - Results and discussions

Full validation of the method SD-1973/1 has been conducted. The method has been shown to be specific for the determination of 1,2-dichloroethane (DCE) in product A18032E and no significant interference was observed. Based on the results for repeatability, recovery, linearity and specificity, precision and accuracy of the method are established. Therefore, the method is suitable for the specific, accurate and precise determination of 1,2-dichloroethane (DCE) in product A18032E. A summary of the validation data is given in Tables 5.2-5 and 5.2-6.

(Meyerhoffer W., 2016 & 2016a)

Table 5.2-5: Validation of Method SD-1973/1 for the determination of DCE in A13789C

	DCE: maximum content in A13789C 0.0375g/L, (1g/kg in technical mesotrione)
Author(s), year	Meyerhoffer W. 2016 (Unpublished Report No. USGR160249)
Principle of method	Headspace gas chromatography with flame ionization detection (HS/GC/FID)
Linearity n = 5 linear range 5 - 20 µg/ml in formulation	r = 0.9997 y = 0.5084x - 0.0408
Precision as repeatability n = 6	S_{rel} (%RSD): 3.9% mean concentration: 10.19 µg/ml in formulation
Accuracy n = 5	mean recovery: 100 % (determined at 5, 8 10, 16 and 20 µg/ml of DCE in formulation)
Interference/ Specificity	No significant interference was observed. The analytical method is able to separate the impurity DCE from the formulation blank with no significant co-elution.
LOQ	The validation data demonstrate that the limit of quantification for DCE is established at concentration of 4 µg/ml.
Comment	The method is suitable for the specific, accurate and precise determination of the relevant impurity DCE in formulation.

Table 5.2-6: Validation of Method SD-1973/1 for the determination of DCE in A18032E

	DCE: maximum content in A18032E 0.15g/kg, (1g/kg in technical mesotrione)
Author(s), year	Meyerhoffer W. 2016a (Unpublished Report No. 300072403)
Principle of method	Headspace gas chromatography with flame ionization detection (HS/GC/FID)
Linearity n = 5 linear range 5 - 20 µg/ml in A18032E	r = 0.9994 y = 0.0047x - 0.0027
Precision as repeatability n = 6	S_{rel} (%RSD): 3.9%[1] mean concentration: 10 µg/g in formulation
Accuracy n = 5	mean recovery: 99.7 % (determined at 5, 8 10, 16 and 20 µg/ml of DCE in formulation)
Interference/ Specificity	No significant interference was observed. The analytical method is able to separate the impurity DCE from the formulation blank with no significant co-elution.
LOQ	The validation data demonstrate that the limit of quantification for DCE is established at concentration of 4ug/ml.
Comment	The method is suitable for the specific, accurate and precise determination of the relevant impurity DCE in A18032E.

[1] precision is quoted for the repeatability data generated in study USGR160249 stated in Table 5.2-5, this is justified based on the fact that headspace analysis involves the analysis of pure DCE, in the vapour phase, and therefore the precision data generated relates to the method only and is independent of the formulation matrix.

Special note in relation to LOQ:

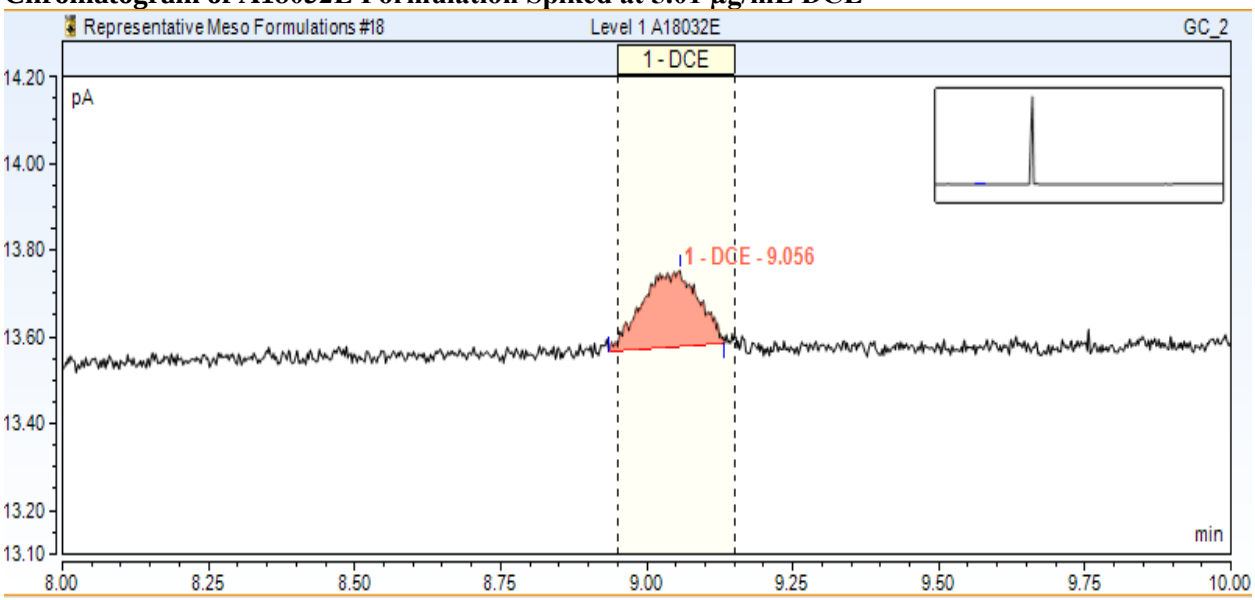
According to SANCO/3030/99 rev.5 2015:

However, in certain cases the content of the active substance in the plant protection product can be too low in order to determine a relevant impurity at the level derived from the maximum content in the technical active substance. In this case, the validation must be performed at the lowest possible concentration, and by means of chromatograms it must be demonstrated that the content of the analyte is below the LOQ.

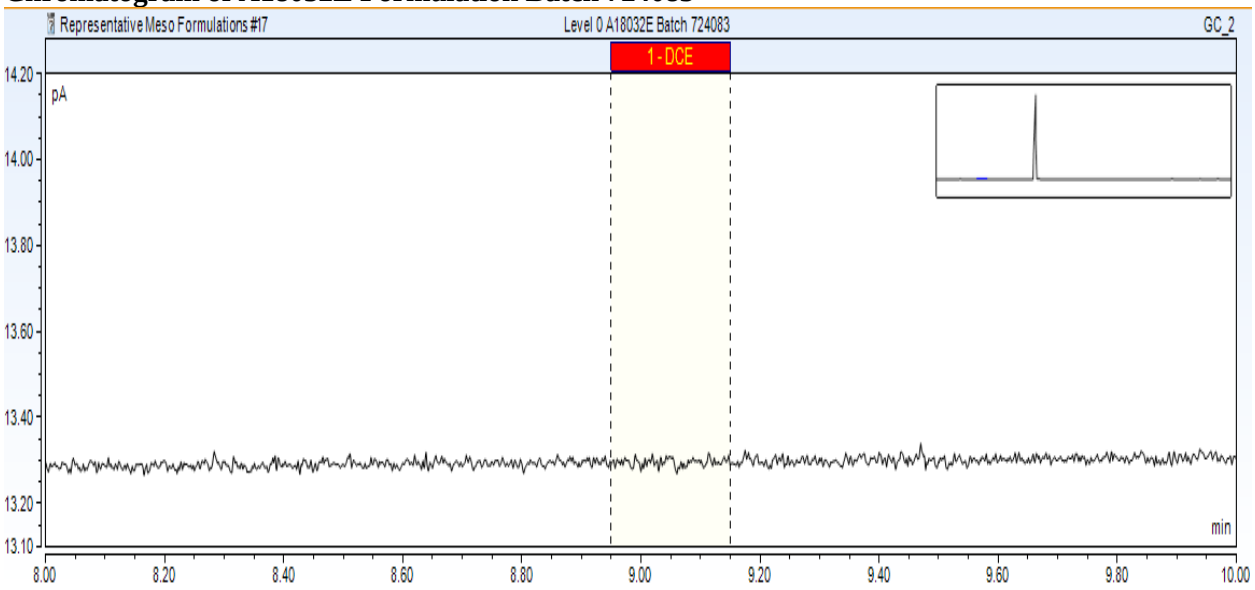
Figure 5.2-4: Chromatogram to demonstrate that DCE is below the LOQ of 4ug/ml (4 ppm) in A18032E

The following chromatograms demonstrate that the concentration of DCE in an unfortified batch of A18032E is below the LOQ (4ug/ml or 4 ppm).

Chromatogram of A18032E Formulation Spiked at 5.01 µg/mL DCE



Chromatogram of A18032E Formulation Batch 724083



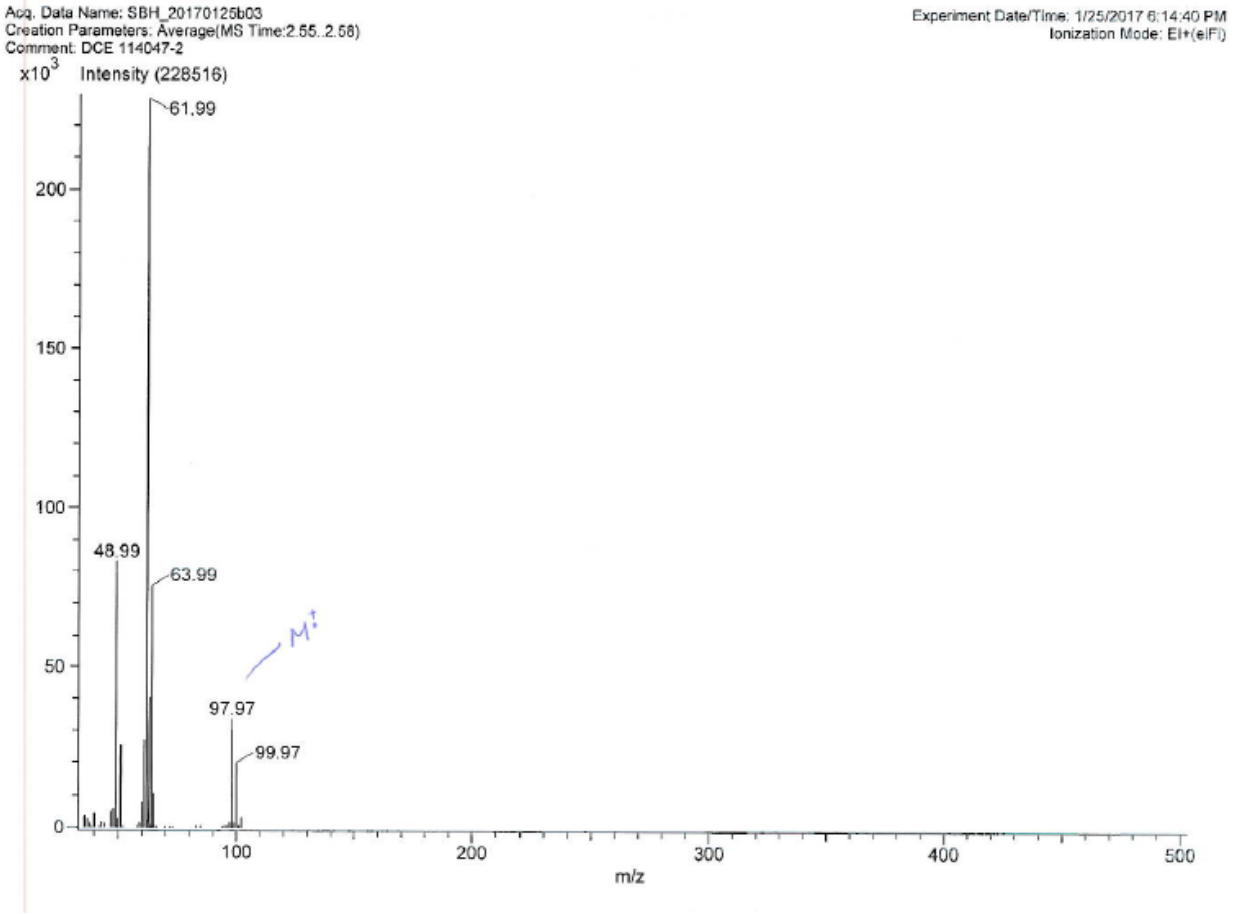
Confirmation of Identity of 1,2-dichloroethane (DCE)

According to SANCO/3030/99: *Confirmatory techniques are required to support identification of the a.s. and significant and/or relevant impurities, when the primary method of determination is not GC-MS or another highly specific method such as HPLC-UV DAD.*

The analytical method SD-1973/1 determines relevant impurity 1,2-dichloroethane (DCE) in mesotrione containing formulations using a multiple point, standard addition calibration based on headspace gas chromatography with flame ionization detection (HS/GC/FID). The analytical technique of headspace analysis requires that the analyte is evaporated from the matrix and is therefore by definition it is presented to the detector as pure phase and therefore the formulation matrix has no inherent influence on the specificity of the method. Furthermore, standard addition is used in analytical method SD-1973/1 which ensures that identity is unequivocally confirmed with each analysis as the analyte samples are fortified with certified pure analytical standard of 1,2-dichloroethane. Therefore, confirmation of identity of 1,2-

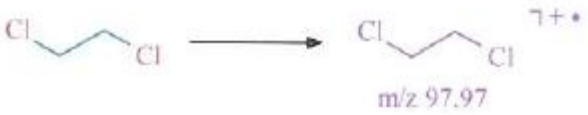
dichloroethane is inherent in the method.
As additional proof of identity i.e. a mass spectrum generated during the characterization of the reference material 1,2-dichloroethane, Batch 114047 is given below.

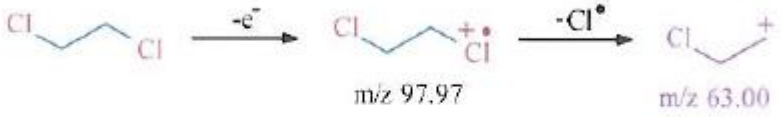
Figure 5.2-5: Mass Spectra for 1,2-dichloroethane Analytical Standard, Batch 114047

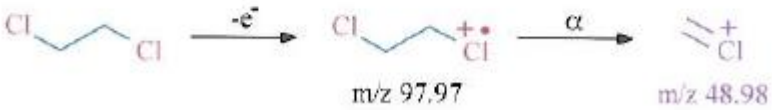


Mass spectrum interpretation:

$C_2H_4Cl_2$
98.96
97.969006
C 24.27% H 4.07% Cl 71.65%







Conclusion
The method is suitable for the specific, accurate and precise determination of the relevant impurity 1,2-dichloroethane (DCE) in A18032E.

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

There are no relevant formulants in A18032E therefore no methods are required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There is no CIPAC method available for the determination of mesotrione, nicosulfuron, and dicamba in mixed WG formulations such as A18032E.

5.2.2 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 tables. For the detailed evaluation of new studies it is referred to Appendix 2.

Table 5.2-7: Validated methods for the generation of pre-authorization data for dicamba in soil, water, air (KCP 5.1.2.1 in support of environmental fate 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 / missing / EU agreed**
REM 193.02 Dicamba EFSA Journal (2011) 9(1), 1965	Soil	0.01 mg/kg	GC-MS	Method: Gasser, 2000a Report: REM 193.02 Validation: Gasser, 2000b Report 301/00 EU agreed: Denmark 2011
REM 193.02 DCSA EFSA Journal (2011) 9(1), 1965	Soil	0.01 mg/kg	GC-MS	Method: Gasser, 2000a Report: REM 193.02 Validation: Gasser, 2000b Report 301/00 EU agreed: Denmark 2011
GRM022.06A Dicamba	Soil	0.0035 mg/kg	LC-MS/MS	Method: Braid & Garcia-Alix, 2013 Report: SAN837_11434 New data KCP 5.1.2.1/01 Validation: Garcia-Alix, 2013 Report: SAN837_11433 New data KCP 5.1.2.1/02
GRM022.06A DCSA	Soil	0.0035 mg/kg	LC-MS/MS	Method: Braid & Garcia-Alix, 2013 Report: SAN837_11434 New data KCP 5.1.2.1/01 Validation: Garcia-Alix, 2013 Report: SAN837_11433 New data KCP 5.1.2.1/02
REM 193.03 Dicamba EFSA Journal (2011) 9(1), 1965	Water	0.1 µg/L	GC-MSD	Method: Gasser, 2000c Report: REM 193.03 Validation: Gasser, 2000d Report 302/00 EU agreed: Denmark 2011
REM 193.03 DSCA EFSA Journal (2011) 9(1), 1965	Water	0.1 µg/L	GC-MSD	Method: Gasser, 2000c Report: REM 193.03 Validation: Gasser, 2000d Report 302/00 EU agreed: Denmark 2011
GRM022.02A Dicamba	Water	0.05 µg/L	GC-MSD	Method: Hargreaves, 2007 Report: SAN837/6654

Component of residue definition: dicamba				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				New data KCP 5.1.2.1/03 Validation: Emburey, 2007 Report: SAN837/6653 New data KCP 5.1.2.1/04 EU agreed: Included in 2008 AIR top up but no evaluation published therefore provided in AIR3 Supplementary dossier and summarised in Appendix 2. ILV: Kotthoff, 2016 Report: SAN837_11602 New data KCP 5.1.2.1/05
GRM022.09A DCSA	Water	0.05 µg/L	LC-MS/MS	Method: Allen & Brooks (2017) Report: NOA414746_10010 New data KCP 5.1.2.1/08 Validation: Allen (2017) Report: NOA414746_100011 New data KCP 5.1.2.1/09
21401 Dicamba EFSA Journal (2011) 9(1), 1965	Air	21 µg/m^3	GC-MSD	Method and validation: Kettner & Karapally 1993 Report: 21401 EU agreed: Denmark 2011
GRM022.01A Dicamba	Air	0.002 µg/L (2.0 µg/m^3)	GC-MSD	Method: Hargreaves, 2007 Report: SAN837/6677 New data KCP 5.1.2.1/06 Validation: Emburey, 2007 Report: SAN837/6678 New data KCP 5.1.2.1/07 **New data** Included in 2008 AIR top up but no evaluation published therefore provided in AIR3 Supplementary dossier and summarised in Appendix 2.

Validated methods for the generation of pre-authorization data for dicamba in soil, water (KCP 5.1.2.2 in support of efficacy studies)

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

Validated methods for the generation of pre-authorization data for dicamba in feed, body fluids and tissues and air (KCP 5.1.2.3 in support of toxicological studies)

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

Validated methods for the generation of pre-authorization data for dicamba in body fluids, air and any additional matrices used (KCP 5.1.2.4 in support of operator, worker, resident and bystander exposure studies)

No new data are submitted in the framework of this application.

Table 5.2-8: Validated methods for the generation of pre-authorization data for dicamba in plant and animal products (KCP 5.1.2.5 in support of residues studies)

Component of residue definition for plant products (risk assessment): Dicamba + 5-OH-dicamba, free and conjugated				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
P-14.063.02	High protein/high starch content (dry) *Oats grain, barley grain, wheat grain, maize cob, maize grain* High water content *Oats whole plant , barley whole plant , wheat whole plant , maize whole plant , maize plant without cob* No group *Oats straw , oats husk , oats rolled , barley straw , wheat straw*	0.01 mg/kg	GC-MS	Method: Schmidt, 1994 (P-14.063.02) Validation: Stolze, 2000 (gr 04398) Beinhauer, 1998a (97 10 47 029) Beinhauer, 1998b (97 10 61 030) Beinhauer, 1998c (97 10 63 031) Beinhauer, 1998d (97 10 61 028) Hertl, 1995 (R 10280) Kaethner, 1996a (R10305) Konig, 1996b (R93042E) Konig, 1996c (R93041F) Konig, 1996d (R93041E) EU agreed (Denmark, 2010)
REM 193.01 (R97-003)[(a)]	High protein/high starch content (dry) *Maize grain* High water content *Maize whole plant, pasture* High oil content *Oilseed rape* High acid content *Orange* No group *Maize straw*	0.01 mg/kg	GC-MS	Method: Gasser, 1997 (R97-003) Gasser, 1998 (REM 193.01) Validation: Maffezzoni, 2004 (SYN/DIC/03041) ILV: Steinhauer, 2004 (ADE-0402V) EU agreed[(b)] (Denmark, 2010)
REM 193.05	*High protein/high starch content (dry)* *Barley grain* *High water content* *Barley whole plant,* *No group* *Barley straw, malt, wort, spent hops and spent yeast*	0.01 mg/kg	GC-MS	Method: Richards, Mackenzie, 2006 (REM 193.05) New data; KCPA 5.1.2.5/01 Richards, Mackenzie, Crook 2008 (REM 193.05b) New data; KCPA 5.1.2.5/02 Validation: Richards, Mackenzie, 2004 (03-7009) New data; KCPA 5.1.2.5/03 Richards, 2004 (03-7013) New data; KCPA 5.1.2.5/04 Richards, Mackenzie,2004a (03-7017)

Component of residue definition for plant products (risk assessment): Dicamba + 5-OH-dicamba, free and conjugated				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				New data; KCPA 5.1.2.5/05
GRM022.07A	High protein/high starch content (dry) *Maize grain, barley grain, maize kernel, sorghum grain, lentils*	0.01 mg/kg	LC-MS/MS	Method: Braid & Crook, 2016 (TK0047496) New data* KCP 5.1.2.5/06 Validation: Kennedy, 2016 (SAN837_11691) New data* KCP 5.1.2.5/07
	High water content *Potato tuber, spinach, cereal forage, carrot tops&leaves, carrot tuber, sugarcane, sorghum whole plant, sorghum forage*	0.01 mg/kg		
	High oil content *Oilseed seed*	0.01 mg/kg		
	High acid content *Orange*	0.01 mg/kg		
	No group *Flour, cereal straw, sorghum stover*	0.01 mg/kg		

* These data are currently under evaluation for the renewal of approval of the active substance, dicamba (Commission Implementing Regulation (EU) No. 844/2012 of 18 September 2012).

Component of residue definition for animal products (risk assessment): Dicamba, free and conjugated				
AM-0938-0994-0	Muscle	0.01 mg/kg	GC-ECD (Confirmation GC-MS)	Method and validation: Formanski, 1994 (AM-0938-0994-0) ILV: Baldi, 1994 (09/94/AM) Not accepted[(c)] (Denmark, 2010)
	Fat	0.01 mg/kg		
	Liver	0.01 mg/kg		
	Kidney	0.01 mg/kg		
	Milk	0.01 mg/kg		
AM-0685	Muscle	1.0 mg/kg	GC-ECD	Method: Cahill & Johnson, 1984 (74) EU agreed (Denmark, 2010)
	Fat	1.0 mg/kg		
	Liver	1.0 mg/kg		
	Eggs	1.0 mg/kg		
AM-0659	Muscle	Dicamba: 0.02 mg/kg DCSA: 0.02	GC-ECD	Method: Anon, 1978 (SAN837_5376) Validation: Gilsdorf & Weissenburger, 1979a (379; SAN837_5246) Gilsdorf & Weissenburger, 1979b (379; SAN837_5387) Tims & Weissenburger, 1979 (379; SAN837_5103) Gilsdorf & Weissenburger, 1979 (379; SAN837_5104)
	Fat	Dicamba: 0.02 mg/kg DCSA: 0.02		
	Liver	Dicamba: 0.02 mg/kg DCSA: 0.02		
	Kidney	Dicamba: 0.02 mg/kg DCSA: 0.02		
	Milk	Dicamba: 0.01		

Component of residue definition for animal products (risk assessment): Dicamba, free and conjugated				
		mg/kg DCSA: 0.02		EU agreed (Denmark, 2007)
REM 193.04	Muscle	5-OH-dicamba: 0.01 mg/kg	HPLC-MS/MS	Method: Gasser, 2001 (REM 193.04) Validation: Gasser, 2001a (309/01)
	Fat	5-OH-dicamba: 0.01 mg/kg		
	Liver	5-OH-dicamba: 0.01 mg/kg		
	Kidney	5-OH-dicamba: 0.01 mg/kg		
	Milk	5-OH-dicamba: 0.005mg/L		
	Blood	5-OH-dicamba: 0.01 mg/L		EU agreed (Denmark, 2007)

(a) Method also used for post-authorisation and monitoring
(b) Hydrolysis step validated (identified as data GAP; EFSA, 2011) in rotational crop metabolism study (CA 6.6.1/01: Swales, 2016)
(c) Not accepted for monitoring purposes (EFSA, 2011)

The analytical methods presented to support the proposed crop use on maize, use a strong acid hydrolysis step in order to release free dicamba from conjugates of dicamba. It is widely acknowledged that analysis of residues of acidic compounds will be underestimated if a hydrolysis step is not included. The current and historical Syngenta methods for dicamba analysis in crops use a strong acid hydrolysis step to release free dicamba from conjugates of dicamba which would otherwise not be quantified.
Extraction and hydrolysis affected by reflux in 1 M HCl for 1 hour. It is clearly indicated in the metabolism report (Vollmin), previously reviewed at EU level, that residues of dicamba in forage are present as free dicamba at 2.3% TRR extracted with 80/20 methanol/water and conjugated dicamba 7.7% TRR extracted by acid hydrolysis. In grain, 16.1% free dicamba was extracted with 80/20 methanol/water and conjugated dicamba 1.7% TRR extracted by acid hydrolysis. In straw 2.3% TRR extracted with 80/20 methanol/water and conjugated dicamba 2.6% TRR extracted by acid hydrolysis. The metabolism report demonstrates that the acidic extraction conditions used in the residue method are appropriate for the determination of dicamba and 5-OH dicamba.
As with the crop commodities, significant residues of dicamba are conjugated in commodities of animal origin. In kidney, free dicamba constitutes 72% TRR and conjugated dicamba 22% TRR. In liver free dicamba constitutes 54% TRR and conjugated dicamba 14% TRR. Extraction and hydrolysis of free and conjugated dicamba residues is affected by heating in 1 M HCl at 95ºC for 90 minutes. The metabolism report demonstrates that the acidic extraction conditions used in the residue method are appropriate for the determination of dicamba.
As a hydrolysis step is required, the usual multi-residue methods (S19, QuEChERS) are generally unsuitable for the determination of residues of dicamba without modification. The Syngenta position is that GRM022.05A is an appropriate monitoring method.

Validated methods for the generation of pre-authorization data for dicamba in plant matrices (KCP 5.1.2.6 in support of ecotoxicological studies)
No analytical methods were used to generate ecotoxicology data for this product.

Validated methods for the generation of pre-authorization data for soil, water (Ecotoxicology)

Please refer to the validated analytical methods for specific matrices. For ecotoxicology studies, the analytical method used was deemed fit for purpose at the time of study conduct and is detailed in each individual report to the extent required at the time. An overview is presented in the tables below. Analyses in ecotoxicology studies typically are dose verifications and therefore serve the purpose of confirming already known concentrations. It is considered that any omissions in the reported analytical methodology compared to current analytical reporting requirements are only due to changing requirements and do not express a lack of scientific diligence of the study directors. Therefore they are highly unlikely to affect measured values and do not change study conclusions.

Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Component of residue definition: Dicamba				
SAN837/5205	Avian diet	50 ppm (43.5 ppm a.s.)	HPLC-UV	Method: Beavers et al., 1994 Report: SAN837/5205 EFSA Conclusion 2011
SAN837/5206	Avian diet	50 ppm (43.5 ppm a.s.)	HPLC-UV	Method: Beavers et al., 1994a Report: SAN837/5206 EFSA Conclusion 2011
SAN837/6142	Water	Not reported	HPLC-UV	Method: Volz, 2004 Report: SAN837/6142 EFSA Conclusion 2011
SAN837/5331	Water	LOD: 0.43 µg/L	HPLC-UV	Method: Scheerbaum, 1990 Report: SAN837/5331 EFSA Conclusion 2011
SAN837/5332	Water	LOD: 0.05 mg /L	HPLC (detector not reported)	Method: Douglas, 1993 Report SAN837/5332 EU agreed (Denmark, 2010)
SAN837/0411	Water	LOD: 0.024 mg/L	HPLC-UV	Method: Smith et al., 1998 Report SAN837/0411 EFSA Conclusion 2011
SAN837/5229	Water	14 µg/L	HPLC-UV	Method: Hoberg, 1993 Report SAN837/5229 EFSA Conclusion 2011
SAN837/5224	Water	4.17 µg/L	HPLC-UV	Method: Hoberg, 1993 Report SAN837/5224 EFSA Conclusion 2011
SAN837/5223	Water	14 µg/L	HPLC-UV	Method: Hoberg, 1993a Report SAN837/5223

Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
				EFSA Conclusion 2011
Component of residue definition: DCSA (NOA414746)				
NOA414746/0003	Water	LOD: 0.1 mg/L	HPLC-UV	Method: Douglas et al., 1993 Report NOA414746/0003 EFSA Conclusion 2011
NOA414746/0005	Water	LOD: 0.05 mg/L	HPLC-UV	Method: Douglas et al., 1993a Report NOA414746/0005 EFSA Conclusion 2011
NOA414746/0013	Water	LOD: 0.006 mg/L	HPLC-UV	Method: Grade, 2002 Report NOA414746/0013 EFSA Conclusion 2011

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

An overview on the acceptable methods and possible data gaps for analysis of residues of mesotrione for the generation of pre-authorization data is given in the following tables. For the detailed evaluation studies it is referred to Appendix 2.

Table 5.2-9: Validated methods for the generation of pre-authorization data for mesotrione in soil, water, air (KCP 5.1.2.1 in support of environmental fate studies)

Component of residue definition: Mesotrione, MNBA and AMBA				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
TMR0661B	Soil	0.005 mg/kg	HPLC-FSD	Method: Alferness (1996) Report TMR0661B EU agreed (UK, 2015, 2015a)
T001200-03	Soil	0.002 mg/kg	LC-MS/MS	Method: Williams (2004) Report T001200-03 New data; KCP 5.1.2.1/08
GRM007.10A	Soil	0.002 mg/kg	LC-MS/MS	Method: Jutsum & Williams, 2013 Report GRM007.10A Validation: Jutsum, 2013 Report CEMR-5657-REG EU agreed (UK, 2015, 2015a)
Component of residue definition: Mesotrione and MNBA				
TMR0707B	Water	0.05 µg/L	GC-MSD	Method: Meyers, 1997 Report TMR0707B EU agreed (UK, 2015, 2015a)
Component of residue definition: Mesotrione, MNBA and AMBA				

Component of residue definition: Mesotrione, MNBA and AMBA				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
6179-04	Water		HPLC-MS/MS	Method: Chamkasem, 2004 Report T006179-04 Validation: McLean, 2005 Report T006450-04 EU agreed (UK, 2015, 2015a)
GRM007.09A	Water	0.05 LOQ µg/L	HPLC-MS/MS	Method: Jutsum & Chamkesam, 2013 Report GRM007.09A Validation: Jutsum, 2013a Report CEMR-5658-REG ILV: Wiesner & Breyer, 2013 Report S13-04185 EU agreed (UK, 2015, 2015a)
Component of residue definition: Mesotrione				
RR 97-031B	Air	0.01 mg/m^3	HPLC-UV	Method: Leung, 1997 Report RR 97-031B EU agreed (UK, 2015, 2015a)
GRM007-08B	Air	4.5 µg/m^3	LC-MS/MS	Method: Jutsum, 2013b Report GRM007.08B Validation: Jutsum, 2013c Report CEMR-5403-REG EU agreed (UK, 2015, 2015a)

Validated methods for the generation of pre-authorization data for mesotrione in soil, water (KCP 5.1.2.2 in support of efficacy studies)

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

Validated methods for the generation of pre-authorization data for mesotrione in feed, body fluids and tissues and air (KCP 5.1.2.3 in support of toxicological studies)

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

Table 5.2-10: Validated methods for the generation of pre-authorization data for mesotrione in body fluids, air and any additional matrices used (KCP 5.1.2.4 in support of operator, worker, resident and bystander exposure studies)

Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Component of residue definition: MNBA				
BFI0148	Aqueous carboxymethylcellulose	1 mg/L	HPLC-UV	Method Bachelor, 2014 Report 11070

Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
				New data, KCP 5.1.2.4/01
BFI0147	Aqueous carboxymethylcellulose	1 mg/L	HPLC-UV	Method Faulkner &Heap, 2013 Report BFI0147 New data, KCP 5.1.2.4/02
BFI0148	Aqueous carboxymethylcellulose	1 mg/L	HPLC-UV	Method Faulkner & Heap, 2013a Report BFI0148 New data, KCP 5.1.2.4/03
BFI0148	Aqueous carboxymethylcellulose	1 mg/L	HPLC-UV	Method Faulkner & Heap, 2013b Report BFI0149 New data, KCP 5.1.2.4/04
Component of residue definition: AMBA				
BFI068MS for blood and BFI074MS for plasma	Blood and plasma of rats	Not given	HPLC-MS/MS	Method xxxxxxxxxxxxxxxx Report BFI0533 New data, KCP 5.1.2.4/05

Table 5.2-11: Validated methods for the generation of pre-authorization data for mesotrione in plant and animal products (KCP 5.1.2.5 in support of residues studies)

Component of residue definition: mesotrione (and MNBA)				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
TMR0643B	High protein/high starch content (dry) *Maize forage, maize grain*	0.01 mg/kg	HPLC-FL	Method: Alferness, 1996 Report: TMR0643B Validation: Bolygo, 1996 Report: RJ0689B EU agreed (UK, 2015, 2015a)
	High water content *Maize fodder*			
RAM 366/01	High protein/high starch content (dry) *Maize grain, maize silage, maize stover*	0.01 mg/kg	HPLC-MS/MS (2 transitions)	Method: Crook, 2002 Report: RAM 366/01 Validation: Hill, 2004 Report: RJ3253B ILV: Bruns *et al.* 2001 Report: S13-02460 EU agreed (UK, 2015, 2015a)
	High water content *Maize whole plant,*			
	High water content *Oilseed rape whole plant*	0.01 mg/kg		Validation: Malet & Allard, 2010 Report: RXCO00307 EU agreed (UK, 2015, 2015a)
	High oil content *Oilseed rape seed*			

Component of residue definition: mesotrione (and MNBA)				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	High oil content *Linseed seed*	0.01 mg/kg		Validation: Simon, 2004 Report: gli58003 EU agreed (UK, 2015, 2015a)
	High oil content *Poppyseed seed*	0.01 mg/kg		Validation: Simon, 2004a Report: gpp067003 EU agreed (UK, 2015, 2015a)
GRM007.11A (update to RAM 366/01)	High protein/high starch content (dry) *Maize grain whole*	0.01 mg/kg	HPLC-MS/MS (2 transitions)	Method: Watson & Crook, 2013 Report: GRM007.11A Validation: Watson, 2013 Report: S12-03629 ILV: Amic, 2013 Report: S13-02460 EU agreed (UK, 2015, 2015a)
	High water content *Maize forage*			
	High oil content *Oilseed rape seed*			
	High acid content *Whole orange*			

Please refer to the validated analytical methods for specific matrices. For ecotoxicology studies, the analytical method used at the time of study conduct was deemed fit for purpose and is detailed in each individual report. Although it is not fully compliant with SANCO/3029/99, it is considered that any omissions in the reported analytical methodology compared to current analytical reporting requirements are highly unlikely to affect measured values and do not change study conclusions.

Table 5.2-12: Validated methods for the generation of pre-authorization data for water (KCP 5.1.2.6 in support of ecotoxicological studies)

Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Component of residue definition: Mesotrione				
S12-02294	Water	0.00156 mg mesotrione/L	HPLC-MS/MS	Method: Weber, 2012 Report S12-02294 New data, KCP 5.1.2.6/01
S12-02296	Water	0.00153 mg mesotrione/L	HPLC-MS/MS	Method: Falk, 2012 Report S12-02296 New data, KCP 5.1.2.6/02
S12-02295	Water	0.00156 mg mesotrione/L	HPLC-MS/MS	Method: Weich, 2012 Report S12-02295 New data, KCP 5.1.2.6/03
S12-02297	Water	0.00156 mg mesotrione/L	HPLC-MS/MS	Method: Weber, 2012 Report S12-02297 New data, KCP 5.1.2.6/04
ACE-12-148	Water	0.001 g	HPLC-DAD (UV)	Method:

Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
		mesotrione/L		Bramby-Gunary, 2013 Report ACE-12-148 New data, KCP 5.1.2.6/05
ACE-12-149	Water	0.001 g mesotrione/L	HPLC-DAD (UV)	Method: Bramby-Gunary, 2013 Report ACE-12-149 New data, KCP 5.1.2.6/06
105731240A	Water	1 µg/L	HPLC-MS/MS	Method: Hengsberger & Wydra, 2015 Report 105731240 New data, KCP 5.1.2.6/07
105732240	Water	1 µg/L	HPLC-MS/MS	Method: Kosak & Wydra, 2016 Report 105732240 New data / KCP 5.1.2.6/08
S16-06273	Water	0.4 µg/L	HPLC-MS/MS	Method: Gonsior, 2017 Report S16-06273 New data, KCP 5.1.2.6/09
Component of residue definition: SYN546974				
D77394	Water	0.0497 mg/L	HPLC-UV	Method: Liedtke, 2013 Report D77394 EU agreed (UK, 2015, 2015a)
Component of residue definition: AMBA				
D55614	Water	0.171 mg/L	HPLC-UV	Method: Liedtke, 2013a Report D55614 EU agreed (UK, 2015, 2015a)
Component of residue definition: MNBA				
D55592	Water	0.161 mg/L	HPLC-UV	Method: Liedtke, 2013b Report D55592 EU agreed (UK, 2015, 2015a)

Validated methods for the generation of pre-authorization data for mesotrione in plant matrices (KCP 5.1.2.6 in support of ecotoxicological studies)

Component of residue definition: Mesotrione and MNBA				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
GRM007.11A	Plants	0.01 mg/kg	HPLC-MS/MS (2 transitions)	Method: North, L., 2016 Report: S15-02057 New data, KCP 5.1.2.6/4

Validated methods for the generation of pre-authorization data for water, buffer solutions (Properties)

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 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 tables. For the detailed evaluation of new studies it is referred to Appendix 2.

Table 5.2-13: Validated methods for the generation of pre-authorization data for nicosulfuron in soil, water, air (KCP 5.1.2.1 in support of environmental fate studies)

Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Component of residue definition: nicosulfuron				
770117	Soil	0.05 µg/kg	HPLC-MS	Method: Wais, 2000 Report 770117 EU agreed: UK, 2006
614340	Soil	0.005 mg/kg	HPLC-UV	Method: Huber, 1996 Report: 614340 EU agreed: UK, 2006
Component of residue definition: ADMP				
614340	Soil	0.01 mg/kg	HPLC-UV	Method: Huber, 1996 Report: 614340 EU agreed: UK, 2006
Component of residue definition: ASDM				
614340	Soil	0.01 mg/kg	HPLC-UV	Method: Huber, 1996 Report: 614340 EU agreed: UK, 2007
Component of residue definition: HMUD, ASDM, ASMP, AUSN, UCSN, MU-466				
28685	Groundwater	0.05 µg/L(for nicosulfuron) 0.1 µg/L (for HMUD, ASDM, ASMP, AUSN, UCSN, MU-466)	HPLC-MS/MS	Method: Schneider & Holzer, 2016 Report: DuPont-28685 New data, KCP 5.1.2.1
Component of residue definition: HMUD, ASDM, ADMP, AUSN, UCSN, MU-466				
40798	Groundwater	0.05 µg/L(for nicosulfuron) 0.1 µg/L (for HMUD, ASDM, ADMP, AUSN, UCSN, MU-466)	HPLC-MS/MS	Method: Ferrari, 2016 Report DuPont-40798 New data, KCP 5.1.2.1
Component of residue definition:MU-466				
854404	Soil	Not given	HPLC-MS	Method: Volkl, 2004 Report: 854404 EU agreed: UK, 2006
854405	Soil	Not given	HPLC-UV (for sandy loam, loam and clay) HPLC-MS/MS (for silty clay loam and silt loam)	Method: Volkel, 2004 Report: 854405 EU agreed: UK, 2006

Method type	Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Component of residue definition:nicosulfuron, ASDM, ADMP				
272237	Soil	0.01 mg/kg	HPLC-UV	Method: Schulz, 1991 Report: 272237 EU agreed: UK, 2006
330682	Soil	0.01 mg/kg	HPLC-UV	Method: Wyss-Benz, 1994 Report: 330682 EU agreed: UK, 2005
F-006-H	Soil	0.01 mg/kg	HPLC-UV	Method: Bonfanti, 1995 Report: F-006-H EU agreed: UK, 2006
Component of residue definition: HMUD				
854406	Soil	Not given	HPLC-UV (for sandy loam, loam and clay) HPLC-MS/MS (for silty clay loam and silt loam)	Method: Volkel, 2004a Report: 854406 EU agreed: UK, 2006

Validated methods for the generation of pre-authorization data for nicosulfuron in soil, water (KCP 5.1.2.2 in support of efficacy studies)

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

Validated methods for the generation of pre-authorization data for nicosulfuron in feed, body fluids and tissues and air (KCP 5.1.2.3 in support of toxicological studies)

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

Validated methods for the generation of pre-authorization data for nicosulfuron in body fluids, air and any additional matrices used (KCP 5.1.2.4 in support of operator, worker, resident and bystander exposure studies)

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

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 residues studies)

Component of residue definition for plant and animal products: Nicosulfuron				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
GRM074.01A	High protein/high starch content (dry) Maize kernels	0.01 mg/kg	LC-MS/MS	Method: Crook, Andrews, 2016 Report: GRM074.01A New Data KCP 5.1.2.5/08 Validation: Andrews, 2016 Report TK0258007-REG New Data, KCP 5.1.2.5/09
	High water content Maize Whole Plant	0.01 mg/kg		

Validated methods for the generation of pre-authorization data for water (Ecotoxicology)
Please refer to the validated analytical methods for specific matrices. For ecotoxicology studies, the analytical method used at the time of study conduct was deemed fit for purpose and is detailed in each individual report. Although it is not fully compliant with SANCO/3029/99, it is considered that any omissions in the reported analytical methodology compared to current analytical reporting requirements are highly unlikely to affect measured values and do not change study conclusions.
The location of the reported analytical methods within the individual reports is indicated in the following tables.

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

Component of residue definition for plant and animal products: Nicosulfuron				
Method type	**Matrix type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
MU-466	Water (test medium)	0.09 mg/L	LC-MS/MS	Method: Obert-Rauser, 2016 Report; S15-05478 New data; KCP 5.1.2.6
HMUD	Water (test medium)	0.01 mg/L	LC-MS/MS	Method: Dengler, 2009 Report; GAB S08-00827 New data; KCP 5.1.2.6

Validated methods for the generation of pre-authorization data for nicosulfuron in water, buffer solutions (KCP 5.1.2.7 in support of physical and chemical properties tests)
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)

Analytical methods and validation reports for the determination of dicamba and mesotrione in the plant protection A18032E are fully described in point 5.2.1 and can be applied for post-authorization control and monitoring purposes.

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

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is understood to be identical.
The Draft Assessment Report provides a residue definition in both plants and animals as “dicamba” whereas in the legal definition this is elaborated to “Dicamba and its salts and conjugated dicamba expressed as dicamba”. From an analytical determination perspective, the definitions are consistent representing the total parent dicamba residue.

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

Matrix	Residue definition	MRL / LOQ	Reference for MRL/level Remarks
Plant, high water content	Dicamba and its salts and conjugated dicamba expressed as dicamba	0.05* mg/kg	Reg. (EU) 2015/845
Plant, high acid content		0.05* mg/kg	Reg. (EU) 2015/845
Plant, high protein/high starch content (dry commodities)		0.05* mg/kg	Reg. (EU) 2015/845
Plant, high oil content		0.05* mg/kg	Reg. (EU) 2015/845
Plant, difficult matrices (hops, spices, tea)		0.05* mg/kg	Reg. (EU) 2015/845
Muscle	Dicamba and its salts and conjugated dicamba expressed as dicamba	0.05* mg/kg	Reg. (EU) 2015/845
Milk		0.2 mg/kg	Reg. (EU) 2015/845
Eggs		0.05* mg/kg	Reg. (EU) 2015/845
Fat		0.04 mg/kg	Reg. (EU) 2015/845
Liver, kidney		0.07 mg/kg	Reg. (EU) 2015/845
Soil (Ecotoxicology)	Dicamba	0.0035 mg/kg	LC_{50} >480 mg a.s./kg dw soil RAC 48 mg/kg (earthworm)
Drinking water (Human toxicology)	Dicamba	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Dicamba	0.05 µg/L	E_rC_{50} >0.45 mg a.s./L RAC 45 µg/L (Myriophyllum spicatum)
Air	Dicamba	2 µg/m^3	$AOEL_{sys}$ 0.3 mg/kg bw/day
Tissue (meat or liver)	Not defined	Not required	Not classified as T / T+
Body fluids		Not required	Not classified as T / T+

5.3.2.1 Description of analytical methods for the determination of residues of dicamba in plant matrices (KCP 5.2.1)

An overview on the acceptable methods and possible data gaps for analysis of dicamba in plant matrices is given in the following tables.
Only single residue methods have been described here although intended for monitoring purposes. Due to the chemical nature of the dicamba residues, strong acid hydrolysis is needed – this procedure is not consistent with multi-residue methodology.

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

Component of residue definition: Dicamba and its salts and conjugated dicamba expressed as dicamba				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary: REM 193.01[(a) (b)]	0.01 mg/kg	HPLC MS/MS	Gasser A., 1998 EU agreed [(c)] (Denmark, 2007)
	Validation: R97-003 SYN/DIC/03041			Gasser A., 1997 Maffezzoni M., 2004 EU agreed [(c)] (Denmark, 2007)
	ILV: ADE-0402V			Steinhauer, S., 2004 EU agreed [(c)] (Denmark, 2007)
High protein/high starch content (dry)	Primary: REM 193.01[(a) (b)]	0.01 mg/kg	HPLC MS/MS	Gasser A., 1998 EU agreed [(c)] (Denmark, 2007)
	Validation: R97-003 SYN/DIC/03041			Gasser A., 1997 Maffezzoni M., 2004 EU agreed [(c)] (Denmark, 2007)
	ILV: ADE-0402V			Steinhauer, S., 2004 EU agreed [(c)] (Denmark, 2007)
High acid content	Primary: REM 193.01[(a) (b)]	0.01 mg/kg	HPLC MS/MS	Gasser A., 1998 EU agreed [(c)] (Denmark, 2007)
	Validation: SYN/DIC/03041			Maffezzoni M., 2004 EU agreed [(c)] (Denmark, 2007)
High oil content	Primary: REM 193.01[(a) (b)]	0.01 mg/kg	HPLC MS/MS	Gasser A., 1998 EU agreed [(c)] (Denmark, 2007)
	Validation: SYN/DIC/03041			Maffezzoni M., 2004 EU agreed [(c)] (Denmark, 2007)

(a) This method is also for use for data generation
(b) The residue definitions for monitoring requires dicamba salts and conjugates to be quantified during analysis. In order to deconjugate residues, a hydrolysis step is required. Residues released following deconjugation are also subject to derivatisation prior to analysis. As a result of these requirements, multi-residue methods are not suitable for the determination of dicamba residues
(c) The EFSA peer review concluded that further information was needed to confirm whether the hydrolysis step is efficient in releasing dicamba conjugates (EFSA, 2011). In the new metabolism in rotational crop study presented in MCA Section 6 (CA 6.6.1/01: Swales, 2016), the hydrolysis step used in analytical methods was fully investigated for efficiency.

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	The extraction procedure has been shown to be efficient within a rotational crop metabolism study Swales, 2016 Report TK0103764 The extraction efficiency data is summarised in Appendix 2 New data; KCP 5.2.1/01

	Method for products of plant origin
	These data are currently under evaluation for the renewal of approval of the active substance, dicamba (Commission Implementing Regulation (EU) No. 844/2012 of 18 September 2012).
Not required, because:	-

Comments of zRMS:

Analytical methods for the determination of residues of dicamba and its metabolites in plant matrices are available and have been presented in the draft Assessment Report for dicamba (Vol.3, Section B.5.2, February 2007) and in the addendum to DAR (Vol.3, Section B.5.2, November 2010). During the peer review under Directive 91/414/EEC, the residue method REM 193.01 was demonstrated to be suitable for the determination of dicamba and its conjugates and was validated in high water- (pasture, maize plant), high starch- (maize grain), high oil- (rape seed), high acid-content matrices (orange) and dry matrices (maize straw), achieving a LOQ of 0.01 mg/kg. Suitable ILV data were provided for high water- (pasture) and high starch-content matrices (maize grain).
No additional data are required.

5.3.2.2 Description of analytical methods for the determination of residues of dicamba in animal matrices (KCP 5.2.2)

Table 5.3-4: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Not Required				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary: GRM022.05A[(a)(b)]	0.01 mg/kg	GC-MSD	Richardson, M. & Braid, S., 2012 New Data; KCP 5.2.2/01
	Validation: T010322-04-REG	0.01 mg/kg	GC-MSD	Heillaut, C., 2008 New Data; KCP 5.2.2/02
	ILV: B 1836 G	0.01 mg/kg	GC-MSD	Class, T. & Kuhn, T., 2010 New Data; KCP 5.2.2/03
Eggs	Primary: GRM022.05A[(a)(b)]	0.01 mg/kg	GC-MSD	Richardson, M. & Braid, S., 2012 New Data; KCP 5.2.2/01
	Validation: T010322-04-REG	0.01 mg/kg	GC-MSD	Heillaut, C., 2008 New Data; KCP 5.2.2/02
	ILV: B 1836 G	0.01 mg/kg	GC-MSD	Class, T. & Kuhn, T., 2010 New Data; KCP 5.2.2/03
Muscle	Primary: GRM022.05A[(a)(b)]	0.01 mg/kg	GC-MSD	Richardson, M. & Braid, S., 2012 New Data; KCP 5.2.2/01
	Validation: T010322-04-REG	0.01 mg/kg	GC-MSD	Heillaut, C., 2008 New Data; KCP 5.2.2/02
Fat	Primary: GRM022.05A[(a)(b)]	0.01 mg/kg	GC-MSD	Richardson, M. & Braid, S., 2012 New Data; KCP 5.2.2/01
	Validation: T010322-04-REG	0.01 mg/kg	GC-MSD	Heillaut, C., 2008 New Data; KCP 5.2.2/02
Kidney, liver	Primary: GRM022.05A[(a)(b)]	0.01 mg/kg	GC-MSD	Richardson, M. & Braid, S., 2012

Component of residue definition: Not Required				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
				New Data; KCP 5.2.2/01
	Validation: T010322-04-REG	0.01 mg/kg	GC-MSD	Heillaut, C., 2008 New Data; KCP 5.2.2/02
	ILV: B 1836 G	0.01 mg/kg	GC-MSD	Class, T. & Kuhn, T., 2010 New Data; KCP 5.2.2/03

(a) This method is also for use for data generation
(b) The residue definitions for monitoring requires dicamba salts and conjugates to be quantified during analysis. In order to deconjugate residues, a hydrolysis step is required. Residues released following deconjugation are also subject to derivatisation prior to analysis. As a result of these requirements, multi-residue methods such as S19 or QuEChERS are not suitable for the determination of dicamba residues.

These data are currently under evaluation for the renewal of approval of the active substance, dicamba (Commission Implementing Regulation (EU) No. 844/2012 of 18 September 2012).

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Animal analytical methods AM-0938-0994-0, GRM022.05A (dicamba), and GRM022.03A and B (dicamba and DCSA) all use the same acid extraction procedure (1M HCl; 95°C; 1.5h) followed by dichloromethane partition to isolate the above analytes from liver, muscle, fat and kidney (dichloromethane fraction analysed). Although the method used to extract these tissues in the supporting cow, goat and hen metabolism studies (see methods summary below) were not identical to that of the residue methods, the metabolism methods include procedures that demonstrate that the efficiency of the residue method extraction would be high. Guirguis & Yu, 1994. Report 28 (MCA 6.2.3) Yu & Atallah, 1983. Report 65 (MCA 6.2.2) Oehler & Ivie, 1980. Report SAN837/5145. (MCA 6.2.3)
Not required, because:	-

Comments of zRMS:

New analytical methods for the determination of dicamba residues in animal matrices have been provided by Applicant. The studies have been submitted for the purpose of renewal of dicamba.
An enforcement method (GRM022.05A) using GC-MSD is available and has been validated for the determination of dicamba in animal commodities (meat, fat, liver, kidney, milk and eggs) with an LOQ of 0.01 mg/kg.
The method has been independently validated in liver, eggs and milk.

Remark:
According to the information provided by Applicant, the independent laboratory validation study was repeated. See additional information in Appendix 2, point A 2.1.2.2.1.

5.3.2.3 Description of methods for residues of dicamba in the analysis of soil (KCP 5.2.4)

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

Table 5.3-6: Validated methods for soil

Component of residue definition: dicamba, DCSA			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
GRM022.06A	0.0035 mg/kg	LC-MS/MS	Method: Braid & Garcia-Alix, 2013 Report: SAN837_11434 New data; KCP 5.2.4/01 Validation: Garcia-Alix, 2013 Report: SAN837_11433 New data; KCP 5.2.4/02 EU agreed: No (new method, refer to Appendix 2)

For any special comments or remarkable points concerning the analytical methods for soil please refer to Appendix 2.

Comments of zRMS:

New analytical methods for the determination of dicamba and NOA414746 residues in soil samples have been provided by Applicant. The studies have been submitted for the purpose of renewal of dicamba.
This analytical method (GRM022.06A) has been successfully validated for the determination of dicamba and NOA414746 residues in soil, with a limit of quantification (LOQ) of 0.0035 mg/kg. It fulfils the requirements of SANCO 3029/99 rev.4 and SANCO/825/00 rev. 8.1.
No further data are required.

5.3.2.4 Description of methods residues of dicamba for the analysis of water (KCP 5.2.5)

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. For the detailed valuation of new studies it is referred to Appendix 2.

Table 5.3-7: Validated methods for water

Component of residue definition: dicamba, DCSA				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	GRM022.02A	0.05 µg/L	GC-MSD	Method: Hargreaves, 2007 Report: SAN837/6654 New data; KCP 5.2.5/01 Validation: Emburey, 2007 Report: SAN837/6653 New data; KCP 5.2.5/02 EU agreed: : Included in 2008 AIR top up but no evaluation published therefore provided in AIR3 Supplementary dossier and summarised in Appendix 2. ILV: Kotthoff, 2016 Report: SAN837_11602

Component of residue definition: dicamba, DCSA				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
				New data; KCP 5.2.5/03 EU agreed: No (new method; refer to Appendix 2)
	ILV	0.05 µg/L	GC-MSD	ILV: Kotthoff, 2016 Report: SAN837_11602 New data; KCP 5.2.5/03 EU agreed: No (new method; refer to Appendix 2)
Surface water	GRM022.02A	0.05 µg/L	GC-MSD	Method: Hargreaves, 2007 Report: SAN837/6654 New data; KCP 5.2.5/01 Validation: Emburey, 2007 Report: SAN837/6653 New data; KCP 5.2.5/02 EU agreed: Included in 2008 AIR top up but no evaluation published therefore provided in AIR3 Supplementary dossier and summarised in Appendix 2.

Component of residue definition: DCSA				
Drinking water Surface water	GRM022.09A	0.05 µg/L	LC-MS/MS	Method: Allen & Brooks (2017) Report: NOA414746_10010 New data KCP 5.1.2.1/08 Validation: Allen (2017) Report: NOA414746_100011 New data KCP 5.1.2.1/09

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

Comments of zRMS:

New analytical methods for the determination of dicamba and dicamba metabolite NOA414746 (DCSA) in water samples have been provided by Applicant. The studies have been submitted for the purpose of renewal of dicamba.
1. The analytical method (GRM022.02A) has been successfully validated for the determination of dicamba residues in water (river, groundwater and drinking water), with a limit of quantification (LOQ) of 0.05 µg/L.
Analytical method GRM022.02A was independent laboratory validated on drinking water samples at the limit of quantification (LOQ) of the method (0.05 µg/L).
It fulfils the requirements of SANCO 3029/99 rev.4 and SANCO/825/00 rev. 8.1.
2. The analytical method GRM022.09A has been successfully validated for the determination of the dicamba metabolite NOA414746 (DCSA) residues in water (groundwater, surface water and seawater), with a limit of quantification (LOQ) of 0.05 µg/L. It fulfils the requirements of SANCO 3029/99 rev.4 and SANCO/825/00 rev. 8.1.
No further data are required.

5.3.2.5 Description of methods residues of dicamba for the analysis of air (KCP 5.2.6)

An overview on the acceptable methods and possible data gaps for analysis of dicamba in air is given in the following tables. For the detailed evaluation of new studies please refer to Appendix 2.

Table 5.3-8: Validated methods for air

Component of residue definition: Open			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
GRM022.01A	0.002 µg/L (2.0 µg/m^3)	GC-MSD	Method: Hargreaves, 2007a Report: SAN837/6677 New data; KCP 5.2.6/01 Validation: Emburey, 2007a Report: SAN837/6678 New data; KCP 5.2.6/02 EU agreed: Included in 2008 AIR top up but no evaluation published therefore provided in AIR3 Supplementary dossier and summarised in Appendix 2.

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 2.

Comments of zRMS:

The analytical method has been validated for the determination of dicamba in air with a LOQ of 2 µg/m^3.
No further data are required.

5.3.2.6 Description of methods residues of dicamba for the analysis of body fluids and tissues (KCP 5.2.3)

Dicamba is not classified as toxic or highly toxic, therefore analytical methods for the determination of residues in body fluids and tissues are not required (EFSA, 2011).

Comments of zRMS:

According to the EFSA Journal 2011;9(1):1965 a method of analysis for body fluids and tissues is not required as the active substance is not classified as toxic or very toxic.
However in Commission Regulation (EU) No 283/2013 it is stated that "…*methods, with a full description, shall be submitted for the analysis in body fluids and tissues for active substance and relevant metabolites*".
In our opinion the analytical method for the determination of residues in body fluids and tissues is required and should be provided at the renewal of the active substance.

5.3.2.7 Other studies/ information

None.

5.3.3 Description of analytical methods for the determination of residues of mesotrione (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 / LOQ	Reference for MRL/level Remarks
High water content *Lettuce*	Mesotrione	0.01 mg/kg	Regulation (EU) No ~~2016/53~~ 2017/626
High acid content *Orange*		0.01 mg/kg	Regulation (EU) No ~~2016/53~~ 2017/626
High oil content *Sunflower seed*		0.01 mg/kg	Regulation (EU) No ~~2016/53~~ 2017/626
High protein/high starch content (dry) *Maize grain, dry broad bean*		0.01 mg/kg	Regulation (EU) No ~~2016/53~~ 2017/626
Difficult matrices		0.01 – 0.05 mg/kg	Regulation (EU) No ~~2016/53~~ 2017/626
Muscle	(Mesotrione)[(a)]	0.01 mg/kg	Regulation (EU) No ~~2016/53~~ 2017/626
Milk		0.01 mg/kg	Regulation (EU) No ~~2016/53~~ 2017/626
Eggs		0.01 mg/kg	Regulation (EU) No ~~2016/53~~ 2017/626
Fat		0.01 mg/kg	Regulation (EU) No ~~2016/53~~ 2017/626
Liver, kidney		0.01 mg/kg	Regulation (EU) No ~~2016/53~~ 2017/626
Soil (Ecotoxicology)	Mesotrione MNBA AMBA	1.18 mg/kg 36 mg/kg 210 mg/kg	EC_{10}/5 for earthworms NOEC/5 for earthworms NOEC/5 for earthworms
Drinking water (Human toxicology)	Active ingredient MNBA AMBA	0.1 µg/L 10 µg/L 10 µg/L	general limit for drinking water (sanco/221/2000 rev 10)
Surface water (Ecotoxicology)	Active ingredient MNBA AMBA	3.1 µg/L 3.8 mg/L 0.94 mg/L	Endpoint covers geometric mean aquatic macrophytes based on E_rC_{50} Lowest EC_{50}/10 from aquatic toxicity studies
Air	Mesotrione	4.5 µg/m^3	AOEL sys: 0.005 mg/kg bw/d (corrected for 50 % oral absorption)d No AOEL inhal.
Tissue (meat or liver)	Mesotrione	0.01 mg/kg	Validation: Watson, 2013b Report S12-03250 EU agreed (UK, 2015, 2015a)
Body fluids		0.01 mg/kg	

(a) No residue definition and MRLs have been set for products of animal origin.

5.3.3.2 Description of analytical methods for the determination of residues of mesotrione in plant matrices (KCP 5.2.1)

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in plant matrices is given in the following tables. No new data are submitted in the framework of this application.

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

Component of residue definition: mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content *Maize forage*	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	QuEChERS Method and validation: Watson, 2013a Report: S12-03251 ILV: Tessier, 2013 Report: S12-04607 EU agreed (UK, 2015, 2015a)
	ILV (QuEChERS)	0.01 mg/kg		
High acid content *Whole orange*	QuEChERS	0.01 mg/kg		
High oil content *Oilseed rape seed*	QuEChERS	0.01 mg/kg		
High protein/high starch content (dry) *Maize kernel*	QuEChERS	0.01 mg/kg		
	ILV (QuEChERS)	0.01 mg/kg		

Table 5.3-11: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Studies on the metabolism of mesotrione in maize incorporate a number of different extraction steps, one of which is extraction with acetonitrile/water in a 1:1 ratio. Residues of mesotrione in grain were extremely low and therefore no data are available to address the extraction efficiency in grain however data are available from samples of maize fodder and forage leaf as these samples contained the majority of the radioactivity. These data indicate that the majority of the total radioactive residue obtained via solvent extraction from these matrices was extracted via use of acetonitrile/water and subsequent characterisation indicated that these extracts contained the residue of mesotrione. This would therefore indicate that the use of acetonitrile/water in a 1:1 v/v ratio is effective for extraction of residues of mesotrione. Wei & Dohn, 1997 Report: RR 96-026B Tarr & van Neste, 1997 Report: RR96-007B EU agreed (UK, 2015)
Not required, because:	-

Comments of zRMS:

The analytical method QuEChERS was validated and independent validated for the determination of mesotrione in plant matrices according to the SANCO 825/00 rev. 8.1.
No further data are required.

5.3.3.3 Description of analytical methods for the determination of residues of mesotrione in animal matrices (KCP 5.2.2)

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in animal matrices is given in the following tables. New data are submitted in the framework of this application and are summarised in detail in Appendix 2.

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

Component of residue definition: (mesotrione)[a]				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Muscle	QuEChERS	0.01 mg/kg	LC-MS/MS (multi-residue)	QuEChERS Method and validation: Watson, 2013b Report: S12-03250 EU agreed (UK, 2015, 2015a) ILV: Bernal, 2013 Report: S12-04608 EU agreed (UK, 2015, 2015a)
Fat	QuEChERS	0.01 mg/kg		
Liver	QuEChERS	0.01 mg/kg		
	ILV	0.01 mg/kg		
Kidney	QuEChERS	0.01 mg/kg		
Milk	QuEChERS	0.01 mg/kg		
	ILV	0.01 mg/kg		
Eggs	QuEChERS	0.01 mg/kg		
	ILV	0.01 mg/kg		
Whole blood	QuEChERS	0.01 mg/kg		

(a) No residue definition and MRLs have been set for products of animal origin.

Table 5.3-13: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	--
Not required, because:	No study on mesotrione metabolism and extraction efficiency in animal matrices is required since intake of mesotrione is not significant. Consequently, no MRLs have been set for products of animal origin and no monitoring method for residues in animal products is necessary.

Comments of zRMS:

The analytical method QuEChERS was validated and independent validated for the determination of mesotrione with LOQs of 0.01 mg/kg in all animal matrices according to the SANCO 825/00 rev. 8.1.
No further data are required.

5.3.3.4 Description of methods residues of mesotrione for the analysis of body fluids and tissues (KCP 5.2.3)

The following methods can be used to determine residue levels of mesotrione in body fluids and tissues. New data are submitted in the framework of this application and are summarised in detail in Appendix 2.

Table 5.3-14: Methods for body fluids and tissues

Component of residue definition: mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / miss-ing**
Whole blood	QuEChERS Method	0.01 mg/kg	HPLC-MS/MS	Validation: Watson, 2013b Report S12-03250

Component of residue definition: mesotrione				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
				EU agreed (UK, 2015, 2015a)

Comments of zRMS:

The multi-residue method was validated for the determination of mesotrione in body fluids and tissues with a LOQ of 0.01 mg/kg.

5.3.3.5 Description of methods for residues of mesotrione in the analysis of soil (KCP 5.2.4)

An overview on the acceptable methods and possible data gaps for analysis of mesotrione, MNBA and AMBA in soil is given in the following tables. No new studies have been submitted.

Table 5.3-15: Validated methods for soil

Component of residue definition: mesotrione, MNBA and AMBA			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
GRM007.10A	0.002 mg/kg	LC-MS/MS	Method: Jutsum & Williams, 2013 GRM007.10A Validation: Jutsum, 2013 CEMR-5657-REG EU agreed (UK, 2015, 2015a)
T001200-03	0.002 mg/kg	HPLC-MS/MS	Method: Williams, 2004 Report T001200-03 New data; KCP 5.2.4/01

Comments of zRMS:

The analytical method (HPLC-MS/MS) with LOQ of 0.002 mg/kg was validated for the determination of mesotrione and its metabolites AMBA and MNBA in soil.
No further data are required.

5.3.3.6 Description of methods for residues of mesotrione in the analysis of water (KCP 5.2.5)

An overview on the acceptable methods and possible data gaps for analysis of mesotrione, MNBA and AMBA in surface and drinking water is given in the following tables. No new studies have been submitted.

Table 5.3-16: Validated methods for water

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Component of residue definition: mesotrione, MNBA and AMBA				
Drinking water	GRM007.09A	0.05 (surface water) µg/L	HPLC-MS/MS	Method: Jutsum & Chamkesam, 2013 Report GRM007.09A Validation: Jutsum, 2013a Report CEMR-5658-REG EU agreed (UK, 2015, 2015a)
	ILV	0.05 µg/L	HPLC-MS/MS	Wiesner & Breyer, 2013 Report S13-04185 EU agreed (UK, 2015, 2015a)
Ground water	GRM007.09A	0.05 µg/L	HPLC-MS/MS	Method: Jutsum & Chamkesam, 2013 Report GRM007.09A Validation: Jutsum, 2013a Report CEMR-5658-REG EU agreed (UK, 2015, 2015a)
Component of residue definition: Mesotrione and MNBA				
Drinking water	TMR0707B	0.05 µg/L	GC-MSD	Method: Meyers, 1997 Report TMR0707B EU Agreed (UK, 2015, 2015a)

Comments of zRMS:

The analytical methods with LOQ of 0.05 µg/L were validated for the determination of mesotrione and its metabolites AMBA and MNBA in ground and surface water.
ILV with LOQ of 0.05 µg/L for drinking water is available and accepted.
No further data are required.

5.3.3.7 Description of methods for residues of mesotrione in the analysis of air (KCP 5.2.6)

An overview on the acceptable methods and possible data gaps for analysis of mesotrione in air is given in the following tables. No new studies have been submitted.

Table 5.3-17: Validated methods for air

Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Component of residue definition: mesotrione			
RR 97-031B	0.01 mg/m^3	HPLC-UV	Method: Leung, 1997 Report RR 97-031B EU agreed (UK, 2015, 2015a)
GRM007-08B	0.45 µg/m^3	HPLC-MS/MS	Method: Jutsum, 2013b GRM007.08B Validation: Jutsum, 2013c CEMR-5403-REG EU agreed (UK, 2015, 2015a)

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 2.

Comments of zRMS:

The analytical method HPLC-MS/MS with LOQ of 0.45 µg/m^3 was validated for the determination of mesotrione in air.
No further data are required.

5.3.3.8 Other studies/ information

No new data submitted in the framework of this application.

5.3.4 Description of analytical methods for the determination of residues of nicosulfuron (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.

Table 5.3-18: 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	Reg. (EU) No 617/2014
Plant, high acid content		0.01* mg/kg	Reg. (EU) No 617/2014
Plant, high protein/high starch content (dry commodities)		0.01* mg/kg	Reg. (EU) No 617/2014
Plant, high oil content		0.02* mg/kg	Reg. (EU) No 617/2014
Plant, difficult matrices (hops, spices, tea)		0.05* mg/kg	Reg. (EU) No 617/2014
Muscle	Not defined	0.02* mg/kg	Reg. (EU) No 617/2014
Milk		0.02* mg/kg	Reg. (EU) No 617/2014
Eggs		0.02 mg/kg	Reg. (EU) No 617/2014
Fat		0.02 mg/kg	Reg. (EU) No 617/2014
Liver, kidney		0.02 mg/kg	Reg. (EU) No 617/2014
Soil (Ecotoxicology)	Nicosulfuron	100 mg/kg dw	Endpoint covered for *Eisenia fetida* LC_{50}/5(safety factor)
Drinking water (Human toxicology)	Nicosulfuron	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Nicosulfuron	0.17 µg/L	Endpoint covered for *Lemna gibba* E_rC_{50}/10(safety factor)
Air	Nicosulfuron	1.2 µg/m^3	$AOEL_{sys}$ 0.8 mg/kg bw/d
Tissue (meat or liver)	Not defined	Not required	Not classified as T / T+
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 5.2.1)

An overview on the acceptable methods and possible data gaps for analysis of nicosulfuron in plant matrices is given in the following tables.
Single and multi-residue methods are described here for monitoring purposes. EFSA (EFSA, 2012) highlighted that a multi-residue QuEChERS using diatomaceous earth clean up in combination with LC-MS/MS method, as described by CEN (2008), is also available to analyse the parent nicosulfuron in dry commodities.
EFSA concluded (EFSA, 2012) that all tentative MRLs still need to be confirmed by the following data:

- Confirmatory data for the HPLC-MS/MS method with an LOQ of 0.01 mg/kg in dry commodities;
- An analytical method, its ILV and a confirmatory method fully validated for the determination of parent nicosulfuron in high water content commodities.

New data are presented below to address these outstanding requirements. For the detailed evaluation of new studies it is referred to Appendix 2. These data are currently under evaluation for the renewal of approval of the active substance, nicosulfuron (Article 14 of Regulation (EC) 1107/2009 and Article 6 of Commission Implementing Regulation (EU) No. 844/2012 of 18 September 2012).

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

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Component of residue definition: nicosulfuron				
High water content *Maize whole plant*	107 NIS	0.01 mg/kg	HPLC-MS/MS	Multiresiude Method: Validation: Steinhilper, 2008[(a)] Report: 107 NIS New data, KCP 5.2.1 ILV: Schwarz, 2008[(a)] Report: 119 NIS New data, KCP 5.2.1
	H-641	0.01 mg/kg (LOD)	HPLC-UV	Kanza et al., 1991 Report: H-641 EU agreed, UK 2007
High water content *Maize sprout*	614351	0.01 mg/kg Confirmatory: 0.025 mg/kg	HPLC-UV Confirmatory: GC-MS or HPLC-MS	Method: Huber, 1996 Report: 614351 Confirmatory method: Mirbach, 1998, Report: 699873 EU agreed, UK 2006
High water content *Corn silage, corn forage cherry*	DuPont-11776	0.01 mg/kg	HPLC-MS/MS	Method: Cabusas & Pentz, 2012 Report: DuPont-11776 New data, KCP, 5.2.1 Validation: McInerney, 2016 Report: 100077587-03 New data, KCP, 5.2.1 ILV: Ducat & Pigeon, 2004 Report: DuPont-12347 New data, KCP 5.2.1
High acid content *Lemon*	DuPont-11776	0.01 mg/kg	HPLC-MS/MS	Method: Cabusas & Pentz, 2012 Report: DuPont-11776 New data, KCP, 5.2.1 Validation: McInerney, 2016 Report: 100077587-03 New data, KCP, 5.2.1 ILV: Not provided*

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
High oil content	DuPont-11776	0.01 mg/kg	HPLC-MS/MS	Method: Cabusas & Pentz, 2012 Report: DuPont-11776 New data, KCP, 5.2.1 Validation: McInerney, 2016a Report: 100077587-03 New data, KCP, 5.2.1 ILV: Not provided*
High protein/high starch content (dry) *Maize grain*	793596	0.01 mg/kg	HPLC-MS/MS	Method: Wolf, 2000 Report: 793596 ILV: Ginzburg, 2000 Report: A-22-00-04 EU Agreed, UK 2006
	107 NIS	0.01 mg/kg	HPLC-MS/MS	Multiresidue Method: Validation: Steinhilper, 2008[(a)] Report: 107 NIS New data, KCP 5.2.1 ILV: Schwarz, 2008[(a)] Report: 119 NIS New data, KCP 5.2.1

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	614351	0.01mg/kg Confirmatory: 0.025 mg/kg	HPLC-UV Confirmatory: GC-MS or HPLC-MS/MS	Method: Huber, 1996 Report: 614351 Confirmatory method: Mirbach, 1998, Report: 613866 EU agreed, UK 2006
High protein/high starch content (dry) *Corn grain*	DuPont-11776	0.01 mg/kg	HPLC-MS/MS	Method: Cabusas & Pentz, 2012 Report: DuPont-11776 New data, KCP, 5.2.1 Validation: McInerney, 2016 Report: 100077587-03 New data, KCP, 5.2.1 ILV: Ducat & Pigeon, 2004 Report: DuPont-12347 New data, KCP 5.2.1
Difficult *Maize straw*	793596	0.01 mg/kg	HPLC-MS/MS	Method: Wolf, 2000 Report: 793596 EU Agreed (UK, 2006) ILV: Ginzburg, 2000 Report: A-22-00-04 EU Agreed, UK 2006
	107 NIS	0.01 mg/kg	HPLC-MS/MS	Multiresiude Method: Validation: Steinhilper, 2008[(a)] Report: 107 NIS New data, KCP 5.2.1 ILV: Schwarz, 2008[(a)] Report: 119 NIS New data, KCP 5.2.1

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Analyte: ADMP, ASDM (nicosulfuron metabolites but not currently a component of the residue definition for food of plant origin)				
High protein/high starch content (dry) *Maize grain*	614351	0.04 mg/kg for ADMP 0.02 mg/kg for ASDM	HPLC-UV	Method: Huber, 1996 Report: 614351 EU agreed, UK 2007
High water content *Maize whole plant*	614351	0.04 mg/kg for ADMP 0.06 mg/kg for ASDM	HPLC-UV	Method: Huber, 1996 Report: 614351 EU agreed, UK 2007
	H-641	0.02 mg/kg (LOD)	HPLC-UV	Method: Kanza et al., 1991 Report: H-641 EU agreed, UK 2006

(a) All data are owned by Cheminova A/S: access is granted to original studies via a letter of access;
* The active substance nicosulfuron was evaluated on EU level according to the old data requirements in 2006 (DAR 2006). No new active substance data will be submitted in this application according to the guidance document on the interpretation of the transitional measures for the data requirements for chemical active substances and plant protection products according to Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013 (SANTE/11509 /2013– rev. 5.2, 9 October 2015).

Table 5.3-20: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	--
Not required, because:	Residues of nicosulfuron are not anticipated to be above LOQ in any matrix

Comments of zRMS:

The analytical methods for the determination of nicosulfuron in plant matrices are available.

Analytical method (Steinhilper D., 2008 / Report No. 107 NIS) and its ILV (Schwarz T., 2008 / Report No. 119 NIS) using LC-MS/MS for the determination of residues of nicosulfuron in maize matrices (plant, grain and stover) has been validated considered as highly specific with a LOQ at 0.01 mg/kg.
No further data are required.

5.3.4.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2.2)

EFSA (EFSA, 2012) concluded that "there is no significant intake of residues by livestock, no residue definition and no MRLs are proposed for commodities of animal origin […]. Therefore, an analytical method for enforcement of residues in food of animal origin is not necessary." Consequently, no method for animal tissues has been reviewed or proposed.

Method type	Method LOQ	Principle of method (*i.e.* GC-MS or HPLC-UV)	Author(s), year / missing
Component of residue definition: nicosulfuron			
90011604	0.01 mg/kg (muscle and liver)	HPLC-MS/MS	Method: Wolf, 2009 Report: Report: 90011604

Method type	Method LOQ	Principle of method (*i.e.* GC-MS or HPLC-UV)	Author(s), year / missing
			New data, KCP 5.2.2

Comments of zRMS:

The analytical method for the determination of nicosulfuron in animal matrices is available.
The analytical method (Wolf, 2009) was successfully validated for the determination of nicosulfuron (DPX-V9360) in animal tissues (milk, egg, muscle and liver) at a limit of quantitation (LOQ) of 0.010 mg/kg egg, muscle and liver and 0.01 g/L for milk according to the SANCO /825/00 rev. 8.1.
No further data are required.

5.3.4.4 Description of methods for the analysis of body fluids and tissues (KCP 5.2.3)

Nicosulfuron is not classified as toxic or highly toxic, therefore analytical methods for the determination of residues in body fluids and tissues are not required (EFSA, 2008).
An overview on the acceptable methods and possible data gaps for analysis of nicosulfuron in body fluids and tissues is given in the following table. For the detailed evaluation of new studies it is referred to Appendix 2.

Table 5.3-21: Methods for body fluids and tissues

Component of residue definition: mesotrione			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
100077587-04	~~0.1~~ 0.05 mg/L (plasma)	HPLC-MS/MS	Method: xxxxxxxxxxxxx Report: 100077587-04 New data, KCP 5.2.3

For any special comments or remarkable points concerning the analytical methods for body fluids and tissues please refer to Appendix 2.

Comments of zRMS:

The analytical method for the determination of nicosulfuron in body fluids is available.
The analytical method (xxxxxxxxxxxxx 2016a) for the determination of nicosulfuron in mouse plasma was successfully validated by achieving a calibration with a coefficient of determination (R^2)> 0.990 and mean recoveries of fortified samples between 70% and 110% with a relative standard deviation < 20% for both transitions monitored (following SANCO/825/00 rev 8.1). The validated LOQ was 0.05 mg/L.
No further data are required.

5.3.4.5 Description of methods for the analysis of soil (KCP 5.2.4)

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

Table 5.3-22: Validated methods for soil

Component of residue definition: nicosulfuron			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
EFSA Scientific Report (2007) 120, 1-91	0.05 µg/kg	LC-MS	Method: Wais, 2000a Report: 770117 EU agreed: UK, 2007
614340	0.005 mg/kg	HPLC-UV	Method: Huber, 1996a Report: Report: 614340

Component of residue definition: nicosulfuron			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
			EU agreed: UK, 2007

Comments of zRMS:

The analytical method with LOQ of 0.05 µg/kg was validated for the determination of nicosulfuron in soil and was accepted (IK, 2007).
No further data are required.

5.3.4.6 Description of methods for the analysis of water (KCP 5.2.5)

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. For the detailed evaluation of additional studies it is referred to Appendix 2.

Table 5.3-23: 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 / missing**
Drinking water	604383	0.05 µg/L	HPLC-UV	Method: Schulz and Ullrich-Mitzel, 1995a Report: 604383 EU agreed: UK, 2007
	Confirmatory	0.05 µg/L	LC- DAD	Method: Wais, 2000b Report: DAR Vol 3, Annex B5, June 2006 EU agreed: UK, 2007
	ILV	-	-	Not required, old data requirements apply
	B25773	0.05 µg/L	HPLC-MS/MS	Method: Wolf, 2007 Report: B25773 EU agreed: UK, 2007
Surface water	GRM042.01A	0.05 µg/L	LC- DAD	Method: Wais, 2000b Report: 770128 EU agreed: UK, 2007
Analyte: ADMP, ASDM, AUSN (nicosulfuron metabolites but not currently a component of the residue definition for food of plant origin)				
Drinking water	604574	0.05 µg/L	HPLC-UV HPLC-MS for AUSN	Method: Wais & Ullrich-Mitzel, 1997 Report 604574 EU agreed: UK, 2007

Comments of zRMS:

The analytical method with LOQ of 0.05 µg/L was validated for the determination of nicosulfuron and its metabolites ADMP, ASDM, AUSN in drinking and surface water and was accepted (UK, 2007).
ILV for drinking water is not available (old data requirements apply).

5.3.4.7 Description of methods for the analysis of air (KCP 5.2.6)

An overview on the acceptable methods and possible data gaps for analysis of nicosulfuron in air is given in the following tables. No new studies have been conducted as part of this assessment.

Table 5.3-24: 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 / missing**
385470	1.2 µg/m^3	HPLC-UV	Method: Schulz & Ullrich-Mitzel, 1995a Report: 385470 EU agreed: UK, 2007
765358	1.2 µg/m^3	HPLC-UV	Method: Wais, 2000b Report: 765358 EU agreed: UK, 2007

Comments of zRMS:

The analytical methods with LOQ of 1.2 µg/m^3 were validated for the determination of nicosulfuron in air and were accepted (UK, 2007).
No further data are required.

5.4 References

Dicamba

Denmark, 2007. Draft assessment report on the active substance dicamba prepared by the rapporteur Member State Denmark in the framework of Council Directive 91/414/EEC, February 2007

Denmark, 2010. Final addendum to the Draft Assessment Report (DAR) – public version prepared by the rapporteur Member State Denmark in the framework of Council Directive 91/414/EEC, November 2010

EFSA (European Food Safety Authority), 2011. Conclusion on the peer review of the pesticide risk assessment of the active substance dicamba. EFSA Journal 2011;9(1):1965, [52 pp.] doi:10.2903/j.efsa.2011.1965

Mesotrione

United Kingdom, 2015. Renewal assessment report (RAR) on the active substance mesotrione prepared by the rapporteur Member State, the United Kingdom, in the framework of Commission Implementing Regulation (EU) No 844/2012, February 2015

United Kingdom, 2015a. Revised renewal assessment report (RAR) on mesotrione, compiled by EFSA, December 2015

Nicosulfuron

EFSA (European Food Safety Authority), 2012: Reasoned opinion on the review of the existing maximum residue levels (MRLs) for nicosulfuron according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2012;10(12):3048. [27 pp.] doi:10.2903/j.efsa.2012.3048

United Kingdom, 2006. Draft assessment report on the active substance nicosulfuron prepared by the rapporteur Member State United Kingdom in the framework of Council Directive 91/414/EEC, June 2006

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted or referred to by the applicant and relied on

Plant Protection Product

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
KCP 5.1.1 / 01	Adolph S.	2012	Analytical Method SF-568/1 - mesotrione/dicamba/nicosulfuron WG (15731.25/10) in formulation, by HPLC Syngenta Syngenta Crop Protection, Münchwilen, Switzerland, 10493506 Not GLP not published Syngenta File No A18032E_10062	N	SYN (ADAMA has LOA)
KCP 5.1.1 / 02	De Benedictis S.	2013	A18032E - Validation of Analytical Method SF-568/1 Syngenta Syngenta Crop Protection, Münchwilen, Switzerland, 10528232 GLP not published Syngenta File No A18032E_10063	N	SYN (ADAMA has LOA)
KCP 5.1.1 / 03	Hager M.	2011	R287431 - Analytical Method SD-977/2 Syngenta Syngenta Crop Protection, LLC, Greensboro, NC, USA, 10427012 Not GLP not published Syngenta File No R287431_10003	N	SYN (ADAMA has LOA)
KCP 5.1.1 / 04	Hager M.	2011a	Validation of method SD-977/2 - R287431 in A14203B, A13789C, A14351BX, A12909Q, A15189G, A12738A, A15901A and A18219B Syngenta Syngenta Crop Protection, Inc., Greensboro, USA, 10427878 GLP not published Syngenta File No R287431_10001	N	SYN (ADAMA has LOA)
KCP 5.1.1 / 05	Hager M.	2013	Mesotrione - Relevant Impurity in A18032E Syngenta	N	SYN (ADAMA

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**
			Syngenta Crop Protection, LLC, Greensboro, NC, USA, 10538107 Not GLP not published Syngenta File No A18032E_10061		has LOA)
KCP 5.1.1 / 06	Hager M.	2017	A18032E- Statement on Validation of Analytical Method SD-977 /2 for the Determination of R287431 (Xan-1) in Formulation A18032E Syngenta Syngenta Crop Protection, LLC, Greensboro, NC, USA, 300074792 Not GLP not published Syngenta File No A18032E_10366	N	SYN (ADAMA has LOA)
KCP 5.1.1 / 07	Hager M., *et al*	2017	A18032E - Response to the Greek Regulatory Authority Concerning Relevant Impurity R287431, Syngenta Syngenta Crop Protection, LLC, Greensboro, NC, USA Not GLP not published Syngenta File N.o. A18032E_10452	N	SYN (ADAMA has LOA)
KCP 5.1.1 / 08	Huang S.	2016	ZA1296 - SD-1990/1 - Determination of R287432 in Mesotrione Related Formulations by Liquid Chromatography/Mass Spectrometry (LC/MS) Syngenta Syngenta Crop Protection, LLC, Greensboro, NC, USA, 300068727 Not GLP not published Syngenta File No A13789C_50005	N	SYN (ADAMA has LOA)
KCP 5.1.1 / 09	Huang S.	2016a	A13789C - Validation of Analytical Method SD-1990/1 Syngenta Syngenta Crop Protection, LLC, Greensboro, NC, USA, USGR160250 GLP not published Syngenta File No A13789C_50004	N	SYN (ADAMA has LOA)
KCP 5.1.1 / 10	Huang S.	2016b	A18032E - Statement on Validation of Analytical Method SD-1990/1 for Determination of R287432 in Formulation A18032E (SAN837/ZA1296/nicosulfuron WG (31.25/15/10)) Syngenta	N	SYN (ADAMA has LOA)

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**
			Syngenta Crop Protection, LLC, Greensboro, NC, USA, 300072567 Not GLP not published Syngenta File No A18032E_10347		
KCP 5.1.1 / 11	Meyerhoffer W., Zhang Y., Patterson J.	2016	ZA1296 - SD-1973/1 - Determination of Impurity DCE (1,2-dichloroethane) in Mesotrione Related Formulations by Headspace Gas Chromatography Syngenta Syngenta Crop Protection, LLC, Greensboro, NC, USA, 300066025 Not GLP not published Syngenta File No A13789C_50002	N	SYN (ADAMA has LOA)
KCP 5.1.1 / 12	Meyerhoffer W.	2016	A13789C - Validation of Analytical Method SD-1973/1 Syngenta Syngenta Crop Protection, LLC, Greensboro, NC, USA, USGR160249 GLP not published Syngenta File No A13789C_50001	N	SYN (ADAMA has LOA)
KCP 5.1.1 / 13	Meyerhoffer W.	2016a	A18032E - Statement on Validation of Analytical Method SD-1973/1 for Determination of 1,2-Dichloroethane (DCE) in Formulation A18032E (SAN837/ZA1296/Nicosulfuron WG (31.25/15/10)) Syngenta Syngenta Crop Protection, LLC, Greensboro, NC, USA, 300072403 Not GLP not published Syngenta File No A18032E_10346	N	SYN (ADAMA has LOA)

Dicamba

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
KCP 5.1.2.1 / 01 & KCP 5.2.4 / 01	Braid S., Garcia-Alix M.	2013	Dicamba - Analytical Method GRM022.06A for the Determination of Dicamba and its Metabolite NOA414746 in Soil Syngenta CEMAS, North Ascot, United Kingdom, GRM022.06A Not GLP not published Syngenta File No SAN837_11434	N	SYN/ BASF (ADAMA has LOA)
KCP 5.1.2.1 / 02 & KCP 5.2.4 / 02	Garcia-Alix M.	2013	Dicamba - Analytical Method GRM022.06A for the Determination of Dicamba and its Metabolite NOA414746 in Soil Syngenta CEMAS, North Ascot, United Kingdom, CEMR-5791-REG GLP not published Syngenta File No SAN837_11433	N	SYN/ BASF (ADAMA has LOA)
KCP 5.1.2.1 / 03 & KCP 5.2.5 / 01	Hargreaves S.	2007	Dicamba - Residue Method for the Determination of Residues in Water Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott's Hill, Bracknell, United Kingdom, GRM022.02A Not GLP not published Syngenta File No SAN837/6654	N	SYN/ BASF (ADAMA has LOA)
KCP 5.1.2.1 / 04 & KCP 5.2.5 / 02	Emburey S.	2007	Dicamba - Validation of an Analytical Method for the Determination of Residues of Dicamba in Water Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott's Hill, Bracknell, United Kingdom, T002102-06-REG GLP not published Syngenta File No SAN837/6653	N	SYN/ BASF (ADAMA has LOA)
KCP 5.1.2.1 / 05 & KCP 5.2.5 / 03	Kotthoff M.	2016	Dicamba - Independent Laboratory Validation of Analytical Method GRM022.02A for the Determination of Residues of Dicamba (SAN837) in Water Syngenta Fraunhofer Institute, Schmallenberg, Germany, SYN-037/6-22 GLP not published Syngenta File No SAN837_11602	N	SYN/ BASF (ADAMA has LOA)

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**
KCP 5.1.2.1 / 06 & KCP 5.2.6 / 01	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 Not GLP not published Syngenta File No SAN837/6677	N	SYN/ BASF (ADAMA has LOA)
KCP 5.1.2.1 / 07 & KCP 5.2.6 / 02	Emburey S.	2007a	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	N	SYN/ BASF (ADAMA has LOA)
KCP 5.1.2.1/08 KCP 5.2.5 / 04	Allen, L. & Brooks S.	2017	Dicamba - Residue Method GRM022.09A for the Determination of the Metabolite NOA414746 (DCSA) in Water. Syngenta Analytical Method GRM022.09A. CEM Analytical Services Ltd (CEMAS), Imperial House, Oaklands Business Centre, Oaklands Park, Wokingham, Berkshire, RG41 2FD UK. Not GLP Not published Syngenta File No. NOA414746_10010	N	SYN (ADAMA has LOA)
KCP 5.1.2.1/09 & KCP 5.2.5 / 05	Allen, L.	2017	Dicamba - Validation of Draft Residue Method GRM022.09A for the Determination of Dicamba Metabolite NOA414746 (DCSA) in Water. CEMAS Report Number CEMR-7878. CEM Analytical Services Ltd (CEMAS), Imperial House, Oaklands Business Centre, Oaklands Park, Wokingham, Berkshire, RG41 2FD UK. GLP Not published Syngenta File No. NOA414746_10011	N	SYN (ADAMA has LOA)
KCP 5.1.2.5 / 01	Richards S., Mackenzie R.	2006	Residue analytical method for the determination of residues of dicamba (SAN837) and 5-hydroxy dicamba (NOA405873) in barley (grain, straw, whole plant) and barley processed fractions (malt, wort, spent hops and spent yeast) Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott's Hill International, Bracknell, Berkshire, United Kingdom, REM 193.05 Not GLP not published Syngenta File No SAN837/6535	**N**	SYN/ BASF (ADAMA has LOA)

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**
KCP 5.1.2.5 / 02	Richards SJ., Mackenzie R., Crook SJ.	2008	Residue analytical method for the determnation of Dicamba (SAN837) and 5-Hydroxy Dicamba (NOA405873) in barley (grain, straw, whole plant) and barley processed fractions (malt, wort, spent hops and spent yeast) Syngenta - Jealott's Hill International, Bracknell, Berkshire, United Kingdom Syngenta - Jealott's Hill International, Bracknell, Berkshire, United Kingdom, REM193.05B Not GLP not published Syngenta File No SAN837/6686	**N**	SYN/ BASF (ADAMA has LOA)
KCP 5.1.2.5 / 03	Richards S., Mackenzie R.	2004	Residue Study with Dicamba (SAN837) in or on Winter Barley in France (South) Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott's Hill, Bracknell, United Kingdom, 03-7009 GLP not published Syngenta File No SAN837/6191	**N**	SYN/ BASF (ADAMA has LOA)
KCP 5.1.2.5 / 04	Richards S.	2004	Residue Study with Triasulfuron (CGA131036) and Dicamba (SAN837) in or on Winter Barley in The United Kingdom Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott's Hill, Bracknell, United Kingdom, 03-7013 GLP not published Syngenta File No CGA131036/1358	**N**	SYN/ BASF (ADAMA has LOA)
KCP 5.1.2.5 / 05	Richards S., Mackenzie R.	2004a	Residue Study with Dicamba (SAN837) in or on Winter Barley and Brewing Fractions in The United Kingdom Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott's Hill, Bracknell, United Kingdom, 03-7017 GLP not published Syngenta File No SAN837/6359	**N**	SYN/ BASF (ADAMA has LOA)
KCP 5.1.2.5 / 06	Braid S., Kennedy S.	2017	Dicamba - Analytical Method GRM022.07A for the Determination of Dicamba and its Metabolite NOA405873 in Crops Syngenta Syngenta - Jealott's Hill, Bracknell, United Kingdom, GRM022.07A Not GLP not published Syngenta File No SAN837_11703	**N**	SYN/ BASF (ADAMA has LOA)

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**
KCP 5.1.2.5 / 07	Kennedy S.	2016	Dicamba - Validation of Analytical Method GRM022.07A for the Determination of Dicamba and its metabolite NOA405873 in Plant Matrices by LC-MS/MS Syngenta CEM Analytical Services Ltd (CEMAS) - Berkshire, UK, CEMR-7414 GLP not published Syngenta File No SAN837_11691	**N**	SYN/ BASF (ADAMA has LOA)
KCP 5.2.1 / 01	Swales S.	2016	SAN837 - Uptake and Metabolism of [14C]-SAN837 in Confined Rotational Crops Syngenta, BASF Corporation, Research Triangle Park, NC, USA Smithers Viscient (ESG) Ltd, Harrogate, UK, AgroChemex Ltd, Manningtree, United Kingdom, 3200368 GLP not published Syngenta File No SAN837_11645	**N**	SYN/ BASF (ADAMA has LOA)
KCP 5.2.2 / 01	Richardson M., Braid S.	2012	Dicamba - Analytical Method for the Determination of Residues of Dicamba (SAN837) in Animal Matrices Final Determination by GC-MSD Syngenta Syngenta - Jealott's Hill, Bracknell, United Kingdom, GRM022.05A Not GLP not published Syngenta File No SAN837_11414	**N**	SYN/ BASF (ADAMA has LOA)
KCP 5.2.2 / 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	**N**	SYN/ BASF (ADAMA has LOA)
KCP 5.2.2 / 03	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	**N**	SYN/ BASF (ADAMA has LOA)

Mesotrione

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**
KCP 5.1.2.1 / 10 & KCP 5.2.4 / 03	Williams R.	2004	Analytical Method 1200-03 for the Determination of Mesotrione and its Metabolites AMBA and MNBA, in Soil, Using Liquid Chromatography - Electrospray Ionization Tandem Mass Spectrometry (Including Validation Data) Syngenta Crop Protection AG, Basel, Switzerland Syngenta Crop Protection, Inc., Greensboro, USA, T001200-03 GLP not published Syngenta File No ZA1296/1567	N	SYN (ADAMA has LOA)
KCP 5.1.2.4 / 01	Bachelor B.	2014	Analytical Method Transfer and Partial Validation for the Determination of CA3511 in Dosing Formulations Syngenta Xenometrics, LLC, Stilwell, KS, USA, 11070 GLP not published Syngenta File No CA3511_50013	N	SYN (ADAMA has LOA)
KCP 5.1.2.4 / 02	Faulkner L., Heap C.	2013	CA3511 - Feasibility of the Assay for the Determination of CA3511 in 1 % w/v Aqueous Carboxymethylcellulose Syngenta Sequani Limited, Ledbury, United Kingdom, BFI0147 Not GLP not published Syngenta File No CA3511_10006	N	SYN (ADAMA has LOA)
KCP 5.1.2.4 / 03	Faulkner L., Heap C.	2013a	CA3511 - Validation of the Assay for the Determination of CA3511 in 1 % w/v Aqueous Carboxymethylcellulose Syngenta Sequani Limited, Ledbury, United Kingdom, BFI0148 GLP not published Syngenta File No CA3511_10007	N	SYN (ADAMA has LOA)
KCP 5.1.2.4 / 04	Faulkner L., Heap C.	2013b	CA3511 - Validation of the Formulation Procedure for CA3511 in 1 % w/v Aqueous Carboxymethylcellulose and Assessment of Formulation Stability Syngenta Sequani Limited, Ledbury, United Kingdom, BFI0149 GLP	N	SYN (ADAMA has LOA)

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**
			not published Syngenta File No CA3511_10009		
KCP 5.1.2.4 / 05	xxxxxxxxxxxx	2016	AMBA - Single Dose Oral (Gavage) Proof of Exposure Study in the Rat Syngenta xxxxxxxxxxxxxxxxxxxx GLP not published Syngenta File No R044276_10012	Y	SYN (ADAMA has LOA)
KCP 5.1.2.6 / 01	Weber K.	2012	Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor (A12127R) - Assessment of Toxic Effects on Daphnia magna using the 48 h Acute Immobilisation Test Syngenta Eurofins Agroscience Services EcoChem GmbH, N-Osch., Germany, S12-02294 GLP not published Syngenta File No A18032E_10008	N	SYN (ADAMA has LOA)
KCP 5.1.2.6 / 02	Falk S.	2012	Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor (A12127R) - Testing of Effects on the Single Cell Green Alga Pseudokirchneriella subcapitata Syngenta Eurofins Agroscience Services GmbH, Niefern-Ã–schel., Germany, S12-02296 GLP not published Syngenta File No A18032E_10002	N	SYN (ADAMA has LOA)
KCP 5.1.2.6 / 03	xxxxxxxxxxxx	2012	Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor (A12127R) - Acute Toxicity Testing in Rainbow Trout (Oncorhynchus mykiss) (Teleostei, Salmonidae) Syngenta xxxxxxxxxxxxxxxxx GLP not published Syngenta File No A18032E_10001	Y	SYN (ADAMA has LOA)
KCP 5.1.2.6 / 04	xxxxxxxxxxxxxxxxxxx	2012a	Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor(A12127R) - Assessment of Toxic Effects on the duckweed Lemna gibba in a Semi-Static Test Syngenta xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx GLP not published	Y	SYN (ADAMA has LOA)

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**
			Syngenta File No A18032E_10009		
KCP 5.1.2.6 / 05	Bramby-Gunary J.	2013	Mesotrione/dicamba/nicosulfuron WG (A18032E) plus A12127R (Adigor adjuvant) - Evaluation of the Phytotoxicity to Non Target Terrestrial Plant Vegetative Vigour Test Syngenta AgroChemex Ltd, Manningtree, United Kingdom, David Norris Analytical Labs Ltd., Dartford, UK, ACE-12-149 GLP not published Syngenta File No A18032E_10025	N	SYN (ADAMA has LOA)
KCP 5.1.2.6 / 06	Bramby-Gunary J.	2013a	Mesotrione/dicamba/nicosulfuron WG (A18032E) plus A12127R (Adigor adjuvant) - Evaluation of the Phytotoxicity to Non Target Terrestrial Plant Seedling Emergence and Seedling Growth Test Syngenta AgroChemex Ltd, Manningtree, United Kingdom, David Norris Analytical Labs Ltd., Dartford, UK, ACE-12-148 GLP not published Syngenta File No A18032E_10024	N	SYN (ADAMA has LOA)
KCP 5.1.2.6 / 07	Hengsberger A., Wydra V.	2015	Mesotrione wet paste (ZA1296) - Toxicity to the aquatic plant Lemna gibba in a reciprocal growth inhibition test Syngenta IBACON GmbH, Rossdorf, Germany, 105731240 GLP not published Syngenta File No ZA1296_10436	N	SYN (ADAMA has LOA)
KCP 5.1.2.6 / 08	Kosak L., Wydra V.	2016	Mesotrione wet paste (ZA1296) - Toxicity to the aquatic plant Lemna gibba in a semi-static growth inhibition test with a subsequent recovery period Syngenta IBACON GmbH, Rossdorf, Germany, 105732240 GLP not published Syngenta File No ZA1296_10438	N	SYN (ADAMA has LOA)
KCP 5.1.2.6 / 09	Gonsior G.	2017	Mesotrione - Growth inhibition of Myriophyllum spicatum in a water/sediment system Syngenta Eurofins Agroscience Services EcoChem GmbH, N-Osch., Germany, S16-06273	N	SYN (ADAMA has LOA)

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**
			GLP not published Syngenta File No ZA1296_10504		
KCP 5.1.2.6 / 10	North L.	2016	Mesotrione - Foliage Decline with A12739A on Maize in Northern France and the United Kingdom in 2015 Syngenta Eurofins Agroscience Services Ltd, Wilson, UK, S15-02057 GLP not published Syngenta File No A12739A_11065	N	SYN (ADAMA has LOA)

Nicosulfuron

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**
KCP 5.1.2.1 (report available from data owner)	Schneider M. Holzer S.	2016	Groundwater Monitoring for Nicosulfuron and Six Metabolites in Four Representative Regions in Germany SGS INSTITUT FRESENIUS GmbH Report: DuPont-28685 GLP Unpublished	N	ADAMA is co-owne
KCP 5.1.2.1 (report available from data owner)	Ferrari F	2016	Groundwater Monitoring for nicosulfuron and 6 Metabolites in Maize Growing Regions of Italy LABCAM s.r.l. Report: DuPont-40798 (Interim Report) GLP Unpublished	N	(ADAMA has LoA from Dupont (1 LoA))
KCP 5.1.2.5 / 08	Crook S., Andrews G.	2016	Nicosulfuron - Analytical Method GRM074.01A for the Determination of Nicosulfuron in Plant Matrices Syngenta Syngenta - Jealott's Hill, Bracknell, United Kingdom, GRM074.01A Not GLP not published	N	SYN (ADAMA has LOA)

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**
			Syngenta File No ASF628_11278		
KCP 5.1.2.5 / 09	Andrews G.	2016	Nicosulfuron and Dicamba - Residue Validation and Study on Maize in Northern France, Germany and Poland in 2015 Syngenta Battelle UK Ltd, Chelmsford, Essex, UK, TK0258007-REG GLP not published Syngenta File No A19658H_10060	N	SYN (ADAMA has LOA)
KCP 5.1.2.6 (report available from data owner)	Obert-Rauser P.	2016	MU-466: Toxicity to the Duckweed *Lemna gibba* under Laboratory Conditions Eurofins Agroscience Services Report: S15-05478 GLP: yes Published: No	N	Nicosulfuron Task Force (ADAMA is member)
KCP 5.1.2.6 (report available from data owner)	Dengler D.	2009	Assessment of Toxic Effects of HMUD on the Duckweed *Lemna gibba* in a Semi Static Test Eurofins-GAB GmbH Report: GAB S08-00827 GLP: yes Published: No	N	Nufarm S.A.S (ADAMA has LoA from Nufarm (2 LoA))
KCP 5.2.1 (report available from data owner)	Steinhilper D	2008	Validation of a Multiresidue method for the determination of Nicosulfuron in maize, Cheminova A/S. Report No.: 107 NIS GLP Unpublished	N	Cheminova (ADAMA has access to equivalent data)(
KCP 5.2.1 (report available from data owner)	Schwarz T.	2008	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, Cheminova A/S. Report No.: 119 NIS GLP Unpublished	N	Cheminova (ADAMA has access to equivalent data)(
KCP 5.2.1 (report available from data owner)	Cabusas, M.E. Pentz, A.	2012	Analytical Method for the Determination of Nicosulfuron and Rimsulfuron in Corn, Cherry, Lemon and Soybean Matrices using HPLC/ESI-MS/MS, Non GLP Unpublished	N	E.I. du Pont de Nemours and Company (Study not protected, non-

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**
					GLP)
5.2.1 (report available from data owner))	McInerney K.	2016	Validation report DuPont-11776 RV2: Extension of the Linearity Range for Nicosulfuron in Oily and Acidic Crop Report No.: 100077587-03 Non GLP Unpublished	N	Nicosulfuron Task Force (ADAMA is member)
5.2.1 (report available from data owner)	Ducat, N., Pigeon O.	2004	Independent Laboratory validation of DuPont-11776, "Analytical Enforcement Method for the Determination of Nicosulfuron in Corn Matrices using HPLC/ESI-MS/MS Report No.: DuPont-12347 GLP Unpublished	N	E.I. du Pont de Nemours and Company (Study should not attract data protection)
KCP 5.2.2 (report available from data owner)	Wolf, S.	2009	Development and Validation of a Residue Analytical Method for Nicosulfuron in Animal Tissues (Milk, Egg, Muscle and Liver) Report No.: 90011604 Non GLP Unpubished	N	ADAMA Agan Chemical Manufacturerers Ltd
KCP 5.2.3 (report available from data owner)	xxxxxxxxxxxxxxxxx	2016a	Method Validation for the Determination of Nicosulfuron in Mouse Plasma Report No.: 100077587-04 GLP Unpubished	Y	Nicosulfuron Task Force (ADAMA is member)

List of data submitted by the applicant and not 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
-	-	-	-	-	-

List of data relied on not submitted by the applicant but necessary for evaluation

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
-	-	-	-	-	-

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for dicamba

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)

Additional studies have been submitted under A 2.1.2.3, A 2.1.2.4 and A 2.1.2.5: Description of Methods for the Analysis of soil, water and air for dicamba as the new methods are for post authorisation monitoring. These methods have also been provided in the dicamba Supplementary AIR 3 dossier submitted to RMS Denmark in June 2016.

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 residues studies (KCP 5.1.2.5)

A 2.1.1.3.1 REM 193.01(modified / REM 193.05 / REM 193.05b

A 2.1.1.3.1.1 Method & Validation

Comments of zRMS:	The residue analytical method described is suitable for the analysis od dicamba and -5-hydroxy dicamba (NOA405873) residues in barley (grain, straw, whole plant) and barley processed fractions (malt, wort, spent hops and spent yeast).The limit od quantification has been set at 0.01 mg/kg with final analysis by GC-MSD for both analytes. Mean recoveries and relative standard deviations for both fortification levels were in the range 70-110% with ≤ 20% RSD). The studies are accepted.

Reference:	KCP 5.1.2.5/01
Report	Richards S, Mackenzie R. 2006 Residue Analytical Method for the Determination of Residues of Dicamba (SAN837) and 5-hydroxy Dicamba (NOA405873) in Barley (Grain, Straw, Whole Plant) and Barley Processed Fractions (Malt, Wort, Spent Hops and Spent Yeast). Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, UK. Syngenta Method Reference: REM 193.05 Syngenta File No: SAN837/6535
Guideline(s):	None stated. Study fulfils requirements as set out in Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).
Deviations:	No
GLP:	No
Acceptability:	Yes

Reference:	KCP 5.1.2.5/02
Report	Richards S, Mackenzie R and Crook S. 2008 Residue Analytical Method for the Determination of Residues of Dicamba (SAN837) and 5-hydroxy Dicamba (NOA405873) in Barley (Grain, Straw, Whole Plant) and Barley Processed Fractions (Malt, Wort, Spent Hops and Spent Yeast). Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, UK. Analytical Method No. REM 193.05b Syngenta File No. SAN837/6686
Guideline(s):	None stated. Study fulfils requirements as set out in Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000).
Deviations:	No
GLP:	No
Acceptability:	Yes

Reference:	KCP 5.1.2.5/03
Report	Richards, S. and Mackenzie, R.. 2004. Residue Study with Dicamba (SAN837) in or on Winter Barley in France (South). Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, UK Unpublished report 03-7009, issued 18 June 2004. Syngenta File N° SAN837/6191
Guideline(s):	Yes FAO Guidelines on Producing Pesticide Residues Data from Supervised Trials (Rome, 1990). EC (European Commission), 1997b. Appendix B. General recommendations for the design, preparation and realization of residue trials. Annex 2. Classification of (minor) crops not listed in the Appendix of Council Directive 90/642/EEC. 7029/VI/95-rev.6. Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Annex I of Directive 91/414/EEC (Article 5.3 and 8.2), 1996
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Reference:	KCP 5.1.2.5/04
Report	Richards, S. 2004 Residue Study with Triasulfuron (CGA131036) and Dicamba (SAN837) in or on Winter Barley in the United Kingdom Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, UK Unpublished report 03-7013, issued 29 June 2004. Syngenta File N° CGA131036/1358
Guideline(s):	Yes

	FAO Guidelines on Producing Pesticide Residues Data from Supervised Trials (Rome, 1990). EC (European Commission), 1997b. Appendix B. General recommendations for the design, preparation and realization of residue trials. Annex 2. Classification of (minor) crops not listed in the Appendix of Council Directive 90/642/EEC. 7029/VI/95-rev.6. Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Annex I of Directive 91/414/EEC (Article 5.3 and 8.2), 1996
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Reference:	KCP 5.1.2.5/05
Report	Richards, S. and Mackenzie, R. 2004a Residue Study with Dicamba (SAN837) in or on Winter Barley and Brewing Fractions in the United Kingdom Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, UK Unpublished report 03-7017, issued 30 November 2004. Syngenta File N° SAN837/6359
Guideline(s):	Yes FAO Guidelines on Producing Pesticide Residues Data from Supervised Trials (Rome, 1990). EC (European Commission), 1997b. Appendix B. General recommendations for the design, preparation and realization of residue trials. Annex 2. Classification of (minor) crops not listed in the Appendix of Council Directive 90/642/EEC. 7029/VI/95-rev.6. Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Annex I of Directive 91/414/EEC (Article 5.3 and 8.2), 1996
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Background
Method REM 193.01 has been previously evaluated under Council Directive 91/414/EEC as a monitoring method for dicamba and 5-OH-dicamba (see EU evaluated data).
For data generation purposes in support of maize, wheat and barley trials, and barley processing studies, it was necessary to adopt the method to improve the analytical procedures. The modifications were considered major, involving changes to the clean-up procedure and derivatisation reagent. The adapted method was referenced as "REM 193.01 Modified" until further validation data were generated in three subsequent residue studies (report references 03-7009, 03-7013 and 03-7017 as presented below). On completion of the validation series, the analytical method was re-issued with the new reference number REM 193.05 to reflect the major changes.
Subsequently to the issue of method REM 193.05, it was noted that certain data from the validation summary had not been included in the written method. This error was corrected and some additional typographical changes were made to improve the phraseology. The method was re-issued with the new reference number REM 193.05b to reflect the minor changes.

The data generated using "REM 193.01 Modified" to support the REM 193.05 method (and hence subsequent REM 193.01b method) are presented below.

Principle of the method

A homogenised sample is extracted and hydrolysed in 1M hydrochloric acid (90°C, 90 minutes). After extraction, the hydrolysed aqueous solution is adjusted to pH>8 with 4M potassium hydroxide. After centrifugation an aliquot is acidified with 6M hydrochloric acid and subjected to clean-up by C8 (EC) SPE cartridge. Dicamba and 5-hydroxy dicamba are then converted to their respective tert-butyl dimethylsilyl derivatives in acetone. Dicamba and 5-hydroxy dicamba derivatives are quantified by gas chromatography with mass spectrometric detection (GC-MS) in the negative ion CI mode using selective ion monitoring (SIM).

Recovery Findings

Full details of recoveries and percent relative standard deviation (RSD) are given in Table A 1.

Table A 1: Recovery Results Obtained During Validation of Method REM193.05 for Dicamba and 5-OH-dicamba in Crops

Matrix	Fortification Level (mg/kg)	Number of Samples	Mean Recovery (%)		RSD (%)	Recovery Range (%)
	Dicamba					
	Richards & Mackenzie, 2004					
Barley grain	0.01	5	82		4.9	77-87
	0.10	5	80		3.0	78-83
	Overall	**10**	**81**		**4**	**77-87**
Barley straw	0.01	5	99		8.0	86-105
	0.10	5	98		4.0	92-102
	Overall	**10**	**99**		**6**	**86-105**
	Richards, 2004					
Barley whole plant	0.01	5	94		3.0	92-99
	0.10	5	100		3.0	96-104
	Overall	**10**	**97**		**4**	**92-104**
	Richards & Mackenzie, 2004a					
Barley malt	0.01	5	100		4.0	94-104
	0.10	5	96		6.0	91-105
	Overall	**10**	**98**		**5**	**91-105**
Barley wort	0.01	5	102		4.0	98-109
	0.10	5	101		3.0	96-104
	Overall	**10**	**101**		**4**	**96-109**
Barley spent hops	0.01	5	86		3.0	83-90
	0.10	5	93		2.0	91-95
	Overall	**10**	**90**		**5**	**83-95**
Barley spent yeast	0.01	5	94		5.0	88-99
	0.10	5	107		2.0	104-110
	Overall	**10**	**100**		**8**	**88-110**
	5-OH-dicamba					
	Richards & Mackenzie, 2004					
Barley grain	0.01	5	75		7.0	70-83
	0.10	5	103		5.0	97-109
	Overall	**10**	**89**		**17**	**70-109**
Barley straw	0.01	5	100		5.0	92-105
	0.10	5	110		2.0	108-112
	Overall	**10**	**105**		**6**	**92-112**
	Richards, 2004					
Barley whole plant	0.01	5	92		4.0	88-96
	0.10	5	106		4.0	99-111
	Overall	**10**	**99**		**9**	**88-111**
	Richards & Mackenzie, 2004a					
Barley malt	0.01	5	99		5.0	94-104
	0.10	5	100		6.0	96-111

Matrix	Fortification Level (mg/kg)	Number of Samples	Mean Recovery (%)		RSD (%)	Recovery Range (%)
	Overall	**10**	**100**		**6**	**94-111**
Barley wort	0.01	5	97		7.0	89-106
	0.10	5	109		3.0	104-114
	Overall	**10**	**103**		**8**	**89-114**
Barley spent hops	0.01	5	90		3.0	88-94
	0.10	5	94		3.0	89-97
	Overall	**10**	**92**		**4**	**88-97**
Barley spent yeast	0.01	5	83		11.0	76-98
	0.10	5	87		5.0	82-91
	Overall	**10**	**85**		**8**	**76-98**

Specificity
The method is suitable to determine parent dicamba and metabolite 5-OH-dicamba in the target crop matrices. Interfering signals from similar compounds or co-extracted matrix components were not observed.

Linearity
Linearity of the method for dicamba was in the range 0.00025 - 0.05 µg/mL with a correlation coefficients > 0.9982.
Linearity of the method for 5-OH-dicamba was in the range 0.00025 - 0.05 µg/mL with a correlation coefficients > 0.9950.

Accuracy
The mean recovery values for both dicamba and 5-OH-dicamba at each fortification level and overall for each crop matrix were between 70% and 110% demonstrating the method has satisfactory accuracy.

Repeatability
The relative standard deviations (RSDs) of dicamba and 5-OH-dicamba at each fortification level and overall for each crop matrices were below 20%. These results demonstrate that the method has satisfactory repeatability.

Limit of 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 limits of quantification (LOQ) for dicamba and its metabolites were established at 0.01 mg/kg in all commodities.

Reproducibility
Method REM 193.05 is a data generation method only and therefore an independent laboratory validation to demonstrate reproducibility is not required.

Conclusion
Method REM 193.05 is considered valid as a data generation method for the determination of dicamba and 5-OH-dicamba residues in crops with an LOQ of 0.01 mg/kg.

A 2.1.1.3.2 GRM022.07A

A 2.1.1.3.2.1 Method & validation

Comments of zRMS:	A new analytical method GRM022.07A has been fully validated for the determination of dicamba and 5-OH-dicamba in plant commodities for data generation purposes according to SANCO/3029/99 rev.4. This method uses LC-MS/MS with 2 ion transitions. The limit of quantification for dicamba and 5-OH-dicamba residues in crop matrices using

	method GRM022.07A was established at 0.01 mg/kg. Mean recoveries and relative standard deviations in most cases were in the range 70-110% with ≤ 20% RSD). Additional, supporting, validation data has also being generated in a separate study of Kennedy, S. 2016 (Syngenta File No. SAN837_11691). The studies are acceptable.

Reference:	KCP 5.1.2.5/06
Report	Braid, S. & Crook, S., 2016 Dicamba - Analytical Method GRM022.07A for the Determination of Dicamba and its Metabolite NOA405873 in Crops. Syngenta Report No. GRM022.07A; Syngenta File No. SAN837_11703
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000). OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17. Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.
Deviations:	Yes; Method is not proposed for post authorisation control – Independent validation according to EC SANCO/825/00 rev 8.1 (2010) has not been conducted.

Reference:	KCP 5.1.2.5/07
Report	Kennedy, S. 2016 Dicamba – Validation of Analytical Method GRM022.07A for the Determination of Dicamba and its metabolite NOA405873 in Plant Matrices by LC-MS/MS CEM Analytical Services Ltd. (CEMAS), Imperial House, Oaklands Business Centre, Oaklands Park, Wokingham, Berkshire RG41 2FD Task No. TK0285785; Syngenta File No. SAN837_11691
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000). OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17. Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.
Deviations:	None

Method validation data was generated during method development and study analysis and is reported in GRM022.07A (Braid & Cook, 2016). Additional validation data are presented in a separate report (Kennedy, 2016).

Principle of the method

10 g sub samples of crop are extracted by refluxing in 1 M HCl for 1 hour. After cooling, acetonitrile is added and samples shaken. Extracts are decanted and centrifuged. Aliquots are cleaned-up by liquid-liquid partition into diethyl ether, followed by a solid phase extraction (SPE) procedure using Waters anion exchange (MAX) cartridges.

Final determination is by high performance liquid chromatography with triple quadrupole mass

spectrometric detection (LC-MS/MS). Two LC-MS/MS transitions are provided for confirmatory analysis and in addition, alternative chromatography conditions are supplied so that any residues of dicamba and 5-OH-dicamba can be reliably confirmed in matrices where low level interference is present in the second transition.
Analytical method GRM022.07A method was validated in a range of crop matrices.

Recovery Findings
Summaries of the results for dicamba and 5-OH-dicamba are presented in the tables below.

Table A 2: Recovery Results From Validation of Method GRM022.07A in Crop: Dicamba Recovery Data (Atlantis T3 HPLC column). Transition m/z = 219→ 145

Matrix	Fortification Level (mg/kg)	Recovery (%)**	n	Mean (%)	RSD (%)	Range (%)
	Braid & Crook, 2016					
Sorghum Whole Plant[#]	0.01*	75, 84, 79	3	79	6	75-84
	0.1	76, 73, 77	3	75	3	73-77
		Overall	6	77	5	73-84
Sorghum Forage[#]	0.01*	90	1	90	-	-
	0.1	77	1	77	-	-
		Overall	2	84		77-90
Sugarcane[^]	0.01*	95, 94, 92, 86, 105	5	94	7	86-105
	0.1	81, 81, 84, 85, 87	5	84	3	81-87
		Overall	10	89	8	81-105
	Kennedy, 2016					
Maize Kernel	0.01*	88, 80, 82, 81, 80	5	82	4.1	80 - 88
	0.1	74, 73, 78, 75, 85	5	77	6.3	73 - 85
		Overall	10	80	6.0	73 - 88
Barley grain	0.01*	85, 83, 82, 88, ***	5	85	3.1	82 - 88
	0.1	85, 82, 77, 87, 86	5	83	4.8	77 - 87
		Overall	10	84	4.0	77 - 88
Lentils	0.01*	90, 92, 91, 95, 98	5	93	3.5	90 - 98
	0.1	84, 80, 83, 80, 82	5	82	2.2	80 - 84
		Overall	10	88	7.4	80 - 98
Whole orange	0.01*	100, 82, 118, 105, 94	5	100	13.3	82 - 118
	0.1	83, 82, 84, 82, 82	5	83	1.1	82 - 84
		Overall	10	91	13.9	82 - 118
Carrots	0.01*	115, 97, 103, 100, 95	5	102	7.7	95 - 115
	0.1	91, 93, 93, 93, 92	5	92	1.0	91 - 93
		Overall	10	97	7.5	91 - 115
Oilseed Rape Seed##	0.01	51, 53, 54, 58, 52	5	54	5.0	51-58
	0.1	59, 60, 59, 63, 60	5	60	2.7	59-63
	Overall	-	10	57	7.1	51-63
Wheat Straw	0.01	61, 64, 72, 64, 72	5	67	7.6	61-72
	0.1	71, 75, 75, 76, 74	5	74	2.6	71-76
	Overall	-	10	70	7.7	61-76

*Limit of quantification, defined by the lowest validated fortification level
**Residues in control samples and reagent blanks were less than 30% of the LOQ.
***One recovery excluded as an outlier using Dixon's Q Test.
^Data reported within GRM022.07A from Brown, 2012, Report no. 66033-1
[#]Data from method development
##Dicamba results calculated using control corrected matrix-matched bracketing standards

Table A 3: Recovery Results From Validation of Method GRM022.07A in Crop: Dicamba Recovery Data (Atlantis T3 HPLC column). Transition *m/z* = 219→ 175

Matrix	Fortification Level (mg/kg)	Recovery (%)**	n	Mean (%)	RSD (%)	Range (%)
	Braid & Crook, 2016					
Cereal Whole Plant[#]	0.01*	77, 68, 73	3	73	6	68-77
	0.1	67, 72, 70	3	70	4	67-72
		Overall	6	71	5	67-77
Sorghum Grain[#]	0.01*	77, 83, 79	3	80	4	77-83
	0.1	84, 84, 87	3	85	2	84-87
		Overall	6	82	4	77-87
Sorghum Stover[#]	0.01*	77, 68, 73	3	73	6	68-77
	0.1	67, 72, 70	3	70	4	78-81
		Overall	6	71	5	68-77
Sugarcane[^]	0.01*	92, 84, 99, 96, 109	5	96	10	84-109
	0.1	83, 84, 88, 82, 88	5	85	3	82-88
		Overall	10	91	10	82-109

*Limit of quantification, defined by the lowest validated fortification level
**Residues in control samples and reagent blanks were less than 30% of the LOQ.
^Data reported within GRM022.07A from Brown, 2012, Report no. 66033-1
[#]Data from method development

Table A 4: Recovery Results From Validation of Method GRM022.07A in Crop: Dicamba Recovery Data (XSelect CSH C18 HPLC column). Transition *m/z* = 219→ 145

Matrix	Fortification Level (mg/kg)	Recovery (%)**	n	Mean (%)	RSD (%)	Range (%)
	Braid & Crook, 2016					
Cereal Grain[^]	0.01*	73, 77, 82, 78, 90, 80	6	80	7	73-90
	0.1	71, 71, 74, 77, 75	5	74	4	71-77
		Overall	11	77	7	71-90
Cereal Forage[^]	0.01*	98, 89, 85, 68, 70	5	82	16	68-98
	0.1	82, 70, 77, 80	4	77	7	70-82
		Overall	9	80	12	68-98
Cereal Straw[^]	0.01*	78, 76, 99	3	84	15	76-99
	0.1	69, 78, 86	3	78	11	69-86
		Overall	6	81	13	69-99
Sorghum Forage[#]	0.01*	80, 75, 93	3	85	10	75-93
	0.1	70, 73, 61	3	70	10	61-77
		Overall	6	77	13	61-93
Sorghum Stover#	0.01*	76, 76, 79	3	77	2	76-79
	0.1	78, 80, 81	3	80	2	78-81
		Overall	6	78	3	76-81
Orange#	0.01*	88, 88, 89	3	88	1	88-89
	0.1	84, 88, 87	3	86	2	84-88
		Overall	6	87	2	84-89
Potato Tuber#	0.01*	78, 52, 85	3	72	24	52-85
	0.1	62, 74, 82	3	73	14	62-82
		Overall	6	72	18	52-85
Spinach[^#]	0.01*	86, 71, 87, 89, 98, 98	6	88	11	71-98
	0.1	81, 89, 74, 94, 98, 99	6	89	11	74-99
		Overall	12	89	11	71-99
Carrot Tops &	0.01*	84	1	84	-	-

Matrix	Fortification Level (mg/kg)	Recovery (%)**	n	Mean (%)	RSD (%)	Range (%)
Leaves ^	0.1	83	1	83	-	-
		Overall	2	83	-	83-84
Carrot Roots^	0.01*	69, 96	2	83	-	69-96
	0.1	73, 90,100, 91	4	89	13	73-100
		Overall	6	87	15	69-100
Flour#	0.01*	89, 89	2	89	-	89-89
	0.1	79, 83, 83	3	82	3	79-83
		Overall	5	85	5	79-89
	Kennedy, 2016					
Maize Kernel	0.01*	79, 72, 86, 83, 82	5	80	6.6	72 - 86
	0.1	70, 71, 73, 70, 81	5	73	6.4	70 - 81
		Overall	10	77	8.0	70 - 86
Barley grain	0.01*	81, 91, 93, 87, ***	5	88	6.0	81 - 93
	0.1	82, 77, 82, 84, 84	5	82	3.5	77 - 84
		Overall	10	85	5.9	77 - 93
Lentils	0.01*	91, 95, 91, 91, 89	5	91	2.4	89 - 95
	0.1	84, 82, 84, 80, 82	5	82	2.0	80 - 84
		Overall	10	87	5.9	80 - 95
Whole orange	0.01*	95, 89, 97, 97, 91	5	94	3.9	89 - 97
	0.1	89, 86, 88, 88, 87	5	88	1.3	86 - 89
		Overall	10	91	4.6	86 - 97
Carrots	0.01*	102, 85, 95, 100, 94	5	95	6.9	85 - 102
	0.1	87, 90, 92, 88, 89	5	89	2.2	87 - 92
		Overall	10	92	6.0	85 - 102
Oilseed Rape Seed##	0.01	55, 63, 64, 68, 58	5	62	8.3	55-68
	0.1	72, 72, 69, 76, 71	5	72	3.5	69-76
	Overall	-	10	67	10.0	55-76
Wheat Straw	0.01	67, 72, 77, 66, 75	5	71	6.8	66-77
	0.1	78, 78, 80, 78, 78	5	78	1.1	78-80
	Overall	-	10	75	6.6	66-80

*Limit of quantification, defined by the lowest validated fortification level
**Residues in control samples and reagent blanks were less than 30% of the LOQ.
***One recovery excluded as an outlier using Dixon's Q Test.
^ Data reported within GRM022.07A from Andrews, 2016, Report no. TK0223573-REG; Austin & Andrews, 2016, Report no. NC14032 (CA 6.6.2)
#Data from method development
##Dicamba results calculated using control corrected matrix-matched bracketing standards

Table A 5: Recovery Results From Validation of Method GRM022.07A in Crop: Dicamba Recovery Data (XSelect CSH C18 HPLC column). Transition *m/z* = 219→ 175 (confirmatory)

Matrix	Fortification Level (mg/kg)	Recovery (%)**	n	Mean (%)	RSD (%)	Range (%)
	Braid & Crook, 2016					
Sorghum Grain#	0.01*	82, 83, 86	3	84	2	82-86
	0.1	83, 85, 81	3	83	1	81-85
		Overall	6	83	2	81-86
Potato Tuber#	0.01*	79, 49, 80	3	69	25	49-80
	0.1	63, 76, 77	3	72	11	63-77
		Overall	6	71	17	49-80
Flour#	0.01*	80, 84	2	82	-	80-84

	0.1	80, 82, 82	3	81	1	80-82
		Overall	5	82	3	80-84
Orange#	0.01*	91, 92, 83	3	89	6	83-92
	0.1	88, 89, 89	3	88	1	88-89
		Overall	6	89	4	83-92
Spinach^	0.01*	77, 71, 73, 80, 79, 93, 102, 96	8	84	14	71-102
	0.1	75, 79, 83, 99, 97, 101, 101	7	91	12	75-101
		Overall	15	87	13	71-102
Carrot Tops & Leaves ^	0.01*	70	1	-	-	-
	0.1	71	1	-	-	-
		Overall	2	71	-	70-71
Carrot Roots^	0.01*	79, 77, 79, 86	4	80	5	77-86
	0.1	84, 80, 89, 97, 89	5	88	7	80-97
		Overall	9	84	8	80-97
	Kennedy, 2016					
Maize Kernels	0.01	74, 70, 78, 72, 74	5	74	4.0	70-78
	0.1	73, 70, 73, 74, 86	5	75	8.3	70-86
	Overall	-		74	6.3	70-86
Barley	0.01	70, 76, 79, 87, ***	5	78	9.1	70-87
	0.1	77, 74, 77, 78, 77	5	77	2.0	74-78
	Overall	-	10	77	5.9	70-87
Lentils	0.01	96, 99, 95, 98, 98	5	97	1.7	95-99
	0.1	87, 82, 85, 80, 82	5	83	3.3	80-87
	Overall	-	10	90	8.5	80-99
Whole Orange	0.01	68, 62, 95, 67, 65	5	71	18.8	62-95
	0.1	89, 84, 86, 85, 82	5	85	3.0	82-89
	Overall	-	10	78	14.9	62-95
Carrots	0.01	92, 83, 89, 87, 84	5	87	4.2	83-92
	0.1	88, 89, 91, 88, 89	5	89	1.4	88-91
	Overall	-	10	88	3.2	83-92
Oilseed Rape Seed##	0.01	60, 58, 62, 66, 58	5	61	5.5	58-66
	0.1	63, 63, 62, 66,63	5	63	2.4	62-66
	Overall	-	10	62	4.5	58-66
Wheat Straw	0.01	63, 72, 65, 65, 66	5	66	5.2	63-72
	0.1	75, 74, 74, 76, 72	5	74	2.0	72-76
	Overall	-	10	70	7.0	63-76

*Limit of quantification, defined by the lowest validated fortification level
**Residues in control samples and reagent blanks were less than 30% of the LOQ.
*** One recovery excluded as an outlier using Dixon's Q Test
^ Data reported within GRM022.07A from Andrews, 2016, Report no. TK0223573-REG; Austin & Andrews, 2016, Report no. NC14032 (CA 6.6.2)
#Data from method development
##Dicamba results calculated using control corrected matrix-matched bracketing standards

Table A 6: Recovery Results From Validation of Method GRM022.07A in Crop: 5-OH-dicamba Recovery Data (Atlantis T3 HPLC Column). Transition *m/z* = 235 → 140

Matrix	Fortification Level (mg/kg)	Recovery (%)**	n	Mean (%)	RSD (%)	Range (%)
	Braid & Crook, 2016					
Cereal Forage#	0.01*	75, 80, 75, 98	4	82	13	75-98
	0.1	74, 78, 77, 79	4	77	3	74-79
		Overall	8	80	10	74-98

Cereal Grain#	0.01*	93	1	93	-	-
	0.1	79	1	79	-	-
		Overall	2	86	-	79-93
Sorghum Whole Plant#	0.01*	100, 100, 98	3	99	1	98-100
	0.1	91, 94, 94	3	93	2	91-94
		Overall	6	96	4	91-100
Sugarcane^	0.01*	101, 96, 107, 98, 109	5	102	6	96-109
	0.1	105, 94, 94, 97, 99	5	98	5	94-105
		Overall	10	100	5	94-109

*Limit of quantification, defined by the lowest validated fortification level
**Residues in control samples and reagent blanks were less than 30% of the LOQ.
*** One recovery excluded as an outlier using Dixon's Q Test
#Data from method development,
^Data reported within GRM022.07A from Brown, 2012, Report no. 66033-1

Table A 7: Recovery Results From Validation of Method GRM022.07A in Crop: 5-OH-dicamba Recovery Data (XSelect CSH C18 HPLC column). Transition *m/z* = 235 → 140 (primary)

Matrix	Fortification Level (mg/kg)	Recovery (%)**	n	Mean (%)	RSD (%)	Range (%)
	Braid & Crook, 2016					
Cereal Grain^#	0.01*	78, 77, 77, 100, 93, 94,	6	87	12	77-100
	0.1	80, 75, 90, 86, 86,	5	83	7	75-90
		Overall	11	85	10	75-100
Cereal Forage^#	0.01*	80, 80, 75, 80, 75	5	78	4	75-80
	0.1	74, 84, 74,78, 77	5	77	5	74-84
		Overall	10	78	4	74-84
Wheat Straw^	0.01*	85, 96	2	91	-	85-96
	0.1	84, 96	2	90	-	84-96
		Overall	4	90	7	84-96
Sorghum Stover#	0.01*	85, 101, 95	3	94	9	85-101
	0.1	101, 100, 102	3	101	1	100-102
		Overall	6	97	7	85-102
Orange#	0.01*	93, 85, 86, 89, 79, 84	6	86	6	79-93
	0.1	89, 92, 87	3	89	3	87-92
		Overall	9	87	5	79-93
Spinach^#	0.01*	71, 72, 72, 77, 90, 88, 89, 95	8	82	12	71-95
	0.1	69, 77, 90, 85, 94, 94, 95	7	86	12	69-94
		Overall	15	84	12	71-90
Carrot Tops & Leaves ^	0.01*	90, 96	2	93	-	90-96
	0.1	82, 93	2	88	-	82-93
		Overall	4	90	7	82-96
Carrot Roots#	0.01*	95	1	-	-	-
	0.1	93, 100, 88	3	94	6	93-100
		Overall	4	94	5	93-100
Flour#	0.01*	82, 84, 82	3	83	1	82-84
	0.1	74, 74, 73	3	74	1	73-74
		Overall	6	78	6	73-84
	Kennedy, 2016					
Maize Kernel	0.01*	90, 92, 91, 88, 86	5	89	2.7	86 - 92
	0.1	90, 86, 83, 80, 94	5	89	2.7	86 - 92
		Overall	10	88	4.9	80 - 94
Barley grain	0.01*	88, 78, 80, 78, ***	5	81	5.9	78 - 88

	0.1	90, 87, 86, 87, 86	5	87	1.9	86 - 90
		Overall	10	84	5.4	78 - 90
Lentils	0.01*	101, 102, 96, 99, 105	5	101	3.3	96 - 105
	0.1	104, 101, 103, 95, 98	5	100	3.7	95 - 104
		Overall	10	100	3.3	95 - 105
Orange	0.01*	73, 73, 81, 85, 85	5	79	7.6	73 - 85
	0.1	91, 91, 89, 90, 88	5	90	1.5	88 - 91
		Overall	10	85	8.1	73 - 91
Carrots	0.01*	118, 113, 110, 102, 100	5	109	6.9	100 - 118
	0.1	115, 112, 110, 108, 110	5	111	2.4	108 - 115
		Overall	10	110	5.0	100 - 118
Oilseed Rape Seed	0.01	81, 93, 96, 102, 83	5	91	9.7	81-102
	0.1	95, 92, 95, 101, 101	5	97	4.2	92-101
	Overall	-	10	94	7.6	81-102
Wheat Straw	0.01	80, 89, 91, 87, 91	5	88	5.2	80-91
	0.1	89, 90, 91, 90, 88	5	90	1.3	88-91
	Overall	-	10	89	3.7	80-91

*Limit of quantification, defined by the lowest validated fortification level
**Residues in control samples and reagent blanks were less than 30% of the LOQ.
***One recovery excluded as an outlier using Dixon's Q Test.
^Data reported within GRM022.07A from Andrews, 2016, Report no. TK0223573-REG; Austin & Andrews, 2016, Report no. NC14032 (CA 6.6.2)
#Data from method development

Table A 8: Recovery Results From Validation of Method GRM022.07A: 5-OH-dicamba Recovery Data (XSelect CSH C18 HPLC column). Transition *m/z* = 235 → 155 (confirmatory)

Matrix	Fortification Level (mg/kg)	Recovery (%)**	n	Mean (%)	RSD (%)	Range (%)
	Braid & Crook, 2016					
Cereal Grain^#	0.01*	91, 96, 99, 97, 104	5	97	5	91-104
	0.1	77, 83, 92, 92, 87	5	86	7	77-92
		Overall	10	92	9	77-104
Cereal Forage^	0.01*	82, 79, 78, 72, 79, 77	6	78	4	72-82
	0.1	79, 100, 76, 77, 78	5	82	12	72-100
		Overall	10	80	9	78-100
Barley Straw^	0.01*	80, 71	2	76	-	71-80
	0.1	82, 72	2	77	-	72-82
		Overall	4	76	7	71-82
Orange#	0.01*	88, 85, 82, 92, 74, 79	6	83	8	74-92
	0.1	89, 94, 89	3	91	3	89-94
		Overall	9	86	8	74-94
Spinach^#	0.01*	76, 80, 87, 94, 90, 95	6	87	8	76-95
	0.1	80, 92, 85, 94, 97, 99	6	91	8	80-99
		Overall	12	89	8	76-99
Carrot Tops & Leaves^	0.01*	76	1	-	-	-
	0.1	80	1	-	-	-
		Overall	2	78	-	76-80
Carrot Roots^#	0.01*	73, 72, 102, 72, 100	5	84	19	72-102
	0.1	74, 86, 80, 93, 99, 90	6	87	10	74-99
		Overall	11	86	14	72-102
Potato Tuber#	0.01*	82, 91, 82	3	85	6	82-91
	0.1	86, 85, 82	3	84	2	82-86

		Overall	6	84	4	82-91
Flour#	0.01*	76, 78, 75	3	76	2	75-78
	0.1	74, 75, 75	3	75	1	74-75
		Overall	6	76	2	74-78
	Kennedy, 2016					
Maize Kernel	0.01*	65, 73, 79, 73, 62	5	70	9.7	62 - 79
	0.1	86, 81, 80, 74, 91	5	82	7.8	74 - 91
		Overall	10	76	11.6	62 - 91
Barley grain	0.01*	88, 83, 88, 79, ***	5	85	5.2	79 - 88
	0.1	89, 81, 79, 86, 85	5	84	4.8	79 - 89
		Overall	10	84	4.6	79 - 89
Lentils	0.01*	99, 96, 95, 94, 97	5	96	2.0	94 - 99
	0.1	106, 102, 102, 97, 100	5	101	3.2	97 - 106
		Overall	10	99	3.8	94 - 106
Orange	0.01*	93, 91, 105, 90, 96	5	95	6.4	90 - 105
	0.1	90, 88, 90, 90, 88	5	89	1.2	88 - 90
		Overall	10	92	5.5	88 - 105
Carrots	0.01*	107, 105, 99, 96, 92	5	100	6.2	92 - 107
	0.1	119, 117, 112, 111, 111	5	114	3.3	111 - 119
		Overall	10	107	8.3	92 - 119
Oilseed Rape Seed	0.01	73, 73, 92, 95, 72	5	81	14.2	72-95
	0.1	97, 93, 98, 103, 101	5	98	3.9	93-103
	Overall	-	10	90	13.6	72-103
Wheat Straw	0.01	72, 83, 79, 79, 87	5	80	7.0	72-87
	0.1	90, 93, 92, 91, 90	5	91	1.4	90-93
	Overall	-	10	86	8.2	72-93

*Limit of quantification, defined by the lowest validated fortification level
**Residues in control samples and reagent blanks were less than 30% of the LOQ.
***One recovery excluded as an outlier using Dixon's Q Test.
^Data reported within GRM022.07A from Andrews, 2016, Report no. TK0223573-REG; Austin & Andrews, 2016, Report no. NC14032 (CA 6.6.2)
Data from method development

Specificity

LC-MS/MS with two transitions is considered to be a highly specific detection technique; however for Dicamba it was not possible to achieve accurate quantification using two transitions due to interfering co-extractives, therefore alternative chromatography conditions were used for reliable confirmation. The same transition (219.1 → 145.0) was monitored on HPLC column 1 (XSelect CSH C18 3.0 x 50 mm, 2.5 µm) and HPLC column 2 (Waters Atlantis T3 3.0 x 100 mm, 3.0 µm).
Since two characteristic LC-MS/MS mass transitions were used to monitor NOA405873 the method achieved a high level of specificity and therefore according to EU guidance (SANCO/825/00 rev.8.1, 16/11/2010) no further confirmatory technique was required

Linearity

The linearity of the LC-MS/MS detector response for dicamba and 5-OH-dicamba was tested in the range from to 1.0 to 100 ng/mL using matrix matched calibration standards and from 0.0005 µg/ml to 0.1 µg/ml using standard solutions. Detector response was found to be linear Linearity was tested in both solvent mixtures and for both MS/MS transitions. Standards of at least five different concentrations were injected and the signal area plotted against concentration for all calibration points. Linear plots with correlation coefficients ranging from 0.986 to 0.9999 were obtained for dicamba and 5-OH-dicamba.

Accuracy and repeatability

Samples fortified with dicamba and 5-OH-dicamba were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at 10x LOQ (0.1 mg/kg). Acceptable mean recoveries of between 70% and 110%

were found for both transitions and using alternative chromatography conditions on all matrices tested apart from oil seed rape seed where the overall recovery for Dicamba was 67% (RSD 10.0%) for the quantitation transition and 62% (RSD 4.5%) for the confirmatory transition. Although these data do not satisfy the criteria specified in EU guidance (*SANCO 3029/99 rev.4 11/7/00*) they do comply with the requirements for monitoring specified in *SANCO/825/00 rev.8.1, 16/11/2010*.
The relative standard deviations (RSDs) of dicamba and 5-OH-dicamba recoveries at each fortification level and overall for each matrix tested during method validation were <20% and therefore demonstrate the method has satisfactory repeatability.

Limit of Quantification
The limit of quantification for dicamba and 5-OH-dicamba residues in crop matrices using method GRM022.07A was established at 0.01 mg/kg. Some low-level interfering peaks at the retention time of dicamba and 5-OH-dicamba at levels of approximately 30% of the limit of quantification were observed in the second LC-MS/MS transition foe some crop matrices. Alternative chromatography conditions can be used in these instances.

Limit of Detection
The limit of detections (LOD) was calculated for Dicamba and NOA405873 for both the quantitation and confirmatory transitions in all validated matrices. The LOD was found to be equivalent to less than 30% of the LOQ (0.003 mg/kg for all matrices) for the quantitation transition and equivalent to less than 0.008 mg/kg for all matrices for the confirmatory transition.

Extractability
Dicamba and 5-OH-dicamba have been shown to be efficiently extracted from crop matrices using the extraction system used in GRM022.07A in a radiolabelled metabolism study[1].

Matrix Effects
Significant matrix effects (enhancement or suppression) were observed for Dicamba in barley and wheat straw and for NOA405873 in the majority of matrices (maize kernels, barley, oilseed rape seed and lentils) tested during method validation, therefore matrix matched linearity standards were used for quantification.

Stability of Final Extracts
The stability of sample extracts fortified with Dicamba and NOA405873 at the LOQ level was checked after a storage period of 4 to 7 days in a refrigerator at 2-8° C against freshly prepared calibration standards. The results proved that the Dicamba residues in the stored fortified samples were not stable in fortified orange samples and NOA405873 was not stable in fortified maize and barley samples. Results are shown in

Table A 9 and

Table A 10.

Table A 9: Recovery Results after Storage of 4 to 7 Days (2 – 8 ºC) From Validation of Method GRM022.07A: Dicamba Recovery Data (XSelect CSH C18 HPLC column). Transition *m/z* = 219→ 145 (primary)

Matrix	Fortification Level (mg/kg)	Storage (days)	Recovery (%)**	n	Mean (%)	RSD (%)	Range (%)
	Kennedy, 2016						
Maize kernel	0.01*	0	79, 72, 86, 83, 82	5	80	6.6	72-86
	0.01*	6	88, 77, 76, 73, 69	5	77	9.3	69-88
Barley	0.01*	0	81, 91, 93, 87, ***	5	88	6.0	81-93
	0.01*	7	84, 77, 80, 77, ***	5	80	4.2	77-84

[1] Swales S (2016): SAN837 – Uptake and Metabolism of [14C]-SAN837 in Confined Rotational Crops. Smithers Viscient Report No. 3200368 (See CA 6.6.1).

Matrix	Fortification Level (mg/kg)	Storage (days)	Recovery (%)**	n	Mean (%)	RSD (%)	Range (%)
	Kennedy, 2016						
Lentils	0.01*	0	91, 95, 91, 91, 89	5	91	2.4	89-95
	89-960.01*	7	85, 89, 94, 94, 91	5	91	4.2	85-94
Whole orange	0.01*	0	95, 89, 97, 97, 91	5	94	3.9	89-97
	0.01*	7	80, 75, 92, 78, 79	5	81	8.1	75-92
Carrots	0.01*	0	102, 85, 95, 100, 94	5	95	6.9	85-102
	0.01*	4	51, 62, 57, 62, 52	5	100	4.2	93-104
Oilseed rape seed	0.01*	0	55, 63, 64, 68, 58	5	62	8.3	55-68
	0.01*	7	51, 62, 57, 62, 52	5	57	9.0	51-62
Wheat straw	0.01*	0	67, 72, 77, 66, 75	5	71	6.8	66-77
	0.01*	7	68, 70, 70, 71, 65	5	69	3.5	65-71

*Limit of quantification, defined by the lowest validated fortification level
**Residues in control samples and reagent blanks were less than 30% of the LOQ.

Table A 10: Recovery Results after Storage of 4 to 7 Days (2 – 8 ºC) From Validation of Method GRM022.07A: 5-OH-dicamba Recovery Data (XSelect CSH C18 HPLC column). Transition *m/z* = 235 → 140 (primary)

Matrix	Fortification Level (mg/kg)	Storage (days)	Recovery (%)**	n	Mean (%)	RSD (%)	Range (%)
	Kennedy, 2016						
Maize kernel	0.01*	0	90, 92, 91, 88, 86	5	89	2.7	86-92
	0.01*	6	107, 112, 99, 102, 97	5	103	5.9	97-112
Barley	0.01*	0	88, 78, 80, 78, ***	5	81	5.9	78-88
	0.01*	7	68, 56, 62, 56, ***	5	61	9.5	56-88
Lentils	0.01*	0	101, 102, 96, 99, 105	5	101	3.3	96-105
	89-960.01*	7	118, 114, 111, 107, 107	5	111	4.2	107-118
Whole orange	0.01*	0	73, 73, 81, 85, 85	5	79	7.6	73-85
	0.01*	7	80, 88, 91, 86, 82	5	85	5.2	80-91
Carrots	0.01*	0	118, 113, 110, 102, 100	5	109	6.9	100-118
	0.01*	4	108, 109, 102, 99, 94	5	102	6.1	94-109
Oilseed rape seed	0.01*	0	81, 93, 96, 102, 83	5	91	9.7	81-102
	0.01*	7	72, 86, 88, 92, 73	5	82	11.1	72-92
Wheat straw	0.01*	0	80, 89, 91, 87, 91	5	88	5.2	80-91
	0.01*	7	77, 84, 89, 81, 86	5	83	5.5	77-89

*Limit of quantification, defined by the lowest validated fortification level
**Residues in control samples and reagent blanks were less than 30% of the LOQ.
***One recovery excluded as an outlier using Dixon's Q Test.

Stability of Standard Solutions

The stability of the stored working standard solutions of Dicamba and NOA405873 at 0.01 mg/kg was checked after a storage period of 175 days in a refrigerator at 2-8 o C against freshly prepared calibration standards. The results demonstrated that Dicamba and NOA405873 residues in the stored working standard solutions were stable.

Conclusion

Analytical method GRM022.07A has been demonstrated to be a reliable and accurate procedure for the determination dicamba and 5-OH-dicamba in crop matrices to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.1.1.3.3 Report TK0103764

Comments of zRMS:	The method GRM022.07A, which uses LC-MS/MS with 2 ion transitions, has been used for the analysis of a new field rotational crop study. Method GRM022.07A is considered sufficiently validated according to SANCO/3029/99 rev.4 for the determination of dicamba and 5-OH-dicamba residues in crops with an LOQ of 0.01 mg/kg. The study is acceptable.

Reference:	KCP 5.2.1/01
Report	Swales S., 2016 SAN837 – Uptake and Metabolism of [C^{14}]-SAN837 in Confined Rotational Crops. Report Number 3200368. Smithers Viscient (ESG), 108 Woodfield Drive, Harrogate, North Yorkshire, HG1 4LS, UK. Study Dates: July 2013 – May 2016. Syngenta Report No. 3200368 (Syngenta File No. SAN837_11645)
Guideline(s):	OECD Guideline for the Testing of Chemicals, 502, Metabolism in Rotational Crops. (January 2007) EPA Residue Chemistry Test Guidelines OPPTS 860.1850, Confined Accumulation in Rotational Crops (August 1996) Commission of the EC, Working document 7524/VI/95 rev. 2 (July 1997) Japanese MAFF Guidelines on the Application for Agricultural Chemicals Registration Nohsan No 8147 (November 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Introduction

In a rotational crop study, samples of forage and hay from the 30 day rotational interval were extracted according to residue analytical method GRM022.07A. Comparable extraction methods are applied in other plant residue methods (AM-0691B, AM-0691B-0593-3, REM193.01, REM193.01 (modified), REM193.05, P-14.063.02, GRM022.04A).

Extractability of the radioactive residue from forage and hay was always ≥ 78.2% TRR whether using the metabolism method or the prescribed residue extraction method. For both commodities, the relative efficiency of the residue extraction method was found to be high (Forage: 114.6%; Hay: 104.1%) when compared to the metabolism extraction procedure (see Table A 11 below).

Table A 11: Comparison of Solvent Extractabilities for Metabolism and Residue Analytical Methods

Commodity	Components	Metabolism Method		Residue Method		Relative efficiency of extraction[1]
		%TRR	mg/kg	%TRR	mg/kg	
Forage	Extract	91.5	0.473	104.9	0.559	114.6
Hay	Extract	78.2	0.619	81.4	0.721	104.1

[1] – (Residue method %TRR/Metabolism method %TRR) x 100

Furthermore when radioactivity extracted by the residue method was analysed by 2D-TLC the levels of parent (SAN837) and metabolites NOA40587 and NOA414746 present were found to be in close agreement in all instances with the corresponding levels determined by the metabolism methodology (relative residue levels 93.8 – 110.1%) (see Table A 12below).

Table A 12: Comparison of Parent (SAN837) and Metabolite (NOA405873 and NOA414746) Residue Levels in Forage and Hay Following Analysis Using Metabolism and Residue Analytical Methods

Commodity	Components	Metabolism Method		Residue Method		Relative residue levels detected[1]
		%TRR	mg/kg	%TRR	mg/kg	

Forage	SAN837	20.7	0.107	21.7	0.116	104.8
	NOA405873	50.4	0.260	55.5	0.296	110.1
	NOA414746	6.4	0.033	6.0	0.032	93.8
Hay	SAN837	13.8	0.109	14.5	0.128	105.1
	NOA405873	43.2	0.342	45.5	0.403	105.3
	NOA414746	6.5	0.052	6.3	0.056	96.9

[1] – (Residue method %TRR/Metabolism method %TRR) x 100

A 2.1.1.4 Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2.6)

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 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)

A 2.1.2.2.1 GRM022.05A

A 2.1.2.2.1.1 Method - Validation & ILV

Comments of zRMS:	The studies have been submitted for the purpose of renewal of dicamba. An enforcement method using GC-MSD is available and has been validated for the determination of dicamba in animal commodities (meat, fat, liver, kidney, milk and eggs) with an LOQ of 0.01 mg/kg. The method has been independently validated in liver, eggs and milk. <u>Remark:</u> According to the information provided by Applicant, the independent laboratory validation study was repeated. See additional information below the studies.

Reference:	KCP 5.2.2/01
Report	Richardson, M. & Braid, S., 2012 Dicamba - Analytical Method for the Determination of Residues of Dicamba (SAN837) in Animal Matrices. Final Determination by GC-MSD Syngenta Method No. GRM022.05A; Syngenta File N° SAN837_11414
Guideline(s):	OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007) 17. Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996. EC (European Commission), 2000. Residue analytical methods. For pre-registration data requirement for Annex II (part A, section 4) and Annex III (part A, section 5 of Directive 91/414. SANCO/3029/99-rev.4.

	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010).
Deviations:	No
GLP:	No
Acceptability:	Yes
Reference:	KCP 5.2.2/02
Report	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). Syngenta Report No. T010322-04-REG; Syngenta File N° SAN837_10997
Guideline(s):	Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996. EC (European Commission), 2000. Residue analytical methods. For pre-registration data requirement for Annex II (part A, section 4) and Annex III (part A, section 5 of Directive 91/414. SANCO/3029/99-rev.4. Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 7, 17/03/2004).
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Reference:	KCP 5.2.2/03
Report	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. Syngenta Report No. B 1836 G; Syngenta File No. SAN837_11330
Guideline(s):	Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996. EC (European Commission), 2000. Residue analytical methods. For pre-registration data requirement for Annex II (part A, section 4) and Annex III (part A, section 5 of Directive 91/414. SANCO/3029/99-rev.4. Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 7, 17/03/2004). EC Council Directive 91/414/EEC
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Introduction

Analytical method GRM022.03A was developed in order to determine residues of dicamba and its associated metabolite (NOA414746) in line with the residue definitions required at the time of generation. Following the publication of the “Final addendum to the Draft Assessment Report (DAR)” compiled by RMS Denmark, November 2010, it was concluded that the pertinent residue definition for monitoring for dicamba in animal tissues is “dicamba, free and conjugates”; the metabolite (NOA414746) is not included

in the residue definition for monitoring.
In order to simplify the proposed monitoring method for dicamba in animal tissues, the method has been reissued as GRM022.05A and relates only to dicamba (free and conjugates). The analytical procedures used in GRM022.03A and GRM022.05A are essentially identical consequently, the validation and independent validation generated for original method (GRM022.03A) are applicable to the re-issued method although the validation data relevant to the metabolite (NOA414746) are not required.

Principle of the method
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 dichloromethane 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 a solid phase extraction procedure - the analytes eluted with 0.1% v/v acetic acid in acetonitrile. The eluates are evaporated to dryness and reconstituted in acetone. Dicamba is 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 dichloromethane after the addition of sodium chloride. The dichloromethane extracts are combined and evaporated to dryness and are then reconstituted in 1M HCl solution. Samples are subjected to a solid phase extraction procedure - the analytes are eluted in 0.1% v/v acetic acid in acetonitrile. The eluates are evaporated to dryness and reconstituted in acetone. Dicamba 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).
The limit of quantification of the method is 0.01 mg/kg for total dicamba residues.

Recovery Findings
Control samples were analysed in duplicate and fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and in quintuplet at ten times the LOQ (0.10 mg/kg). Acceptable mean recoveries between 70% and 110% with a relative standard deviation of <20% were typically found for the target and both qualification ions on all animal matrices tested. The mean recovery in the third qualifier ion (m/z 186) in liver was 65% in the independent laboratory validation procedure; this was considered to be acceptable. No residues were detected in the control samples or reagent blanks at or above 30% of the LOQ. The recoveries obtained are detailed in the following tables.

Table A 13: Recovery Results Obtained During Validation of Method GRM022.05A (as GRM022.03A) for Dicamba

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Dicamba – m/z 184						
Milk	0.01	75, 84, 86, 87, 86	5	84	6	75-87
	0.10	83, 86, 86, 94, 86	5	87	5	83-94
	Overall		**10**	**85**	**5**	**75-94**
Eggs	0.01	94, 97, 100, 96, 94	5	96	3	94-100
	0.10	89, 83, 86, 88, 79	5	85	5	79-89
	Overall		**10**	**91**	**8**	**79-100**
Muscle	0.01	80, 85, 89, 88, 92	5	87	5	80-92
	0.10	92, 93, 95, 95, 91	5	93	2	91-95
	Overall		**10**	**90**	**5**	**80-95**
Fat	0.01	99, 96, 96, 94, 96	5	96	2	94-99
	0.10	80, 79, 83, 81, 87	5	82	4	79-87
	Overall		**10**	**89**	**9**	**79-99**
Liver	0.01	86, 91, 84, 86,84	5	86	3	84-91
	0.10	104, 97, 99, 95, 93	5	98	4	93-104
	Overall		**10**	**92**	**8**	**84-104**
Kidney	0.01	89, 93, 96, 99, 96	5	95	4	89-99
	0.10	89, 96, 98, 98, 95	5	95	4	89-98

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
	Overall		**10**	**95**	**4**	**89-99**
Milk (Independent Laboratory)	0.01	83, 80, 74, 77, 67	5	76	8	67-83
	0.10	92, 83, 97, 83, 96	5	90	8	83-97
	Overall		**10**	**83**	**12**	**67-97**
Eggs (Independent Laboratory)	0.01	74, 64, 75, 70, 72	5	71	6	64-75
	0.10	49(2), 70, 68, 67, 85	4	73	11	67-85
	Overall		**9**	**72**	**8**	**64-85**
Liver (1) (Independent Laboratory)	0.01	71, 77, 71, 74, 71	5	73	3	71-77
	0.10	70, 69, 77, 81, 70	5	73	7	69-81
	Overall		**10**	**73**	**5**	**69-81**
Dicamba – m/z 185						
Milk	0.01	64, 72, 76, 76, 75	5	73	7	64-76
	0.10	84, 86, 88, 94, 86	5	88	4	84-94
	Overall		**10**	**80**	**11**	**64-94**
Eggs	0.01	91, 94, 97, 92, 91	5	93	3	91-97
	0.10	90, 83, 86, 89, 79	5	85	5	79-90
	Overall		**10**	**89**	**6**	**79-97**
Muscle	0.01	78, 82, 88, 87, 88	5	85	5	78-88
	0.10	92, 92, 95, 95, 90	5	93	2	90-95
	Overall		**10**	**89**	**6**	**78-95**
Fat	0.01	97, 95, 96, 94, 95	5	95	1	94-97
	0.10	91, 79, 82, 81, 87	5	82	4	79-87
	Overall		**10**	**89**	**8**	**79-97**
Liver	0.01	86, 93, 85, 87, 82	5	87	4	82-93
	0.10	102, 96, 99, 94, 94	5	97	4	94-102
	Overall		**10**	**92**	**7**	**82-102**
Kidney	0.01	90, 92, 96, 97, 94	5	94	3	90-97
	0.10	88, 94, 97, 98, 93	5	94	4	88-98
	Overall		**10**	**94**	**3**	**88-98**
Milk (Independent Laboratory)	0.01	84, 82, 75, 78, 72	5	78	6	72-84
	0.10	92, 84, 101, 81, 92	5	90	9	81-101
	Overall		**10**	**84**	**10**	**72-101**
Eggs (Independent Laboratory)	0.01	84, 69, 84, 79, 77	5	79	7	69-84
	0.10	51(2), 74, 71, 73, 89	4	77	11	71-89
	Overall		**9**	**78**	**8**	**69-89**
Liver (Independent Laboratory)	0.01	66, 79, 66, 77, 69	5	71	9	66-79
	0.10	70, 68, 77, 80, 82	5	75	9	68-82
	Overall		**10**	**73**	**8**	**66-82**
Dicamba – m/z 186						
Milk	0.01	73, 81, 85, 85, 85	5	82	6	73-85
	0.10	84, 86, 88, 94, 87	5	88	4	84-94
	Overall		**10**	**85**	**6**	**73-94**
Eggs	0.01	92, 95, 100, 93, 94	5	95	3	92-100
	0.10	91, 84, 87, 89, 80	5	86	5	80-91
	Overall		**10**	**91**	**6**	**80-100**
Muscle	0.01	78, 81, 87, 87, 87	5	84	5	78-87
	0.10	92, 92, 96, 95, 91	5	93	3	91-96
	Overall		**10**	**89**	**7**	**78-96**
Fat	0.01	96, 93, 96, 93, 95	5	95	1	93-96
	0.10	80, 80, 83, 82, 88	5	83	4	80-88
	Overall		**10**	**89**	**8**	**80-96**
Liver	0.01	85, 90, 84, 87, 83	5	86	3	83-90
	0.10	106, 99, 101, 97, 96	5	100	4	96-106
	Overall		**10**	**93**	**9**	**83-106**
Kidney	0.01	91, 93, 97, 98, 96	5	95	3	91-98
	0.10	91, 94, 97, 98, 94	5	95	3	91-98
	Overall		**10**	**95**	**3**	**91-98**
Milk (Independent Laboratory)	0.01	82, 79, 74, 78, 68	5	76	7	68-82
	0.10	92, 83, 98, 81, 93	5	89	8	81-98
	Overall		**10**	**83**	**11**	**68-98**

Matrix	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Eggs (Independent Laboratory)	0.01	80, 67, 78, 74, 77	5	75	7	67-80
	0.10	51[(2)], 73, 70, 70, 86	4	75	10	70-86
	Overall		**9**	**75**	**8**	**67-86**
Liver (Independent Laboratory)	0.01	65, 70, 58, 62, 56	5	62	9	56-70
	0.10	71, 65, 71, 67, 66	5	68	4	65-71
	Overall		10	**65**	**8**	**56-71**

(1) Minor Modification: The original method uses 184 m/z for evaluation, because of the high matrix interference for liver, 183 m/z was used instead of 184 m/z

(2) Value determined to be outlier by Dixon Test and not used in subsequent calculations

Specificity

During method development no significant suppression or enhancement of instrument response was observed, indicating that non-matrix calibration standards can be used for quantification. For dicamba, a matrix effect was observed for egg, muscle, fat and liver (results for the last two matrices were corrected due to a low signal level for solvent-prepared standards).

In the ILV, fortified sample extracts were evaluated with a multi point calibration obtained from matrix-matched standards and for all animal matrices, recovery calculations were carried out using matrix matched standards to compensate any significant effects.

Linearity

During the validation study, the linearity of the GC-MSD detector responses for dicamba was tested over the range 5 pg to 200 pg injected on column (equivalent to 0.005 µg/mL to 0.2 µg/mL standards when using a 1 µL injection volume), corresponding to corresponding to 0.5 x LOQ to 20 x LOQ. The coefficients of determination (R^2) of the calibration curves were 0.9958 to 0.9999 and hence deemed to be linear for dicamba target ion (m/z 184), qualifier ion 1 (m/z 185) and qualifier ion 2 (m/z 186) on all matrices tested.

During the independent laboratory validation, the linearity of the GC-MSD detector responses for dicamba was tested over the range 2.5 pg to 160 pg injected on column (equivalent to 0.0025 µg/mL to 0.16 µg/mL standards when using a 1 µL injection volume), corresponding to 0.2 x LOQ to 12 x LOQ. The coefficients of determination (R^2) of the calibration curves were 0.9805 to 0.9987 and hence deemed to be linear for dicamba target ion (m/z 184), qualifier ion 1 (m/z 185) and qualifier ion 2 (m/z 186) on all matrices tested

Accuracy

The mean recoveries at each fortification level and overall for each animal matrix tested during method validation studies were in the acceptable range of 70% and 110% (except for the third qualifier ion (m/z 186) in the liver analysis in the independent laboratory validation study). Sufficient evidence is available to demonstrate that the method is accurate in animal tissues; the minor deviation in mean recovery in one difficult to analyse tissue in the third qualifier ion is not considered significant. The accuracy of the method has been demonstrated and the method fulfils the EU guidelines SANCO 3029/99 rev.4 and SANCO 825/00 rev 8.1.

Repeatability

The relative standard deviations (RSDs) of dicamba recoveries at each fortification level and overall for each animal matrix tested during method validation studies were all below 20%. The repeatability of the method has been demonstrated and the method fulfils the EU guidelines SANCO 3029/99 rev.4 and SANCO 825/00 rev 8.1.

Limit of 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 for dicamba residues in animal matrices using method GRM022.05A was established at 0.01 mg/kg. Residues of dicamba measured in the control samples were < 30% of the LOQ during method validation.

Reproducibility
An independent laboratory validation was conducted and demonstrated acceptable reproducibility as required in the EU guidance (SANCO 825/00 rev.8.1).

Stability of extracts
The stability of dicamba residues 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%) and are presented below:

Table A 14: Summary of Stability of Dicamba Residues in Final Matrix Extracts (Egg) of Method GRM022.05A (as GRM022.03A) for Dicamba

Storage Interval (days)	Fortification Level (mg/kg)	Recovery (%)	n	Mean (%)	RSD (%)	Range (%)
Dicamba (m/z 184)						
1	0.01	94, 97, 100, 96, 94	**5**	96	3	94-100
12	0.01	77, 82, 81, 76, 77	**5**	79	4	76-82

Extractability of Residues
The techniques used to extract dicamba from animal matrices are unchanged from the residue analytical method previously evaluated (Method AM-0938-0994-0, "Determination of Dicamba and Dichlorosalicylic Acid Residues in Beef Tissues (GC)", Formanski, L.J., 1994).

Conclusion
Method GRM022.05A was successfully validated for the analysis of dicamba residues in animal matrices and an LOQ of 0.01 mg/kg was established. Method GRM022.05A is suitable as a method for monitoring animal commodities and has been suitably validated for that purpose.

ADDITIONAL INFORMATION
For the purposes of clarity, it is highlighted that the independent laboratory validation study was repeated. The initial ILV study did not demonstrate suitable reproducibility; in some tissues studied, repeatability experiments demonstrated relative standard deviation of greater than 20%. In some studied tissue type, lower than acceptable accuracy was demonstrated. The independent laboratory consulted with the specifying laboratory but the issues observed were not resolved satisfactorily.

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

The following residue analytical methods on soil and associated validation studies have not previously been submitted for review under Regulation (EC) 1107/2009 and are provided in support of this assessment.

The main reason for the method update were:

i) to provide a lower limit of quantification , to allow quantification of dicamba residues to 0.0035 mg/kg in soil based on the most susceptible crop for re-cropping and/or succeeding crops
ii) to provide updated confirmatory conditions

A 2.1.2.3.1 GRM022.06A

Comments of zRMS:	The studies have been submitted for the purpose of renewal of dicamba. This analytical method (GRM022.06A) has been successfully validated for the determination of dicamba and NOA414746 residues in soil, with a limit of quantification (LOQ) of 0.0035 mg/kg. It fulfils the requirements of SANCO 3029/99 rev.4 and SANCO/825/00 rev. 8.1. The limit of quantification for dicamba and DCSA residues in soil using method GRM022.06A was established at 0.0035 mg/kg. Residues of all analytes measured in the control samples were always below 30% of the LOQ during method validation.

	Acceptable mean recoveries of between 70% and 120% with a relative standard deviation lower than 20% were found for each analyte for both the primary and confirmatory transitions. The studies are acceptable.

Method for the Determination of Dicamba and its Metabolite NOA414746 in Soil (GRM022.06A)

Reference:	KCP 5.1.2.1/01 & KCP 5.2.4/01
Report	Braid S., Garcia-Alix M, 2013 Analytical Method GRM022.06A for the Determination of Dicamba and its Metabolite NOA414746 in Soil - Analytical Method. Report No. GRM022.06A. Syngenta File Nº SAN837_11434.
Guideline(s):	EPA OCSPP 850.6100 (2012); EC SANCO/3029/99 rev 4 (2000); EC SANCO/825/00 rev 8.1 (2010)
Deviations:	none
GLP:	no
Acceptability:	Yes
Reference:	KCP 5.1.2.1/02 & KCP 5.2.4/02
Report	Garcia-Alix M., 2013 Analytical Method GRM022.06A for the Determination of Dicamba and its Metabolite NOA414746 in Soil - Method Validation. Report No. CEMR-5791-REG. Syngenta File Nº SAN837_11433
Guideline(s):	EPA OCSPP 850.6100 (2012); EC SANCO/3029/99 rev 4 (2000); EC SANCO/825/00 rev 8.1 (2010)
Deviations:	none
GLP:	yes
Acceptability:	Yes

Principle of the method

Soil samples (10 g) are extracted by heating at reflux with 0.5 M potassium hydroxide solution. The extracts are allowed to cool to room temperature then centrifuged. An aliquot of the extract equivalent to 1 g is acidified and partitioned four times with diethyl ether. The combined diethyl ether fractions are evaporated to dryness and re-dissolved in 0.1 M hydrochloric acid. Samples are then taken through a solid phase extraction (SPE) procedure. Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification of the method is 0.0035 mg/kg (0.0035 ppm, 3.5 ppb).

Recovery Findings

Acceptable mean recoveries of between 70% and 120% with a relative standard deviation lower than 20% were found for each analyte for both the primary and confirmatory transitions. Since two characteristic mass transitions were used to monitor dicamba and NOA414746, the method achieves a high level of specificity and no confirmation on a different detector was necessary.

Full details of the recoveries are given below.

Table A 15: Dicamba Recovery Data (Primary Transition *m/z* 219 → 35)

Soil Type	Fortification Level (mg/kg)	Recovery (%)**	n	Mean (%)	RSD (%)	Range (%)
Soil (Gartenacker)	0.0035*	90,96,97,98,88	5	94	4.8	88-98

	0.035	76,76,84,75,74	5	77	5.2	74-84
	Overall		10	85	11.4	74-98
Soil (18 Acres)	0.0035*	81,81,96,98,80	5	87	10.3	80-98
	0.035	76,77,80,87,79	5	80	5.4	76-87
	Overall		10	84	9.2	76-98

*Limit of quantification, defined by the lowest validated fortification level
**Residues in control samples and reagent blanks were less than 30% of the LOQ.

Table A 16: Dicamba Recovery Data (Confirmatory Transition *m/z* 221→ 37)

Soil Type	Fortification Level (mg/kg)	Recovery (%)**	n	ean (%)	RSD (%)	Range (%)
Soil (Gartenacker)	0.0035*	88,89,103,96,91	5	93	6.6	88-103
	0.035	74,79,83,77,76	5	78	4.4	74-83
	Overall		10	86	11.1	74-103
Soil (18 Acres)	0.0035*	79,87,102,101,76	5	89	13.6	76-102
	0.035	74,78,84,89,80	5	81	7.1	74-89
	Overall		10	85	11.6	74-102

*Limit of quantification, defined by the lowest validated fortification level
**Residues in control samples and reagent blanks were less than 30% of the LOQ

Table A 17: NOA414746 Recovery Data (Primary Transition m/z 205 → 125)

Soil Type	Fortification Level (mg/kg)	Recovery (%)**	n	Mean (%)	RSD (%)	Range (%)
Soil (Gartenacker)	0.0035*	84,91,89,95,91	5	90	4.4	84-95
	0.035	78,81,79,94,91	5	85	8.7	78-94
	Overall		10	87	7.2	78-95
Soil (18 Acres)	0.0035*	72,79,72,83,74	5	76	6.4	72-83
	0.035	88,72,89,85,90	5	85	8.7	72-90
	Overall		10	80	9.3	72-90

*Limit of quantification, defined by the lowest validated fortification level
**Residues in control samples and reagent blanks were less than 30% of the LOQ.

Table A 18: NOA414746 Recovery Data (Confirmatory Transition *m/z* 205→ 161)

Soil Type	Fortification Level (mg/kg)	Recovery (%)**	n	Mean (%)	RSD (%)	Range (%)
Soil (Gartenacker)	0.0035*	91,88,93,97,97	5	93	4.2	88-97
	0.035	85,79,81,90,87	5	84	5.3	79-90
	Overall		10	89	6.9	79-97
Soil (18 Acres)	0.0035*	73,82,70,84,72	5	76	8.3	70-84
	0.035	76,71,89,81,88	5	81	9.5	71-89
	Overall		10	79	9.1	70-89

*Limit of quantification, defined by the lowest validated fortification level
**Residues in control samples and reagent blanks were less than 30% of the LOQ

Specificity

LC-MS/MS as a detection technique with primary and confirmatory ion transitions is considered to be highly specific and therefore according to the guidance (see guidance section of this summary) further confirmation is not required. No residues of dicamba or its metabolite were detected in any of the control specimens indicating that no interferences were present at the retention time of either analyte in the test systems. This is in accordance with the level specified in SANCO guideline 825/00 Rev. 8.1, which requires a control blank level of less than 30% of the LOQ.

Linearity

The linearity of the LC-MS/MS detector response for dicamba and NOA414746 was tested in the range from 0.02 ng to 1.0 ng injected on column (equivalent to 0.0005 µg/mL to 0.025 µg/mL standards when using a 40 µL injection volume) and was found to be linear when using linear regression.

Accuracy
Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.0035 mg/kg) and at ten times the LOQ (0.035 mg/kg). Acceptable mean recoveries of between 70% and 120% were found for both transitions on all matrices tested and therefore, according to EU guidance (SANCO 3029/99 rev.4 11/7/00), demonstrate the method has satisfactory accuracy**.**

Matrix Effect
Significant suppression of detector response was observed for dicamba and NOA414746 in the presence of soil matrices from both soil types; the measured matrix effects were greater than 20%. It is therefore appropriate to use matrix-matched standards for calibration and quantification for all analytes in both soil types.

Repeatability
The repeatability of the method, expressed as the relative standard deviation (RSD), is <20% for all analytes and fortification levels examined.

Final Extract Stability
The stability of each analyte in fortified soil sample extracts stored at between 2 and 8 °C was assessed. Sample extracts were re-analysed after 7 and 9 days of storage and found to be stable for dicamba and NOA414746. The results demonstrated the stability of dicamba and NOA414746 in the fortified soil sample extracts when stored in vials between 2 and 8°C for at least 9 days.

Limit of 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% – 120% with a relative standard deviation (RSD) of ≤ 20% has been obtained.
The limit of quantification for dicamba and DCSA residues in soil using method GRM022.06A was established at 0.0035 mg/kg. Residues of all analytes measured in the control samples were always below 30% of the LOQ during method validation.

Conclusion
This analytical method has been successfully validated and demonstrated to be a reliable and accurate procedure for the determination of dicamba and NOA414746 residues in soil, with a limit of quantification (LOQ) of 0.0035 mg/kg.

A 2.1.2.3.1.1 Confirmatory method

LC-MS/MS as a detection technique with primary and confirmatory ion transitions is considered to be highly specific and therefore according to the guidance (see guidance section of this summary) further confirmation is not required.

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

Additional data on methods/validation in water for dicamba have been developed and have been provided below. The methodology has been updated to meet current guidance.
The repeatability and specificity of the method have been independently validated and analytical method GRM022.02A is therefore considered valid for the determination of residues of dicamba and its metabolite DCSA in drinking water to a limit of quantification of 0.05 µg/L, using commercially available laboratory equipment and reagents. All data are considered adequate.
DCSA is not considered to be an environmentally relevant metabolite (i.e. does not exceed 0.1µg/L in EU GW modelling scenarios) and therefore no monitoring method is required for the metabolite. An updated method has been provided and independently validated for parent dicamba (see below).

A 2.1.2.4.1 GRM022.02A

Comments of zRMS:	The studies have been submitted for the purpose of renewal of dicamba. The analytical method has been successfully validated for the determination of dicamba residues in water (river, groundwater and drinking water), with a limit of quantification (LOQ) of 0.05 µg/L. It fulfils the requirements of SANCO 3029/99 rev.4 and SANCO/825/00 rev. 8.1. Residues of dicamba measured in the control samples were always below 30% of the LOQ during method validation. Acceptable mean recoveries of between 70% and 120% with a relative standard deviation lower than 20% were found for dicamba in all water matrices tested for both the primary and confirmatory transitions. The studies are considered acceptable.

Method for the Determination of Dicamba in Water (GRM022.02A)

Reference:	KCP 5.1.2.1/03 & KCP 5.2.5/01
Report	Hargreaves S. 2007 Dicamba: Residue Method (GRM022.02A) for the Determination of Residues in Water. Report No. GRM022.02A. Syngenta File Nº SAN837/6654
Guideline(s):	EPA OPPTS 850.7100; EC Guidance Document SANCO/3029/99 rev 4; EC Guidance Document SANCO/825/00 rev 7
Deviations:	none
GLP:	no
Acceptability:	Yes

Reference:	KCP 5.1.2.1/04 & KCP 5.2.5/02
Report	Emburey S. 2007 Validation of an Analytical Method (GRM022.02A) for the Determination of Residues of Dicamba in Water. Report No. T002102-06-REG. Syngenta File Nº SAN837/6653
Guideline(s):	EPA OPPTS 850.7100; EC Guidance Document SANCO/3029/99 rev 4; EC Guidance Document SANCO/825/00 rev 7
Deviations:	none
GLP:	yes
Acceptability:	Yes

Principle of the method
Acidified water samples are passed through C18 solid phase extraction (SPE) cartridges. Dicamba is eluted from the SPE cartridge with acetonitrile. Aliquots are derivatised to form the tert-butyl dimethylsilyl ester using *N*-(*tert*-Butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA). Final determination is by negative-ion chemical ionisation gas liquid chromatography with mass selective detection (NICI GC-MSD). The limit of quantification (LOQ) of the method is 0.05 µg/L.

Recovery Findings
Acceptable mean recoveries of between 70% and 120% with a relative standard deviation lower than 20% were found for both the primary and qualifier ions. Since two qualifier ions were used to monitor dicamba, the method achieves a high level of specificity and no confirmation on a different detector was necessary. Full details of the recoveries are given below.

Table A 19: Dicamba Recovery Data Obtained During Method Validation (Target Ion *m/z* = 184)

Water type	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range
River water	Control	< LOQ, < LOQ			
	0.05*	85, 88, 102, 89, 98	92	8	85 - 102
	0.5	84, 91, 101, 92, 101	94	8	84 - 101
		Overall	93	7	84 - 102
Groundwater	Control	< LOQ, < LOQ			
	0.05*	105, 125, 112, 91, 93	105	13	91 - 125
	0.5	94, 78, 83, 82, 75	83	9	75 - 94
		Overall	94	17	75 - 125
Drinking water	Control	< LOQ, < LOQ			
	0.05*	94, 103, 89, 110, 100	99	8	89 - 110
	0.5	102, 88, 100, 94, 101	97	6	88 - 102
		Overall	98	7	88 - 102

* Limit of quantification, defined by the lowest validated fortification level.

Table A 20: Dicamba Recovery Data Obtained During Method Validation (Qualifier Ion *m/z* = 185)

Water type	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range
River water	Control	< LOQ, < LOQ			
	0.05*	85, 82, 96, 86, 96	89	7	82 - 96
	0.5	83, 89, 103, 93, 103	94	10	83 - 103
		Overall	92	9	82 - 103
Groundwater	Control	< LOQ, < LOQ			
	0.05*	120, 105, 118, 96, 86	105	14	86 - 120
	0.5	88, 78, 79, 79, 78	80	5	78 - 88
		Overall	93	18	78 - 120
Drinking water	Control	< LOQ, < LOQ			
	0.05*	79, 103, 94, 94, 103	95	10	79 - 103
	0.5	85, 83, 91, 78, 85	85	6	78 - 85
		Overall	90	10	78 - 103

* Limit of quantification, defined by the lowest validated fortification level.

Table A 21: Dicamba Recovery Data Obtained During Method Validation (Qualifier Ion *m/z* = 186)

Water type	Fortification Level (µg/L)	Recovery (%)	Mean (%)	RSD (%)	Range
River water	Control	< LOQ, < LOQ			
	0.05*	77, 84, 94, 79, 93	85	9	77 - 94
	0.5	81, 84, 93, 89, 95	88	7	81 - 95
		Overall	87	8	77 - 95
Groundwater	Control	< LOQ, < LOQ			
	0.05*	104, 93, 83, 103, 81	93	12	81 - 104
	0.5	81, 70, 64, 68, 64	69	10	64 - 81
		Overall	81	18	64 - 104
Drinking water	Control	< LOQ, < LOQ			
	0.05*	86, 94, 92, 110, 86	94	11	86 - 110
	0.5	74, 75, 87, 74, 81	78	7	74 - 87
		Overall	86	13	74 - 110

* Limit of quantification, defined by the lowest validated fortification level.

Specificity
NICI GC-MSD as a detection technique with target and qualifier ion transitions 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 above 30% of the lower limit of quantification, arising from and of the water matrices, the lab ware, reagents or solvents tested have been observed at the retention time of interest for dicamba.

Linearity
The linearity of the NICI GC-MSD detector response for dicamba was tested 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) and was found to be linear.

Accuracy
Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.05 (µg/L) and at ten times the LOQ (0.5 µg/L). Acceptable mean recoveries of between 70% and 120% were found for all ions in all water matrices tested and therefore, according to EU guidance (SANCO 3029/99 rev.4 11/7/00), demonstrate the method has satisfactory accuracy**.**

Matrix Effect
The effect of water matrices on the GC/MS signal was assessed by preparing standards in the presence of each water sample and comparing the peak areas of dicamba against non-matrix standards at an equivalent concentration. Acceptable procedural recovery data were obtained using non-matrix standards for river and groundwater samples. For drinking water, acceptable procedural recovery data were obtained using matrix matched standards. It is recommended that matrix effects are determined for all individual analytical water samples.

Repeatability
The repeatability of the method, expressed as the relative standard deviation (RSD), is <20% for dicamba and fortification levels examined.

Limit of Quantification
The limit of quantification for dicamba residues in water using method GRM022.02A was established at 0.05 µg/L. Residues of dicamba measured in the control samples were always below 30% of the LOQ during method validation.

Conclusion
This procedure has been demonstrated to be a reliable and accurate procedure for the determination of concentrations of dicamba in water with a limit of quantification (LOQ) of 0.05 µg/L.

A 2.1.2.4.1.1 Confirmatory method

LC-MS/MS as a detection technique with primary and confirmatory ion transitions is considered to be highly specific and therefore according to the guidance (see guidance section of this summary) further confirmation is not required.

A 2.1.2.4.2 ILV of GRM022.09A

Comments of zRMS:	The studies have been submitted for the purpose of renewal of dicamba. Analytical method GRM022.02A was independent laboratory validated on drinking water samples at the limit of quantification (LOQ) of the method (0.05 µg/L). It fulfils the requirements of SANCO/825/00 rev. 8.1. The method is considered acceptable.

Independent Laboratory Validation of the Method for the Analysis of Water

Reference:	KCP 5.1.2.1/05 & KCP 5.2.5/03
Report	Kotthoff, M. 2016. Dicamba - Independent Laboratory Validation of Analytical Method GRM022.02A for the Determination of Dicamba (SAN837) in Water. Report No. SYN-037/6-22. Syngenta File No. SAN837_11602
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000)
Deviations:	none
GLP:	yes
Acceptability:	Yes

Principle of the Method
Dicamba is extracted from acidified water samples by solid phase extraction, followed by derivatisation. Final determination is by negative-ion chemical ionisation gas liquid chromatography with mass selective detection (NICI GC-MSD).

Recovery Findings
Analytical method GRM022.02A was independent laboratory validated on drinking water samples by fortifying with dicamba at the limit of quantification (LOQ) of the method
(0.05 µg/L) and at 10 x LOQ (0.5 µg/L). The recoveries obtained for dicamba, are presented below.

Table A 22: Recovery Results Obtained During Independent Laboratory Validation of Method GRM022.02A for Dicamba in Drinking Water

Matrix	Analyte (Ion)	Fortification Level (µg/L)*	Number of Analysis (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Drinking water	Dicamba (Primary ion; 184)	0.05	5	97	4	94 - 102
		0.5	5	99	3	94 - 102
		Overall	10	98	3	94 - 102
	Dicamba (Confirmatory ion; 185)	0.05	5	90	3	87 - 93
		0.5	5	97	3	92 - 101
		Overall	10	94	5	87 - 101
	Dicamba (Confirmatory ion; 186)	0.05	5	98	3	94 - 102
		0.5	5	99	3	94 - 103
		Overall	10	98	3	94 - 103

Specificity
GC-MSD with three ions is considered to be a highly specific detection technique and therefore according to the guidance (see guidance section of this summary) no further confirmatory technique is required. No significant interferences, above 30% of the LOQ, arising from the drinking water matrix, the labware, reagents or solvents have been observed at the retention time of interest.

Linearity
The linearity of the GC-MS detector response was tested using calibration standard solutions over the range 0.625 µg/L to 50.0 µg/L (equivalent to 0.625 pg to 50 pg of analyte injected on to the column, based on a 1 µL injection). Standards at seven different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients ranging from 0.9995 to 0.9996 were obtained for dicamba in drinking water.

Accuracy
The mean dicamba recoveries, for both the primary and confirmatory ions, at each fortification level and overall for the drinking water matrix tested during independent laboratory method validation were between 90% and 99%. These values are all between 70% and 110% and therefore according to the guidance (see guidance section of this summary) demonstrate the method has satisfactory accuracy.

Repeatability
The relative standard deviations (RSDs) of the dicamba recoveries, for both the primary and confirmatory ions, at each fortification level and overall for the drinking water matrix tested during independent laboratory validation were between 3% and 5%. These values are all below 20% and therefore according to the guidance (see guidance section of this summary), demonstrate the method has satisfactory repeatability.

Limit of Quantification
The LOQ for dicamba in drinking water using method GRM022.02A was confirmed at 0.05 µg/L in the independent laboratory validation. No interfering peaks around the retention time of dicamba in drinking water were found in any of the control samples at levels above 30% of the LOQ.

Conclusion
Analytical method GRM022.02A has been demonstrated to be a reliable and accurate procedure for the determination of dicamba in drinking water to a limit of quantification of 0.05 µg/L, using commercially available laboratory equipment and reagents, in an independent laboratory validation study.

A 2.1.2.4.5 GRM022.09A

Comments of zRMS:	The analytical method GRM022.09A has been successfully validated for the determination of the dicamba metabolite NOA414746 (DCSA) residues in water (groundwater, surface water and seawater), with a limit of quantification (LOQ) of 0.05 µg/L. It fulfils the requirements of SANCO 3029/99 rev.4 and SANCO/825/00 rev. 8.1.

	Residues of NOA414746 (DCSA) measured in the control samples were always below 30% of the LOQ during method validation. Acceptable mean recoveries of between 70% and 120% with a relative standard deviation lower than 20% were found for NOA414746 (DCSA) in all water matrices tested for both primary and confirmatory transitions. The method is considered acceptable.

Reference:	KCP 5.1.2.1/08
Report	Allen, L. & Brooks, S. 2017 Dicamba - Residue Method GRM022.09A for the Determination of the Metabolite NOA414746 (DCSA) in Water. Syngenta Analytical Method GRM022.09A. Report No. GRM022.09A. Syngenta File N° NOA414746_10010
Guideline(s):	SANCO/825/00 rev. 8.1 (2010) SANCO/3029/99 rev. 4 (200) OCSPP 850.6100 (2012)
Deviations:	none
GLP:	no
Acceptability:	Yes

Reference:	KCP 5.1.2.1/09
Report	Allen, L. 2017 Dicamba - Validation of Draft Residue Method GRM022.09A for the Determination of Dicamba Metabolite NOA414746 (DCSA) in Water Report No. CEMR-7878. Syngenta File N° NOA414746_10011
Guideline(s):	SANCO/825/00 rev. 8.1 (2010) SANCO/3029/99 rev. 4 (200) OCSPP 850.6100 (2012)
Deviations:	none
GLP:	~~No~~ Yes
Acceptability:	Yes

Principle of the method

A 5 mL aliquot of the water sample was acidified with 50 µL concentrated hydrochloric acid. Aliquots were cleaned-up and concentrated by a solid phase extraction (SPE) procedure using reversed phase (Phenomenex Strata-X) cartridges. Samples were evaporated to dryness and re-dissolved in acetonitrile/ultra-pure water (20/80, v/v). Final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).

Recovery Findings

The mean procedural recoveries for NOA414746 fortified at 0.05 µg/L and 0.5 µg/L were between 70% and 110% with relative standard deviation (RSD) values of less than 20% for both the target and confirmatory ions.

Table A 23: Recovery results from validation of GRM022.09A for NOA414746 in surface water, groundwater and seawater: primary transition *m*/z 204.8 → 125.0

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Surface Water	0.05	97, 76, 96, 97, 98	5	93	10.1	76-98
	0.5	92, 91, 92, 91, 91	5	91	0.6	91-92
	Overall	-	10	92	6.9	76-98
Groundwater	0.05	85, 80, 84, 82, 90	5	84	4.5	80-90
	0.5	72, 74, 77, 73, 69	5	73	4.0	69-77
	Overall	-	10	79	8.5	69-90
Seawater	0.05	90, 82, 90, 85, 92	5	88	4.7	82-92
	0.5	94, 92, 92, 92, 86	5	91	3.3	86-94
	Overall	-	10	90	4.3	82-94

Table A 24: Recovery results from validation of GRM022.09A for NOA414746 in surface water, groundwater and seawater: confirmatory transition *m*/z 204.8 → 160.9

Matrix	Fortification Level (µg/L)	Recovery (%)	Number of Analysis (n)	Mean (%)	RSD (%)	Range (%)
Surface Water	0.05	94, 77, 99, 97, 94	5	92	9.5	77-99
	0.5	93, 94, 93, 94, 94	5	94	0.6	93-94
	Overall	-	10	93	6.3	77-99
Groundwater	0.05	85, 80, 82, 83, 87	5	83	3.2	80-87
	0.5	72, 75, 74, 75, 70	5	73	3.0	70-75
	Overall	-	10	78	7.5	70-87
Seawater	0.05	86, 93, 94, 92, 98	5	93	3.7	86-98
	0.5	92, 93, 93, 92, 87	5	91	4.7	87-93
	Overall	-	10	92	2.7	86-98

Specificity

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (see guidance section of this summary) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. The strongest ions/transitions were identified using mass spectra data available in the analytical method (GRM022.09A). No significant interferences arising from the matrices tested, the labware, reagents or solvents have been observed at the retention time of interest. Chromatograms for each transition in each matrix at both the LOQ and 10 × LOQ are provided in the validation report.

Linearity

The response of the LC-MS/MS was confirmed to be linear by injecting at least 8 matrix-matched standard solutions covering the working range. The lower margin of the linearity test was at least 30% of the LOQ and the upper margin was at least 20% above the highest fortification concentration in the final extracts. These margins cover the range as demanded in SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4. Straight lines were obtained with correlation coefficients ranging between 0.9996 and 0.9998 (primary transition) and between 0.9972 and 1.0000 (confirmatory transition).

Accuracy and repeatability

Control samples were analysed in duplicate and fortified samples were analysed in quintuplet at the limit of quantification (LOQ) of 0.05 µg/L and 10 ×LOQ (0.5 µg/L) levels. A reagent blank sample was also analysed with each matrix batch.

Acceptable mean recoveries of between 70% and 110% with a relative standard deviation lower than 20% at each fortification level (0.05 and 0.5 µg/L) and overall with a relative standard deviation lower than 20% were found for NOA414746 both primary and confirmatory transitions.

The repeatability and specificity of the method GRM022.09A has been demonstrated for the determination of residues of NOA414746 in water at a limit of quantification (LOQ) of 0.05 µg/L.

Matrix Effect

No significant suppression or enhancement of detector response was observed for NOA414746 in the presence of surface water or groundwater. Significant suppression of detector response was observed in the presence of seawater and therefore matrix-matched standards were used for all water types tested.

Limit of Quantification
The LOQ of the analytical method GRM022.09A was established as 0.05 µg/L for NOA414746 in water. No interfering peaks around the retention time were found in any of the control samples and reagent blank samples at levels above 30% of the LOQ.

Conclusion
Analytical method GRM022.09A has been demonstrated to be a reliable and accurate procedure for the determination of NOA414746 in water to a limit of quantification of 0.05 µg/L, using commercially available laboratory equipment and reagents.

A 2.1.2.4.5.1 Confirmatory method

LC-MS/MS as a detection technique with primary and confirmatory ion transitions is considered to be highly specific and therefore according to the guidance (see guidance section of this summary) further confirmation is not required.

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

Additional data on methods/validation in air for dicamba have been developed and have been provided below. The methodology has been updated to meet current guidance.
The repeatability and specificity of the method have been independently demonstrated and analytical method GRM022.01A is therefore considered valid for the determination of residues of dicamba in air to a limit of quantification of 0.002 µg/L in air (or 2.0 µg/m^3), using commercially available laboratory equipment and reagents. All data are considered adequate.

A 2.1.2.5.1 GRM022.01A

Comments of zRMS:	The analytical method has been successfully validated for the determination of dicamba in air with a LOQ of 2 µg/m^3. Mean recoveries and relative standard deviations for both the target and confirmatory ions were in the range 70-110% with ≤ 20% RSD). The study is aaceptable.

Reference:	KCP 5.1.2.1/06 & KCP 5.2.6/01
Report	Hargreaves, S. L. 2007a GRM022.01A - Dicamba: Residue Method for the Determination of Residues in Air. Report No. GRM022.01A. Syngenta File N° SAN837/6677
Guideline(s):	none
Deviations:	none
GLP:	no
Acceptability:	Yes

Reference:	KCP 5.1.2.1/07 & KCP 5.2.6/02
Report	Emburey, S. N. 2007a Dicamba - Validation of an Analytical Method for the Determination of Residues of Dicamba in Air. . Report No. T010135-04. Syngenta File N° SAN837/6678
Guideline(s):	EC Guidance Document SANCO/3029/99 rev.4; EC Guidance Document SANCO/825/00 rev. 7; EPA OPPTS 850.7100 and 860.1340
Deviations:	none
GLP:	~~No~~ Yes

Acceptability: Yes

Principle of the method

OVS (Occupational Safety and Health Administration (OSHA) Versatile Sampler) Tenax air sampling tubes were fortified with dicamba standard solution. Air was drawn through the tubes at 33-34ºC and 77-85 % humidity for 6 hours, using a flow rate of 0.25 L/min. Dicamba was desorbed separately from the upper and lower sorbent layers of the tubes by ultrasonication with acidified acetonitrile. An aliquot of the acidified acetonitrile was then evaporated to dryness and the residues redissolved in acetone. The 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). The limit of quantification (LOQ) of the method was established as 0.002 µg/L in air (or 2.0 µg/m^3 air) based on 0.25 L/min air flow and 6 hour sampling, equivalent to 0.18 µg dicamba being applied to the tube.

Recovery Findings

The mean procedural recoveries for dicamba fortified at 2.0 ng/L and 20 ng/L in air based on 0.25 L/min air flow and 6 hour sampling, were between 70% and 110% with relative standard deviation (RSD) values of less than 20% for both the target and confirmatory ions.

Table A 25: Dicamba Recovery Data Obtained During Method Validation (Target Ion, m/z = 184)

Conditions	Fortification Level (ng/L)	Recovery (%)	Mean (%)	RSD (%)	Range
33-34ºC and 77-85% humidity 6 hour monitoring	6 Control	7 <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

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

Table A 26: Dicamba Recovery Data Obtained During Method Validation (Confirmatory Ion, m/z= 185)

Conditions	Fortification Level (ng/L)	Recovery (%)	Mean (%)	RSD (%)	Range
33-34ºC and 77-85% humidity 6 hour monitoring	8 Control	9 <LOQ, <LOQ*			
	2.0**(Upper layer)	90, 93, 88, 85, 81	87	5	81 - 93
	20 (Upper layer)	79, 93, 91, 92, 82	87	7	79 - 93
			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

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

Table A 27: Dicamba Recovery Data Obtained During Method Validation (Confirmatory Ion, m/z = 186)

Conditions	Fortification Level (ng/L)	Recovery (%)	Mean (%)	RSD (%)	Range
33-34ºC and 77-85% humidity 6 hour monitoring	10 Control	11 <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
			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 LOD in any of the samples.
** LOQ defined by the lowest validated fortification level.
ND - Not Determined (insufficient data points)
All recovery data were generated using non-matrix matched standards.

Specificity
NICI GC-MSD as a detection technique with target and qualifier ion transitions 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 above 30% of the lower limit of quantification, arising from either the air sampled, the lab ware, or the reagents and solvents used have been observed at the retention time of interest for dicamba.

Linearity
The GC-MSD detector response for dicamba was shown to be linear over a standard concentration range of 0.625 ng/mL to 50 ng/mL.

Accuracy
The limit of detection (LOD) was estimated to be 0.037 ng/L for the dicamba target ion, 0.067 ng/L and 0.052 ng/L for the dicamba confirmatory ions. No residues of dicamba were detected in the control samples above the LOD i.e. residues were less than 30 % of the LOQ.

Matrix Effect
Negative ion chemical ionisation GC-MSD is a highly specific detection technique. Interference arising from the OVS Tenax air sampling tubes tested has not been observed.

Repeatability
The repeatability of the method, expressed as the relative standard deviation (RSD), is <20% for all analytes and fortification levels examined.

Limit of Quantification
The limit of quantitation of the method is defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70-110% with a relative standard deviation of ≤ 20% has been obtained. Generally, for accurate quantitation, the response for an analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time.
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 (10 x LOQ) fortifications.

Conclusion
This procedure has been demonstrated to be a reliable and accurate procedure for the determination of dicamba in air with a limit of quantification (LOQ) of 0.002 µg/L.

A 2.1.2.5.1.1 Confirmatory method

LC-MS/MS as a detection technique with primary and confirmatory ion transitions is considered to be

highly specific and therefore according to the guidance (see guidance section of this summary) further confirmation is not required.

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.

Analytical methods for mesotrione

A 2.2.1.1 Methods used for the generation of pre-authorization data (KCP 5.1) Description of analytical methods for the determination of residues in support of environmental fate studies (KCP 5.1.2.1)

New studies have been submitted and are described in details below.

A 2.2.1.1.1 Analytical method 1200-03

A 2.2.1.1.1.1 Method validation

Comments of zRMS:	The analytical method 1200-03 (HPLC-MS/MS) for the determination of mesotrione and its metabolites AMBA and MNBA in soil was validated with regard to specificity, linearity, precision and accuracy according to the guideline SANCO/3029/99 rev. 4, 11/07/2000. The limit of quantification (LOQ) of the analytical method was 2.0 µg test item/kg. The study has been accepted.

Reference:	KCP 5.1.2.1/10 & KCP 5.2.4/03
Report	Williams R. 2004 Analytical Method 1200-03 for the Determination of mesotrione and its Metabolites AMBA and MNBA, in Soil, Using Liquid Chromatography–Electrospray Ionization Tandem Mass Spectrometry (Including Validation Data) Syngenta File No ZA1296/1567
Guideline(s):	EPA Guideline No. 164-1
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

A. Materials

1. Standards

Reference item:	Mesotrione
CAS No.:	104206-82-8
Purity:	Not stated
Lot/batch No.:	Not stated
Expiry date:	Not stated
Standard for calibration:	As above.

Reference item:	MNBA
CAS No.:	110964-79-9
Purity:	Not stated
Lot/batch No.:	Not stated
Expiry date:	Not stated
Standard for calibration:	As above.

Reference item:	AMBA
CAS No.:	393085-45-5
Purity:	Not stated
Lot/batch No.:	Not stated
Expiry date:	Not stated

Standard for calibration: As above.

2. Test item Not applicable

3. Test medium: Soil

B. Sample preparation and processing

Soil samples (10 g) are extracted three times by shaking with solvent (once with 0.05M NH_4OH, once with 50:50 (v/v) 0.05M NH_4OH: acetone and finally with acetone) at room temperature. The extracts are combined and centrifuged to settle suspended solids. An aliquot of extract is taken and the organic solvent removed by evaporation (N-Evap). The samples are acidified and diluted with formic acid to precipitate soil acids. After centrifugation, an aliquot of the extract is transferred to an LC sample vial. Final determination is by high performance liquid chromatography with tandem mass spectrometric detection (HPLC/MS/MS). External standards in 10:90 (v/v) methanol:ultra-pure water are used for calibration.
Validation of the method was carried out for mesotrione, AMBA and MNBA in soil at fortification levels of 2.0 and 50 ppb (0.002 and 0.05 mg/kg).

C. Analytical instrumentation and analysis

1. HPLC parameters - mesotrione

Instrumentation:	Waters Alliance Model 2695
Column:	Polymer Laboratories PLRP-S, (50 x 4.6 mm)
Column temp.:	30-35 °C
Mobile phase:	A: 0.1% Acetic acid in HPLC grade water B: 0.1% Acetic acid in acetonitrile

For AMBA and MNBA

Instrumentation:	Waters Alliance Model 2695
Column:	Phenomenex Synergi Fusion-RP (75 x 4.6 mm)
Column temp.:	30-35 °C
Mobile phase:	A: 0.1% Acetic acid in HPLC grade water B: 0.1% Acetic acid in acetonitrile

2. MS parameters

Instrumentation:	Micromass Quattro Ultima	
Transitions monitored	1: m/z 338.2 → m/z 291.000	(Mesotrione)
	1: m/z 244.0 → m/z 199.800	(MNBA)
	1: m/z 214.0→ m/z 169.900	(AMBA)

D. Calibration

Principle: Six-point linear

Results and discussions

An HPLC-MS/MS method was used to determine concentrations of Mesotrione, AMBA and MNBA.

Table A 28: Procedural recovery data for mesotrione, AMBA and MNBA

Matrix	Analyte	Fortification level (~~mg~~ µg test item/kg)	Mean recovery (%)	RSD (%)	Comments
Soil	Mesotrione	2.0*	99	1.3	Range : 97-100
		50.0	96	3.2	Range :92-98
	~~MNBA~~ AMBA	2.0*	93	4.2	Range :88-97
		50.0	93	1.8	Range :91-94
	~~AMBA~~ MNBA	2.0*	106	1.6	Range :104-108
		50.0	110	1.8	Range :107-112

*Limit of quantification, defined as the lowest validated fortification level

Table A 29: Characteristics for the analytical method used for validation of mesotrione AMBA and MNBA residues in Maize whole plant

	Mesotrione and MNBA
Calibration (type, number of data points)	A six-point linear calibration curve was used for target analytes quantification and is presented in the study. The equation of the calibration curve is: Mesotrione : y = 2147.83x + 341.076 r = 0.9991 MNBA : y = 7809.13x + 1380.95 r = 0.9994 AMBA : y = 1305.70x + 182.4770 r = 0.9996 Individual recovery data provided in the report R^2 >0.999 for all three analytes
Calibration range	1.0 - 50 µg reference items/L
Limit of quantification	LOQ: 2 ~~mg~~ µg test item/kg

Conclusion
The method was fully validated according to the requirements of EPA Guideline 164-1. Procedural recoveries are provided in the study summarised above and the original method is therefore acceptable for the determination of mesotrione in soil.

A 2.2.1.2 Description of analytical methods for the determination of operator, worker, resident and bystander exposure studies (KCP 5.1.2.4)

New studies have been conducted and summarised below

A 2.2.1.2.1 Analytical method BFI0148

A 2.2.1.2.1.1 Method validation

Comments of zRMS:	The analytical method HPLC-UV for the determination of 2-nitro-4-methylsulfonylbenzoic acid (MNBA) in aqueous carboxymethylcellulose was successfully validated according to SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was 1 mg/mL. The study is acceptable.

Reference:	KCP 5.1.2.4/01
Report	Bachelor, B., 2014 2-Nitro-4-Methylsulfonyl benzoic acid (CA3511) analytical method transfer and partial validation for the determination of CA3511 in dosing formulations. Study No.: 11070.
Guideline(s):	None stated in report
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

A. Materials

1. Standards

Reference item:	2-nitro-4-methylsulfonylbenzoic acid (CA3511)
Synonym:	MNBA
CAS No.:	110964-79-9
Purity:	99.8%
Lot/batch No.:	SM03C0689
Expiry date:	Recertification Date: May 2016
Standard for calibration:	As above.

2. Test item:	2-nitro-4-methylsulfonylbenzoic acid (CA3511)
Synonym:	MNBA
3. Test medium:	Aqueous carboxymethylcellulose

B. Sample preparation and processing

A solution of acetonitrile (ACN):Purified Water (50:50, v/v) was prepared for the partial method validation. Stock solutions of the test substance, were prepared at a concentration of 500 µg/ml of test item in acetonitrile and then further diluted (with vehicle and diluent) to provide three fortification levels at 1.0, 35.0 and 120 mg/mL. Aliquots of the samples were individually transferred to separate HPLC vials and analysed using the instrumentation detailed below.

C. Analytical instrumentation and analysis

1. HPLC parameters

Instrumentation	Agilent 1220 Infinity Gradient Compact LC
Column:	Waters XBridge C8 (100 mm x 4.6 mm I.D., 5 µm particle size)
Mobile phase:	A: Purified Water:Trifluoroacetic Acid (100:0.2, v/v) B: Acetonitrile:Trifluoroacetic Acid (100:0.2, v/v)

2. UV parameters

Detector	UV fixed wavelength
Detector wave length:	270 nm

D. Calibration

Principle:	No information provided

Results and discussions

An HPLC-UV method was used to determine concentrations of MNBA (2-nitro-4-methylsulfonylbenzoic acid) in aqueous carboxymethylcellulose. The LOQ of the method is 1 mg mestrione/mL. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Well-labelled chromatograms are provided in the original study report.

Table A 30: Recovery results from method validation of MNBA using the analytical method

Matrix	Analyte	Fortification level (mg/mL)	Mean recovery (%)	RSD (%)	Comments
Aqueous carboxymethylcellulose	MNBA	1.00 (n=6)	102.0	0.352	-
		35.0 (n=6)	98.6	1.99	-

Matrix	Analyte	Fortification level (mg/mL)	Mean recovery (%)	RSD (%)	Comments
		120 (n=6)	108.8	0.984	-

Table A 31: Characteristics for the analytical method used for validation of 2-nitro-4-methylsulfonylbenzoic acid residues in water

	2-nitro-4-methylsulfonylbenzoic acid
Specificity	Analyte was not detected in any controls. (n=2)
Calibration (type, number of data points)	No information provided
Calibration range	No information provided
Limit of quantification (LOQ)	1 mg mesotrione/mL

Conclusion
The method detailed above was used to determine concentrations of MNBA in aqueous carboxymethylcellulose in support of a toxicological study. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4. The method used well-established analytical systems and techniques and should therefore be considered suitable for the determination of MNBA in aqueous carboxymethylcellulose.

A 2.2.1.2.2 Analytical method BFI0147

A 2.2.1.2.2.1 Method validation

Comments of zRMS:	The analytical method HPLC-UV (BFI0147) for the determination of 2-nitro-4-methylsulfonyl benzoicacid (MNBA) in 1% w/v aqueous carboxymethylcellulose is acceptable. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4 The limit of quantification (LOQ) of the analytical method was 1 mg/mL.

Reference:	KCP 5.1.2.4/02
Report	Faulkner, L., Heap, C., 2013 CA3511 - Feasibility of the Assay for the Determination of CA3511 in 1 % w/v Aqueous Carboxymethylcellulose. Project No.: BFI0147.
Guideline(s):	None stated in report
Deviations:	None stated in report
GLP:	No
Acceptability:	Yes

Materials and methods

A. Materials

1. Standards

Reference item:	2-nitro-4-methylsulfonylbenzoic acid (CA3511)
Synonym:	MNBA
CAS No.:	110964-79-9
Purity:	99.8
Lot/batch No.:	SMO3C0689
Expiry date:	End of May 2016
Standard for calibration:	As above.

2. Test item: 2-nitro-4-methylsulfonylbenzoic acid (CA3511)
Synonym: MNBA

3. Test medium: Aqueous carboxymethylcellulose

B. Sample preparation and processing

Fortified samples were prepared at concentrations of 1 mg/mL, 10 mg/mL and 100 mg/mL CA3511 in vehicle. Samples were individually transferred to separate HPLC vials and analysed using the instrumentation below.

C. Analytical instrumentation and analysis

1. HPLC parameters

Instrumentation: Sartorius HPLC
Column: Hichrom HiRPB (150 mm x 4.6 mm I.D., 5 µm particle size)
Mobile phase: 200 mL of acetonitrile and 800 mL of UHP water with 2 mL trifluoroacetic acid

2. UV parameters

Detector: Sartorius UV-detector
Detector wave length: 270 nm

D. Calibration

Principle: Six-poin linear

Results and discussions

An HPLC-UV method was used to determine concentrations of MNBA (2-nitro-4-methylsulfonylbenzoic acid) in aqueous carboxymethylcellulose. The LOQ of the method is 1 mg mestrione/mL. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Chromatograms are provided in the original study report.

Table A 32: Recovery results from method validation of MNBA using the analytical method

Matrix	Analyte	Fortification level (mg/mL)	Mean recovery (%)	RSD (%)	Comments
Aqueous carboxymethylcellulose	MNBA	1 (n=3)	91	1.2	-
		10 (n=3)	100	7.7	-
		100 (n=3)	100	1.6	-

Table A 33: Characteristics for the analytical method used for validation of MNBA residues in water

	2-nitro-4-methylsulfonylbenzoic acid
Specificity	No co-chromatographic peaks, with the same retention time as CA3511, were detected in the diluted control samples or reagent blanks.
Calibration (type, number of data points)	Six-point linear used for target analyte quantification and is presented in the study. Individual calbration data are provided.

	2-nitro-4-methylsulfonylbenzoic acid
	The equation of the calibration curve is: y = 3.935417e+003 -2.876764e+003; r2 = > 0.995
Calibration range	5.465 - 15.71 µg/mL
Limit of quantification (LOQ)	1 mg/mL

Conclusion
The method detailed above was used to determine concentrations of MNBA in aqueous carboxymethylcellulose in support of a toxicological study. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4. The method used well-established analytical systems and techniques and should therefore be considered suitable for the determination of MNBA in aqueous carboxymethylcellulose.

A 2.2.1.2.3 Analytical method BFI0148

A 2.2.1.2.3.1 Method validation

Method validationComments of zRMS:	The analytical method HPLC-UV (BFI0148) for the determination of 2-nitro-4-methylsulfonylbenzoic acid (MNBA) in 1% w/v aqueous carboxymethylcellulose is acceptable. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4 The limit of quantification (LOQ) of the analytical method was 1 mg/mL.

Reference:	KCP 5.1.2.4/03
Report	Faulkner, L., Heap, C., 2013a CA3511 - Validation of the Assay for the Determination of CA3511 in 1% w/v Aqueous Carboxymethylcellulose - Method Validation.. Project No.: BFI0148.
Guideline(s):	Not given
Deviations:	None reported
GLP:	Yes
Acceptability:	Yes

Materials and methods

A. Materials

1. Standards

Reference item:	2-nitro-4-methylsulfonylbenzoic acid (CA3511)
Synonym:	MNBA
CAS No.:	110964-79-9
Purity:	99.8
Lot/batch No.:	SMO3C0689
Expiry date:	Re-certification date: End of May 2016
Standard for calibration:	As above.

2. Test item:	2-nitro-4-methylsulfonylbenzoic acid (CA3511)
Synonym:	MNBA
3. Test medium:	Aqueous carboxymethylcellulose

B. Sample preparation and processing

Fortified samples were prepared at concentrations of 1 mg/mL, 10 mg/mL and 100 mg/mL CA3511 in vehicle. Samples were individually transferred to separate HPLC vials and analysed using the instrumentation below.

C. Analytical instrumentation and analysis

1. HPLC parameters

Instrumentation	Sartorius HPLC
Column:	Hichrom HiRPB (150 mm x 4.6 mm I.D., 5 µm particle size)
Mobile phase:	200 mL of acetonitrile and 800 mL of UHP water with 2 mL trifluoroacetic acid

2. UV parameters

Detector	Sartorius UV-detector
Detector wave length:	270 nm

D. Calibration

Principle:	Six-point linear

Results and discussions

An HPLC-UV method was used to determine concentrations of MNBA (2-nitro-4-methylsulfonylbenzoic acid) in aqueous carboxymethylcellulose. The LOQ of the method is 1 mg mestrione/mL. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Chromatograms are provided in the original study report.

Table A 34: Recovery results from method validation of MNBA using the analytical method

Matrix	Analyte	Fortification level (mg/mL)	Mean recovery (%)	RSD (%)	Comments
Aqueous carboxymethylcellulose	MNBA	1 (n=6)	100	0.5	-
		10 (n=6)	97	0.7	-
		100 (n=6)	97	0.5	-

Table A 35: Characteristics for the analytical method used for validation of 2-nitro-4-methylsulfonylbenzoic acid residues in water

	2-nitro-4-methylsulfonylbenzoic acid
Specificity	No co-chromatographic peaks, with the same retention time as CA3511, were detected in the diluted control samples or reagent blanks.
Calibration (type, number of data points)	Six-point linear used for target analyte quantification and is presented in the study. Individual calbration data are provided. The equation of the calibration curve is: y = 7.678763e+003 -2.326498e+003; r2 = > 0.999
Calibration range	5.190 - 15.83 µg/mL
Limit of quantification (LOQ)	1 mg/mL

Conclusion
The method detailed above was used to determine concentrations of MNBA in aqueous carboxymethylcellulose in support of a toxicological study. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4. The method used well-established analytical systems and techniques and should therefore be considered suitable for the determination of MNBA in aqueous carboxymethylcellulose.

A 2.2.1.2.4 Analytical method BFI0148

A 2.2.1.2.4.1 Method validation

Comments of zRMS:	The analytical method HPLC-UV (BFI0148) for the determination of 2-nitro-4-methylsulfonylbenzoic acid (MNBA) in aqueous carboxymethylcellulose was validated according to SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was 1 mg/mL. The study has been accepted.

Reference:	KCP 5.1.2.4/04
Report	Faulkner, L., Heap, C., 2013b CA3511 - Validation of the Formulation Procedure for CA3511 in 1 % w/v Aqueous Carboxymethylcellulose and Assessment of Formulation Stability - Method Validation. Project No.: BFI0149.
Guideline(s):	Not given
Deviations:	None reported
GLP:	Yes
Acceptability:	Yes

Materials and methods

A. Materials

1. Standards

Reference item:	2-nitro-4-methylsulfonylbenzoic acid (CA3511)
Synonym:	MNBA
CAS No.:	Not stated
Purity:	99.8
Lot/batch No.:	SMO3C0689
Expiry date:	Re-certification date: End of May 2016
Standard for calibration:	As above.

2. Test item:	2-nitro-4-methylsulfonylbenzoic acid (CA3511)
Synonym:	MNBA

3. Test medium:	Aqueous carboxymethylcellulose

B. Sample preparation and processing

Triplicate samples were taken from the top, middle and bottom of each formulation, diluted to approximately 10 µg/mL with acetonitrile, and analysed using reversed phase HPLC with UV detection has detailed below.

C. Analytical instrumentation and analysis

1. HPLC parameters

Instrumentation	Sartorius HPLC
Column:	Hichrom HiRPB (150 mm x 4.6 mm I.D., 5 µm particle size)

Mobile phase: 200 mL of acetonitrile and 800 mL of UHP water with 2 mL trifluoroacetic acid

2. UV parameters
Detector Sartorius UV-detector
Detector wave length: 270 nm

D. Calibration
Principle: No further information provded

Results and discussions
An HPLC-UV method was used to determine concentrations of MNBA (2-nitro-4-methylsulfonylbenzoic acid) in aqueous carboxymethylcellulose. The LOQ was found at 1 mg/mL. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Chromatograms are provided in the original study report.

Table A 36: Recovery results from method validation of MNBA using the analytical method

Matrix	Analyte	Fortification level (mg/mL) nominal	Mean recovery (%)	RSD (%)	Comments
Aqueous carboxymethylcellulose	MNBA	1 (n=9)	103	1.7	-
		10 (n=9)	93	2.2	-
		100 (n=9)	95	4.5	-

Table A 37: Characteristics for the analytical method used for validation of 2-nitro-4-methylsulfonylbenzoic acid residues in water

	2-nitro-4-methylsulfonylbenzoic acid
Specificity	No information provided
Calibration (type, number of data points)	No information provided
Calibration range	No information provided
Limit of quantification (LOQ)	1 mg/mL

Conclusion
The method detailed above was used to determine concentrations of MNBA in aqueous carboxymethylcellulose in support of a toxicological study. Mean recoveries and associated RSD's meet the requirements of SANCO/3029/99 rev. 4. The method used well-established analytical systems and techniques and should therefore be considered suitable for the determination of MNBA in aqueous carboxymethylcellulose.

A 2.2.1.2.5 Analytical method BFI068MS and BFI074MS

A 2.2.1.2.5.1 Method validation

Comments of zRMS:	The analytical method HPLC-MS/MS for the determination of 2-amino-4-methylsulfonylbenzoic acid (AMBA) in blood and plasma of rats samples is considered acceptable with LOQ at 10 ng/mL.

Reference:	KCP 5.1.2.4/05
Report	xxxxxxxxxxx. 2016 AMBA - Single dose oral (gavage) proof of exposure study in the rat. xxxxxxxxxxxxxx 2016. Project No.: BFI0533.
Guideline(s):	Not given
Deviations:	None reported
GLP:	Yes
Acceptability:	Yes

Materials and methods

A. Materials

1. Standards

Reference item:	2-amino-4-methylsulfonylbenzoic acid
Synonym:	AMBA
Purity:	98.6%
Lot/batch No.:	924777
Expiry date:	Re-certification date: End of March 2017
Standard for calibration:	As above.
Internal standard:	Tolbutamide
Purity:	100%
Lot/batch No.:	SLBN2252V
Expiry date:	21 April 2020
2. Test item:	2-amino-4-methylsulfonylbenzoic acid (CA3511)
Synonym:	AMBA
3. Test medium:	blood and plasma of rats

B. Sample preparation and processing

The test item was formulated on the day of dosing as a suspension in the vehicle, 1.0 % (w/v) carboxymethylcellulose with 0.1 % (v/v) Tween 80. Three male rats of the Crl:WI(Han) strain were allocated to the study and dosed once with 2000 mg/kg AMBA, by gavage, at a dose volume of 10 mL/kg body weight. Blood samples were taken on Day 1 at 1, 4 and 24 hours after dosing for proof of exposure. Samples (Blood/Plasma) were diluted with water (1:1) and individually transferred to separate HPLC vials and analysed using the instrumentation below.

C. Analytical instrumentation and analysis

1. HPLC parameters

Instrumentation	Agilent HPLC pump
Column:	ACE Phenyl (50 mm x 2.1 mm I.D., 5 µm particle size)
Mobile phase:	A: 0.01 % Formic acid B: Methanol

2. MS parameters

Instrumentation:	AB Sciex API4000 mass spectrometer
Ionisation mode:	Electro spray
Heater gas Temp.:	500°C
Transitions monitored	AMBA: m/z 214 → m/z 470 AMBA: m/z 214 → m/z 155 AMBA: m/z 214 → m/z 79 Tolbutamide: m/z 269 → m/z 170

D. Calibration

Principle: Eight-point

Results and discussions

An HPLC-MS/MS method was used to determine concentrations of AMBA (2-amino-4-methylsulfonylbenzoic acid) in blood and plasma of rats.

Table A 38: Recovery results from method validation of AMBA using the analytical method

Matrix	Analyte	Fortification level (~~mg~~ng/mL)	Mean recovery (%)	RSD (%) (between-run precision)	Comments
Blood	AMBA	30	65.63%	8.16	
		4000	65.69%	4.67	
Plasma	AMBA	30	73.31	9.46	
		4000	78.68	7.64	

Table A 39: Characteristics for the analytical method used for validation of 2-amino-4-methylsulfonylbenzoic acid residues in water

	2-amino-4-methylsulfonylbenzoic acid
Specificity	Method highly specific, three transitions monitored for each analysis.
Calibration (type, number of data points)	Linear calibration (quadratic regression), 8 concentrations, 4 replicates per concentration, calibration coefficient ≥0.9945 (blood) and ≥0.9954 (plasma)
Calibration range	10 - 5000 ng/mL
Limit of quantification (LOQ)	10 ng/mL

Conclusion

The method detailed above was used to determine concentrations of AMBA (2-amino-4-methylsulfonylbenzoic acid) in rat whole blood and plasma in support of a toxicological study. The method used well-established analytical systems and techniques and should therefore be considered suitable for the determination of AMBA (2-amino-4-methylsulfonylbenzoic acid) in rat whole blood and plasma.

A 2.2.1.3 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 2.2.1.4 Description of analytical methods for the determination of residues in support of residues studies (KCP 5.1.2.5)

No new data have been submitted in the framework of this application.

A 2.2.1.5 Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2.6)

New studies have been submitted and are described in detail below.

A 2.2.1.5.1 Analytical method S12-02294

A 2.2.1.5.1.1 Method validation

Comments of zRMS:	The analytical method for the determination of mesotrione in water was fully validated with regard to recovery (accuracy), linearity of detector response, repeatability (precision) and specificity according to the guideline SANCO/3029/99 rev. 4, 11/07/2000. Mean recoveries and relative standard deviations per fortification were in the range 70-110% with ≤ 20% RSD). The limit of quantification was 0.1 mg/L of test item (0.0156 mg/L of mesotrione). The study is acceptable.

Reference:	KCP 5.1.2.6/01
Report	Weber, K., 2012 Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor (A12127R) – Assessment of Toxic Effects on Daphnia magna using the 48 h Acute Immobilisation Test. Project No.: S12-02294.
Guideline(s):	SANCO/3029/99 rev.4 11/07/00
Deviations:	None reported
GLP:	Yes
Acceptability:	Yes

Materials and methods

A. Materials

1. Standards

Reference item:	Mesotrione
CAS No.:	104206-82-8
Purity:	99.9%
Lot/batch No.:	SZBB046XV
Expiry date:	15.02.2015
Standard for calibration:	As above.

2. Test item: A18032E

Test item code:	2012-002761
CAS No.:	Mesotrione 104206-82-8 Dicamba 1918-00-9 Nicosulfuron 111991-09-4
Purity:	15.6% w/w mesotrione, 31.3% w/w dicamba and 10.1 % nicosulfuron
Lot/batch No.:	SMU2BP001
Expiry date:	30.09.2014

3. Adjuvant added to test item: A12127R Adigor

Adjuvant code:	2012-001016
CAS No.:	68920-66-1; alcohols, C16-C18 and C18 unsaturated ethoxylated
Density:	925 kg/m^3
Appearance/colour:	Clear liquid/yellow
Purity:	27.1% w/w methyl oleate, 8.80% w/w methyl linoleate, 3.63% w/w methyl linolenate, 2.59% w/w methyl palmitate and 1.04% w/w methyl stearate
Lot/batch No.:	EEM0FA0610
Expiry date:	30.11.2015

4. Test medium: Water

B. Sample preparation and processing

A stock solution of A18032E (388 mg/L; additionally 964 mg/L of A12127R) was prepared in tap water/ demineralized water (1/1, v/v). Dilutions of the stock solution (10 mg/L, 1 mg/L) based on A18032E were prepared in tap water/ demineralized water (1/1, v/v) and used for fortification of recovery samples.
Samples of the test medium were fortified with the test item (plus adjuvant) to provide recovery samples at two levels equivalent to 0.0156 and 0.468 mg mesotrione/L. The samples were homogenised using a Vortex mixer, diluted with acetonitrile/water (1/1, v/v) if required and transferred to glass vials for analysis by HPLC-MS/MS using the instrumentation below.

C. Analytical instrumentation and analysis

1. HPLC parameters

Instrumentation:	Thermo Surveyor MS pump with Thermo Surveyor autosampler	
Column:	Phenomenex Luna 5u Phenyl-Hexyl, 150 mm x 2.0 mm i.d., 5 µm mean particle size (No. 00F-4257-B0) with 4 mm guard column	
Injection volume:	20 µL	
Column temp.:	40 °C	
Mobile phase:	A: water B: acetonitrile C: 1% formic acid	

2. MS parameters

Instrumentation:	Thermo TSQ Quantum triple quadrupole system	
Interface/Ionization mode:	ESI	
Source polarity:	negative	
Spray voltage:	3500 V	
Transitions monitored	1: m/z 337.8 → m/z 290.9	(quantification)
	2: m/z 337.8 → m/z 211.9	(confirmation)

D. Calibration

Principle: eight-point linear

Results and discussions

An HPLC-MS/MS method was used to determine concentrations of mesotrione in water containing the test item (A18032E together with adjuvant A12127R) and validated according to SANCO/3029/99 rev. 4.
The detector response was linear within the range 0.5 – 100 µg mesotrione/L. The method is highly specific with two mass transitions monitored per analysis.
The target analyte was not detected in any controls (n=2). No interference was observed at the retention time of the target analyte. The LOQ for the method was 0.1 mg/L of test item (0.0156 mg mesotrione/L). All recovery data meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Well-labelled chromatograms are provided in the original study report.

Table A 40: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (mg/L)	Mean recovery (%)	RSD (%)	Comments
Water	Mesotrione	0.0156 (n=5)	107	5	-
		0.468 (n=5)	110	2	-

Table A 41: Characteristics for the analytical method used for validation of mesotrione residues in water

	Mesotrione
Specificity	HPLC-MS/MS is considered to be highly specific with two mass transitions which were monitored during each analysis. No interference was observed in any controls. Concentration of mesotrione in controls (blanks) was < 30% LOQ (n=2).
Calibration (type, number of data points)	Eight-point linear used for target analyte quantification and is presented in the study. The equation of the calibration curve is: y=29608.2+16133.7x Individual calbration data are provided. r2 = > 0.999
Calibration range	0.5 – 100 µg/L
Limit of quantification (LOQ) Limit of detection (LOD)	~~0.00156~~ 0.0156 mg mesotrione/L ~~0.000468~~ 0.00468 mg mesotrione/L

Conclusion
The method detailed above was used to determine concentrations of mesotrione in water in support of an ecotoxicological study. The method detailed above was fully validated according to the requirements of SANCO/3029/99 rev. 4. This method is therefore acceptable for the determination of mesotrione in water.

A 2.2.1.5.2 Analytical method S12-02296

A 2.2.1.5.2.1 Method validation

Comments of zRMS:	The analytical method for the determination of mesotrione in water was fully validated with regard to recovery (accuracy), linearity of detector response, repeatability (precision) and specificity according to the guideline SANCO/3029/99 rev. 4, 11/07/2000. Mean recoveries and relative standard deviations for both fortification levels were in the range 70-110% with ≤ 20% RSD). The limit of quantification was 0.00978 mg/L of test item (0.00153 mg/L of mesotrione). The study is acceptable.

Reference:	KCP 5.1.2.6/02
Report	Falk, S., 2012 Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor (A12127R) – Testing of Effects on the Single Cell Green Alga *Pseudokirchneriella subcapitata*. Project No: S12-02296
Guideline(s):	SANCO/3029/99 rev. 4, 11/07/2000
Deviations:	No major deviations

GLP:	Yes
Acceptability:	Yes

Materials and methods

A. Materials

1. Standards

Reference item:	Mesotrione
CAS No.:	104206-82-8
Purity:	99.9%
Lot/batch No.:	SZBB046XV
Expiry date:	15.02.2015
Standard for calibration:	As above.

2. Test item: A18032E

Test item code:	2012-002761
CAS No.:	Mesotrione 104206-82-8 Dicamba 1918-00-9 Nicosulfuron 111991-09-4
Purity:	15.6% w/w mesotrione, 31.3% w/w dicamba and 10.1 % nicosulfuron
Lot/batch No.:	SMU2BP001
Expiry date:	30.09.2014

3. Adjuvant added to test item:	A12127R Adigor
Adjuvant code:	2012-001016
CAS No.:	68920-66-1; alcohols, C16-C18 and C18 unsaturated ethoxylated
Density:	925 kg/m3
Appearance/colour:	Clear liquid/yellow
Purity:	27.1% w/w methyl oleate, 8.80% w/w methyl linoleate, 3.63% w/w methyl linolenate, 2.59% w/w methyl palmitate and 1.04% w/w methyl stearate
Lot/batch No.:	EEM0FA0610
Expiry date:	30.11.2015
4. Test medium:	Water

B. Sample preparation and processing

A stock solution of A18032E (442 mg/L; additionally 1105 mg/L of A12127R) was prepared in tap water/ demineralized water (1/1, v/v). Dilutions of the stock solution (9.78 mg/L, 0.0978 mg/L) based on A18032E were prepared in tap water/ demineralized water (1/1, v/v) and used for fortification of recovery samples. Samples of the test medium (0.5 mL) were fortified with the test item (plus adjuvant) to provide recovery samples. The samples were homogenised using a Vortex mixer, diluted with acetonitrile/water (1/1, v/v) if required and transferred to glass vials for analysis by HPLC-MS/MS using the instrumentation below.

C. Analytical instrumentation and analysis

1. HPLC parameters

Instrumentation:	Thermo Surveyor MS pump with Thermo Surveyor autosampler
Column:	Phenomenex Luna 5u Phenyl-Hexyl, 150 mm x 2.0 mm i.d., 5 µm mean particle size (No. 00F-4257-B0) with 4 mm guard column

Injection volume:	20 µL
Column temp.:	40 °C
Mobile phase:	A: water B: acetonitrile C: 1% formic acid

2. MS parameters

Instrumentation:	Thermo TSQ Quantum triple quadrupole system	
Interface/Ionization mode:	ESI	
Source polarity:	negative	
Spray voltage:	3500 V	
Transitions monitored	1: m/z 337.8 → m/z 290.9	(quantification)
	2: m/z 337.8 → m/z 211.9	(confirmation)

D. Calibration

Principle:	Eight-point linear

Results and discussions

An HPLC-MS/MS method was used to determine concentrations of mesotrione in water containing the test item (A18032E together with adjuvant A12127R) and validated according to SANCO/3029/99 rev. 4.
The detector response was linear within the range 0.3 – 30 µg mesotrione/L. The method is highly specific with two mass transitions monitored per analysis.
The target analyte was not detected in any controls (n=2). No interference was observed at the retention time of the target analyte. The LOQ for the method was 0.00978 mg/L of the test item (equivalent to 0.00153 mg mesotrione/L). All recovery data meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Well-labelled chromatograms are provided in the original study report.

Table A 42: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (mg/L)	Mean recovery (%)	RSD (%)	Comments
Water	Mesotrione	0.00153 (n=5)	78	4	-
		0.122 (n=5)	98	5	-

Table A 43: **Characteristics for the analytical method used for validation of mesotrione residues in water**

	Mesotrione
Specificity	HPLC-MS/MS is considered to be highly specific with two mass transitions which were monitored during each analysis. No interference was observed in any controls. Concentration of mesotrione in controls (blanks) was < 30% LOQ (n=2).
Calibration (type, number of data points)	An eight-point linear calibration curve was used for target analyte quantification and is presented in the study. The equation of the calibration curve is: y = -1700.91+17608.5x Individual calbration data are provided. r^2 = > 0.9991
Calibration range	0.3 – 30 µg reference item/L
Limit of quantification (LOQ)	0.00153 mg mesotrione/L

	Mesotrione
Limit of detection (LOD)	0.000459 mg mesotrione/L

Conclusion
The method detailed above was used to determine concentrations of mesotrione in water in support of an ecotoxicological study. The method detailed above was fully validated according to the requirements of SANCO/3029/99 rev. 4. This method is therefore acceptable for the determination of mesotrione in water.

A 2.2.1.5.3 Analytical method S12-02295

A 2.2.1.5.3.1 Method validation

Comments of zRMS:	The analytical method for the determination of mesotrione in water was fully validated with regard to recovery (accuracy), linearity of detector response, repeatability (precision) and specificity according to the guideline SANCO/3029/99 rev. 4, 11/07/2000. Mean recoveries and relative standard deviations per fortification were in the range 70-110% with ≤ 20% RSD). The limit of quantification was 0.1 mg/L test item (0.0156 mg mesotrione/L). The study is acceptable.

Reference:	KCP 5.1.2.6/03
Report	Weich, M., 2012 Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor (A12127R) – Acute Toxicity Testing in Rainbow Trout (*Oncorhynchus mykiss*) (Teleostei, Salmonidae). Project No.: S12-02295.
Guideline(s):	SANCO/3029/99 rev.4 11/07/00
Deviations:	none
GLP:	Yes
Acceptability:	Yes

Materials and methods
A. Materials

1. Standards

Reference item:	Mesotrione
CAS No.:	104206-82-8
Purity:	99.9%
Lot/batch No.:	SZBB046XV
Expiry date:	15.02.2015
Standard for calibration:	As above.

2. Test item:	A18032E
Test item code:	2012-002761
CAS No.:	Mesotrione 104206-82-8 Dicamba 1918-00-9 Nicosulfuron 111991-09-4
Purity:	15.6% w/w mesotrione, 31.3% w/w dicamba and 10.1 % nicosufuron
Lot/batch No.:	SMU2BP001
Expiry date:	30.09.2014

3. Adjuvant added to test item: A12127R (containing)	Adigor
Adjuvant code:	2012-001016
CAS No.:	68920-66-1; alcohols, C16-C18 and C18 unsaturated ethoxylated
Density:	925 kg/m^3
Appearance/colour:	Clear liquid/yellow
Purity:	27.1% w/w methyl oleate, 8.80% w/w methyl linoleate, 3.63% w/w methyl linolenate, 2.59% w/w methyl palmitate and 1.04% w/w methyl stearate
Lot/batch No.:	EEM0FA0610
Expiry date:	30.11.2015
4. Test medium:	Water

B. Sample preparation and processing

A stock solution of A18032E (988 mg/L; additionally 2472 mg/L of A12127R) was prepared in test medium. Dilutions of the stock solution (100 mg/L, 10 mg/L, 1 mg/L and 0.1 mg/L) based on A18032E were prepared in test medium. The 100 mg/L and 1 mg/L dilutions were used for fortification of recovery samples.

Samples of the test medium were fortified with the test item (plus adjuvant) to provide recovery samples at two levels equivalent to 0.0156 and 1.25 mg mesotrione/L. The samples were homogenised using a Vortex mixer, diluted with acetonitrile/water (1/1, v/v) if required and transferred to glass vials for analysis by HPLC-MS/MS using the instrumentation below.

C. Analytical instrumentation and analysis

1. HPLC parameters

Instrumentation:	Thermo Surveyor MS pump with Thermo Surveyor autosampler	
Column:	Phenomenex Luna 5u Phenyl-Hexyl, 150 mm x 2.0 mm i.d., 5 µm mean particle size (No. 00F-4257-B0) with 4 mm C18 guard column	
Injection volume:	20 µL	
Column temp.:	40 °C	
Mobile phase:	A: water B: acetonitrile C: 1% formic acid	

2. MS parameters

Instrumentation:	Thermo TSQ Quantum triple quadrupole system	
Interface/Ionization mode:	ESI	
Source polarity:	negative	
Spray voltage:	3500 V	
Transitions monitored	1: m/z 337.8 → m/z 290.9	(quantification)
	2: m/z 337.8 → m/z 211.9	(confirmation)

D. Calibration

Principle: nine-point linear

Results and discussions

An HPLC-MS/MS method was used to determine concentrations of mesotrione in water containing the test item (A18032E together with adjuvant A12127R) and validated according to SANCO/3029/99 rev. 4.

The detector response was linear within the range 0.3 – 100 µg mesotrione/L. The method is highly specific with two mass transitions monitored per analysis.

The LOQ for the method was 0.1 mg/L test item (0.0156 mg mesotrione/L).
The retention time of mesotrione in solvent matched the retention time in test medium samples. No peak interferences occurred at the retention time of mesotrione.
The target analyte was not detected in any controls (n=2). No interference was observed at the retention time of the target analyte. All recovery data meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Well-labelled chromatograms are provided in the original study report.

Table A 44: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (mg/L)	Mean recovery (%)	RSD (%)	Comments
Water	Mesotrione	0.0156 (n=5)	86	1	-
		1.25 (n=5)	101	4	-

Table A 45: Characteristics for the analytical method used for validation of mesotrione residues in water

	Mesotrione
Specificity	HPLC-MS/MS is considered to be highly specific with two mass transitions which were monitored during each analysis. No interference was observed in any controls. Concentration of mesotrione in controls (blanks) was < 30% LOQ (n=2).
Calibration (type, number of data points)	Nine-point linear used for target analyte quantification and is presented in the study. The equation of the calibration curve is: y = -1199+30538.6x Individual calbration data are provided. r2 = > 0.997
Calibration range	0.3 – 100 µg/L
Limit of quantification (LOQ) Limit of detection (LOD)	0.0156 mg mesotrione/L 0.00468 mg mesotrione/L

Conclusion
The method detailed above was used to determine concentrations of mesotrione in water in support of an ecotoxicological study. The method detailed above was fully validated according to the requirements of SANCO/3029/99 rev. 4. This method is therefore acceptable for the determination of mesotrione in water.

A 2.2.1.5.4 Analytical method S12-02297

A 2.2.1.5.4.1 Method validation

Comments of zRMS:	The analytical method for the determination of mesotrione in water was fully validated with regard to recovery (accuracy), linearity of detector response, repeatability (precision) and specificity according to the guideline SANCO/3029/99 rev. 4, 11/07/2000. Mean recoveries and relative standard deviations per fortification were in the range 70-110% with ≤ 20% RSD). The limit of quantification was 1.0 µg/L test item (0.156 µg/L of mesotrione). The study is acceptable.

Reference: KCP 5.1.2.6/04

Report	Weber, K., 2012 Mesotrione/nicosulfuron/dicamba WG (A18032E) plus Adigor (A12127R) – Assessment of Toxic Effects on the duckweed *Lemna gibba* in a Semi-Static Test.. Project No.: S12-02297.
Guideline(s):	SANCO/3029/99 rev.4 11/07/00
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

A. Materials

1. Standards

Reference item:	Mesotrione
CAS No.:	104206-82-8
Purity:	99.9%
Lot/batch No.:	SZBB046XV
Expiry date:	15.02.2015
Standard for calibration:	As above.

2. Test item:	A18032E
Test item code:	2012-002761
CAS No.:	Mesotrione 104206-82-8 Dicamba 1918-00-9 Nicosulfuron 111991-09-4
Purity:	15.6% w/w mesotrione, 31.3% w/w dicamba and 10.1 % nicosulfuron
Lot/batch No.:	SMU2BP001
Expiry date:	30.09.2014

3. Adjuvant added to test item:	A12127R Adigor
Adjuvant code:	2012-001016
CAS No.:	68920-66-1; alcohols, C16-C18 and C18 unsaturated ethoxylated
Density:	925 kg/m^3
Appearance/colour:	Clear liquid/yellow
Purity:	27.1% w/w methyl oleate, 8.80% w/w methyl linoleate, 3.63% w/w methyl linolenate, 2.59% w/w methyl palmitate and 1.04% w/w methyl stearate
Lot/batch No.:	EEM0FA0610
Expiry date:	30.11.2015

4. Test medium:	Water

B. Sample preparation and processing

A stock solution of A18032E (1025 mg/L; additionally 2655 mg/L A12127R) was prepared in test medium. Dilutions of the stock solution (1 mg/L, 0.01 mg/L) based on A18032E were prepared in test medium and used for fortification of recovery samples.

Samples of the test medium were fortified with the test item (plus adjuvant) to provide recovery samples at two levels equivalent to 0.156 and 15.6 µg mesotrione/L. The samples were homogenised using a Vortex mixer, diluted with acetonitrile/water (1/1, v/v) if required and transferred to glass vials for analysis by HPLC-MS/MS using the instrumentation below.

C. Analytical instrumentation and analysis

1. HPLC parameters

Instrumentation:	Agilent 1290 infinity
Column:	Phenomenex Luna Phenyl-Hexyl, 150 mm x 2 mm i.d., 5 µm mean particle size (No. 00F-4257-B0) with 4 mm C 18 guard column
Injection volume:	20 µL
Column temp.:	40 °C
Mobile phase:	A: water + 0.1% acetic acid B: acetonitrile + 0.1% acetic acid

2. MS parameters

Instrumentation:	Applied Biosystems API5000	
Interface:	ESI	
Source polarity:	negative	
Spray voltage:	-4500 V	
Transitions monitored	1: m/z 338.2 → m/z 291.0	(quantification)
	2: m/z 338.2 → m/z 211.9	(confirmation)

D. Calibration

Principle:	Eight-point linear

Results and discussions

No peak interference was observed at the retention time of mesotrione. Target analyte concentrations in controls (blanks) were < 30% of the LOQ (n=2). The LOQ for the method was 1 µg test item/L (0.156 µg/L of mesotrione). No interference was observed at the retention time of the target analyte. All recovery data meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Well-labelled chromatograms are provided in the original study report.
An HPLC-MS/MS method was used to determine concentrations of mesotrione in water containing the test item (A18032E together with adjuvant A12127R) and validated according to SANCO/3029/99 rev. 4.
The detector response was linear within the range 0.02 – 10 µg mesotrione/L. The method is highly specific with two mass transitions monitored per analysis.

Table A 46: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (µg mesotrione /L)	Mean recovery (%)	RSD (%)	Comments
Water	Mesotrione	0.156 (n = 5)	98	5	-
		15.6 (n = 5)	98	10	-

Table A 47: Characteristics for the analytical method used for validation of mesotrione residues in water

	Mesotrione
Specificity	HPLC-MS/MS is considered to be highly specific with two mass transitions which were monitored during each analysis. No interference observed in controls. Concentration of mesotrione in controls (blanks) was < 30% LOQ (n=2).
Calibration (type, number of data points)	An eight-point calibration curve was used for target analyte quantification and is presented in the study. The regression equation of the calibration curve is: y = 7.23e+004x-63.3 r = 0.9996

	Mesotrione
Calibration range	0.02 – 10 µg reference item/L
Limit of quantification Limit of detection	LOQ: 0.156 µg mesotrione/L LOD: 0.0468 µg mesotrione/L

Conclusion
The method detailed above was fully validated according to the requirements of to SANCO/3029/99 rev. 4. This method is therefore acceptable for the determination of mesotrione in water.

A 2.2.1.5.5 Analytical method S12-02297

A 2.2.1.5.5.1 Method validation

Comments of zRMS:	The method validation was not fully performed according to the requirements of SANCO/3029/99 rev. 4. The method is not specific since detection was conducted solely at 254 nm. The target analyte was not detected in any controls (n=2). The LOQ for the method was 0.001 g/L of the test item.

Reference:	KCP 5.1.2.6/05
Report	Bramby-Gunary, J., 2013 Mesotrione/dicamba/nicosulfuron WG (A18032E) plus Adigor (A12127R) – Evaluation of the Phytotoxicity to Non Target Terrestrial Plant Seedling Emergence and Seedling Growth Test. Project No.: ACE-12-148.
Guideline(s):	Not SANCO guideline
Deviations:	No
GLP:	Yes
Acceptability:	No

Materials and methods

A. Materials

1. Standards

Reference item:	Mesotrione
CAS No.:	104206-82-8
Purity:	99.9%
Lot/batch No.:	SZBB046XV
Expiry date:	15.02.2015
Standard for calibration:	As above.

2. Test item: A18032E

CAS No.:	Mesotrione 104206-82-8 Dicamba 1918-00-9 Nicosulfuron 111991-09-4
Purity:	15.6% w/w mesotrione, 31.3% w/w dicamba and 10.1 % nicosulfuron
Appearance/colour:	Solid/beige
Lot/batch No.:	SMU2BP001
Expiry date:	30.09.2014

3. Adjuvant added to test item: A12127R Adigor

CAS No.:	68920-66-1; alcohols, C16-C18 and C18 unsaturated

	ethoxylated
Density:	925 kg/m^3
Appearance/colour:	Clear liquid/yellow
Purity:	27.1% w/w methyl oleate, 8.80% w/w methyl linoleate, 3.63% w/w methyl linolenate, 2.59% w/w methyl palmitate and 1.04% w/w methyl stearate
Lot/batch No.:	EEM0FA0610
Expiry date:	30.11.2015
4. Test medium:	Water

B. Sample preparation and processing

For the mesotrione analysis, the water samples were extracted by measuring 10mL of each homogenous sample into a 25mL volumetric flask. The volume for each was adjusted to just below 25mL with acetonitrile and then sonicated for 15 minutes. The samples were shaken and allowed to settle back to volume at room temperature. The samples were made to volume with acetonitrile, and then shaken and further diluted 1:2 with acetonitrile into vials, in order for the mesotrione concentration to be determined by HPLC.

1. HPLC parameters

Instrumentation:	Agilent HPLC
Column:	Varian Pursuit 5 (250mm x 4.6mm), packing C18 5µm
Injection volume:	5 µL
Flow rate:	1 mL/min
Mobile phase:	A: 40% acetonitrile B: 60% water at pH3 adjusted with formic acid

2. UV parameters

Detector	DAD
Detector wave length:	254 nm

D. Calibration

Principle: Nine-point calibration curve

Results and discussions

An HPLC-DAD (UV) method was used to determine concentrations of mesotrione in water samples from the phytotoxicity test. The detector response was linear within the range 0.0002 – 2 mg mesotrione/mL. The method is not specific since detection was conducted solely at 254 nm.

The target analyte was not detected in any controls (n=2). The LOQ for the method was 0.001 g/L of the test item.

Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (g mesotrione /L)	Mean Recovery (%)	RSD (%)	Comments
Water	Mesotrione	0.001 (n = 2)	91.69	-	-
		4.5 (n = 2)	100.2	-	-

Table A 48: Characteristics for the analytical method used for validation of mesotrione residues in water

	Mesotrione
Specificity	Not provided
Calibration (type, number of data points)	A nine-point calibration curve was used for target analyte quantification and is presented in the study. The equation of the calibration curve is: y = 8e-05x-0.0012 Individual calbration data are provided. $r^2 = 1$
Calibration range	0.0002 – 2 mg mesotrione/mL
Limit of quantification (LOQ)	0.001 g mesotrione/L

Conclusion
The validation of the method detailed above was not fully provided.

A 2.2.1.5.6 Analytical method S12-02297

A 2.2.1.5.6.1 Method validation

Comments of zRMS:	The method validation was not fully performed according to the requirements of SANCO/3029/99 rev. 4. The method is not specific since detection was conducted solely at 254 nm. The target analyte was not detected in any controls (n=2). The LOQ for the method was 0.001 g/L of the test item.

Reference:	KCP 5.1.2.6/06
Report	Bramby-Gunary, J., 2013[a] Mesotrione/dicamba/nicosulfuron WG (A18032E) plus A12127R (Adigor adjuvant) – Evaluation of the Phytotoxicity to Non Target Terrestrial Plant vegetative Vigour Test. Project No.: ACE-12-149.
Guideline(s):	Not SANCO guideline
Deviations:	No
GLP:	Yes
Acceptability:	No

Materials and methods
A. Materials
1. Standards

Reference item:	Mesotrione
CAS No.:	104206-82-8
Purity:	99.9%
Lot/batch No.:	SZBB046XV
Expiry date:	15.02.2015
Standard for calibration:	As above.

2. Test item: A18032E

CAS No.:	Mesotrione 104206-82-8 Dicamba 1918-00-9 Nicosulfuron 111991-09-4

Purity:	15.6% w/w mesotrione, 31.3% w/w dicamba and 10.1 % nicosulfuron
Appearance/colour:	Solid/beige
Lot/batch No.:	SMU2BP001
Expiry date:	30.09.2014

3. Adjuvant added to test item: A12127R Adigor

CAS No.:	68920-66-1; alcohols, C16-C18 and C18 unsaturated ethoxylated
Density:	925 kg/m^3
Appearance/colour:	Clear liquid/yellow
Purity:	27.1% w/w methyl oleate, 8.80% w/w methyl linoleate, 3.63% w/w methyl linolenate, 2.59% w/w methyl palmitate and 1.04% w/w methyl stearate
Lot/batch No.:	EEM0FA0610
Expiry date:	30.11.2015

4. Test medium: Water

B. Sample preparation and processing

For the mesotrione analysis, the water samples were extracted by measuring 10mL of each homogenous sample into a 25mL volumetric flask. The volume for each was adjusted to just below 25mL with acetonitrile and then sonicated for 15 minutes. The samples were shaken and allowed to settle back to volume at room temperature. The samples were made to volume with acetonitrile, and then shaken and further diluted 1:2 with acetonitrile into vials, in order for the mesotrione concentration to be determined by HPLC.

1. HPLC parameters

Instrumentation:	Agilent HPLC
Column:	Varian Pursuit 5 (250mm x 4.6mm), packing C18 5µm
Injection volume:	5 µL
Flow rate:	1 mL/min
Mobile phase:	A: 40% acetonitrile B: 60% water at pH3 adjusted with formic acid

2. UV parameters

Detector	DAD
Detector wave length:	254 nm

D. Calibration

Principle: Nine-point calibration curve

Results and discussions

An HPLC-DAD (UV) method was used to determine concentrations of mesotrione in water samples from the phytotoxicity test. The detector response was linear within the range 0.0002 – 2 mg mesotrione/mL. The method is not specific since detection was conducted solely at 254 nm.

The target analyte was not detected in any controls (n=2). The LOQ for the method was 0.001 g/L of the test item.

Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (g mesotrione /L)	Mean Recovery (%)	RSD (%)	Comments
Water	Mesotrione	0.001 (n = 2)	93.47	-	-

Matrix	Analyte	Fortification level (g mesotrione /L)	Mean Recovery (%)	RSD (%)	Comments
		4.5 (n = 2)	100.7	-	-

Table A 49: Characteristics for the analytical method used for validation of mesotrione residues in water

	Mesotrione
Specificity	Concentration of mesotrione in controls (blanks) was < 30% LOQ (n=2).
Calibration (type, number of data points)	A nine-point calibration curve was used for target analyte quantification and is presented in the study. The equation of the calibration curve is: y = 8e-05x+0.0004 Individual calbration data are provided. $r^2 = 1$
Calibration range	0 – 2 mg mesotrione/mL
Limit of quantification (LOQ)	<0.001 g mesotrione/L

Conclusion
The validation of the method detailed above was not fully provided.

A 1.1.1.1.1 Analytical method 105731240A

A 1.1.1.1.1.1 Method validation

Comments of zRMS:	The analytical method 105731240A for the determination of mesotrione in test water supplemented with AAP medium was successfully validated according to SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was 1 µg test item/L. The method is highly specific with two mass transitions monitored per analysis. The study is acceptable.

Reference:	KCP 5.1.2.6/07
Report	Hengsberger, A & Wydra, V. 2015 Mesotrione wet paste (ZA1296) - Toxicity to aquatic plant Lemna gibba in a reciprocal growth inhibition test.. Report No. 105731240
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

A. Materials
1. Standards

Reference item:	Mesotrione
CAS No.:	104206-82-8
Purity:	99.5% ± 0.5% (wt/wt)

Lot/batch No.:	492970
Expiry date:	Recertification Feb 2016
Standard for calibration:	As above.

2. Test item

Name:	Mesotrione wet paste (ZA1296)
Active ingredient:	Mesotrione 86.1% (wt/wt)
Batch No.:	631795 (SMO7F333)
Expiry data:	Recertification Feb 2016

3. Test medium:	Water (containing AAP-growth medium)

B. Sample preparation and processing

Stock solutions were prepared by diluting the test item in acetonitrile. Fortified samples were prepared by further diluting the stock solutions with the test medium to provide fortified samples at 1, 5 and 75 µg test item/L. The fortified samples were homogenised, diluted with acetonitrile by a factor of two and analysed by HPLC-MS/MS using the parameters detailed below.

C. Analytical instrumentation and analysis

1. HPLC parameters

Instrumentation:	Agilent Series 1200
Column:	Synergi 4µ Polar RP 80A (150 × 3 mm)
Column temp.:	20 °C
Mobile phase:	A: 40% HPLC water containing 5mM ammonium acetate B: 60% Methanol containing 5mM ammonium acetate

2. MS parameters

Instrumentation:	API 4000 Mass spectrometer
Interface:	ESI
Source polarity:	positive
Spray voltage:	4000 V
Transitions monitored	1: m/z 357.071 → m/z 228.000 (quantification) 2: m/z 357.071 → m/z 104.000 (confirmation)

D. Calibration

Principle:	Seven-point linear

Results and discussions

An HPLC-MS/MS method was used to determine concentrations of Mesotrione in water supplemented with AAP-growth medium and validated according to SANCO/3029/99 rev. 4. The detector response was linear within the range 0.25 – 40 µg mesotrione/L. The method is highly specific with two mass transitions monitored per analysis. No interference was observed at the retention time of the target analyte. Target analyte concentrations in controls (blanks) were < 30% of the LOQ. The LOQ for the method was 1 µg test item/L. All recovery data meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Well-labelled chromatograms are provided in the original study report.

Table A 50: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (ug test item/L)	Mean recovery (%)	RSD (%)	Comments
Water	Mesotrione	1 (n = 5)	107	5	-
		5 (n = 5)	114	2	-

Matrix	Analyte	Fortification level (ug test item/L)	Mean recovery (%)	RSD (%)	Comments
		75 (n = 5)	105	2	-

Table A 51: Characteristics for the analytical method used for validation of mesotrione residues in water supplemented with APPAAP -medium

	Mesotrione
Specificity	Two mass transitions were monitored during each analysis. No interference observed in controls Concentration of mesotrione in controls (blanks) was < 30% LOQ.
Calibration (type, number of data points)	A seven-point linear calibration curve was used for target analyte quantification and is presented in the study. The equation of the calibration curve is: y = 2597x - 474 r = > 0.999
Calibration range	0.25 – 40 µg reference item/L
Limit of quantification Limit of detection	LOQ: 1 µg test item/L LOD: 0.15 µg test item/L

Conclusion
The method detailed above was fully validated according to the requirements of to SANCO/3029/99 rev. 4. This method is therefore acceptable for the determination of mesotrione in water supplemented with APPAAP -medium.

A 1.1.1.1.2 Analytical method 105732240A

A 1.1.1.1.2.1 Method validation

In the following study the method validated above (105731240A) was used for analysis of mesotrione in water.

Comments of zRMS:	The analytical method 105732240A for the determination of mesotrione in test water supplemented with AAP medium was successfully validated according to SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was 1 µg test item/L. The study is acceptable.

Reference:	KCP 5.1.2.6/08
Report	Kosak, L & Wydra, V. 2016 Mesotrione wet paste (ZA1296) - Toxicity to aquatic plant Lemna gibba in a semi-static growth inhibition test with subsequent recovery period. Final report Amendment 2. Report No. 105732240
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

A. Materials

1. Standards

Reference item:	Mesotrione
CAS No.:	104206-82-8
Purity:	99.5% ± 0.5% (wt/wt)
Lot/batch No.:	492970
Expiry date:	Recertification Feb 2016
Standard for calibration:	As above.

2. Test item

Name:	Mesotrione wet paste (ZA1296)
Active ingredient:	Mesotrione 86.1% (wt/wt)
Batch No.:	631795 (SMO7F333)
Expiry data:	Recertification Feb 2016

3. Test medium:	Water (containing AAP-growth medium)

B. Sample preparation and processing

Stock solutions were prepared by diluting the test item in acetonitrile. Fortified samples were prepared by further diluting the stock solutions with the test medium to provide fortified samples at 1, 5 and 75µg test item/L. The fortified samples were homogenised, diluted with acetonitrile by a factor of two and analysed by HPLC-MS/MS using the parameters detailed below.

C. Analytical instrumentation and analysis

1. HPLC parameters

Instrumentation:	Agilent Series 1200
Column:	Synergi 4µ Polar RP 80A (150 × 3 mm)
Column temp.:	20 °C
Mobile phase:	A: 40% HPLC water containing 5mM ammonium acetate B: 60% Methanol containing 5mM ammonium acetate

2. MS parameters

Instrumentation:	API 4000 Mass spectrometer
Interface:	ESI
Source polarity:	positive
Spray voltage:	4000 V
Transitions monitored	1: m/z 357.071 → m/z 228.000 (quantification)

D. Calibration

Principle:	Nine-point linear

Results and discussions

An HPLC-MS/MS method was used to determine concentrations of Mesotrione in water supplemented with AAP-growth medium and validated according to SANCO/3029/99 rev. 4. The detector response was linear within the range 0.25 – 40 µg mesotrione/L. No interference was observed at the retention time of the target analyte. Target analyte concentrations in controls (blanks) were < 30% of the LOQ. The LOQ for the method was 1 µg test item/L. All recovery data meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Well-labelled chromatograms are provided in the original study report.

Table A 52: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (ug test item/L)	Mean recovery (%)	RSD (%)	Comments
Water	Mesotrione	1 (n = 5)	107	5	-
		5 (n = 5)	105	6	-
		75 (n = 5)	104	5	-

Table A 53: Characteristics for the analytical method used for validation of mesotrione residues in water supplemented with APPAAP -medium

	Mesotrione
Specificity	Only one mass transition was measured in this report, but confimation was done under: 105731240A No interference observed in controls Concentration of mesotrione in controls (blanks) was < 30% LOQ.
Calibration (type, number of data points)	A nine-point linear calibration curve was used for target analyte quantification and is presented in the study. The equation of the calibration curve is: y = 2450x +109 r = 1.00
Calibration range	0.25 – 40 µg reference item/L
Limit of quantification Limit of detection	LOQ: 1 µg test item/L LOD: 0.15 µg test item/L

Conclusion
The method detailed above was fully validated according to the requirements of to SANCO/3029/99 rev. 4. This method is therefore acceptable for the determination of mesotrione in water supplemented with APPAAP -medium.

A 1.1.1.1.3 Analytical method S16-06273

A 1.1.1.1.4 Method validation

Comments of zRMS:	The analytical method S16-06273 for the determination of mesotrione in water supplemented with modified Andrews solution was successfully validated according to SANCO/3029/99 rev. 4. The limit of quantification (LOQ) of the analytical method was 0.4 µg test item/L. The study has been accepted.

Reference:	KCP 5.1.2.6/09
Report	Gonsior, G. 2017 Mesotrione - Growth inhibition of Myrophyllum spicatum in a water/sediment system: Final Report Amendment 1. Report No. S16-06273
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

A. Materials

1. Standards

Reference item:	Mesotrione technical
CAS No.:	104206-82-8
Purity:	84.6% (wt/wt)
Lot/batch No.:	675385
Expiry date:	Recertification Feb 2019
Standard for calibration:	As above.
2. Test item	As above
3. Test medium:	Water supplemented with modified Andrews solution

B. Sample preparation and processing

Stock solutions were prepared by diluting the test item in methanol. Validation samples were prepared from diluted stock solutions to provide fortified samples at 0.4 and 800 µg test item/L and frozen below -18 °C until required for analysis. Frozen fortified (10 ml) samples were thawed, 10 ml acetonitrile were added to each sample and the samples were vortexed. If necessary, samples were further diluted with acetonitrile/water (1:1, v/v) and analysed by HPLC-MS/MS using the parameters detailed below.

C. Analytical instrumentation and analysis

1. HPLC parameters

Instrumentation:	Shimadzu LC-30 AD
Column:	Phenomenex Luna 5µ phenyl-hexyl, 150 mm × 2 mm i.d., 5 µm mean particle size and fitted with a 4 mm guard column.
Column temp.:	30 °C
Mobile phase:	A: Water + 0.5% formic acid B: Methanol + 0.5% formic acid

2. MS parameters

Instrumentation:	Applied Biosystems API 5500 Mass spectrometer
Interface:	ESI
Source polarity:	negative
Spray voltage:	-3500 V
Transitions monitored	1: m/z 337.845 → m/z 291.0 (quantification) 2: m/z 337.8451 → m/z 212.00 (confirmation)

D. Calibration

Principle:	Eight-point curvilinear

Results and discussions

An HPLC-MS/MS method was used to determine concentrations of Mesotrione in water supplemented with modified Andrews solution and validated according to SANCO/3029/99 rev. 4. The method is highly specific with two mass transitions monitored per analysis. Residues were not detectable in untreated test medium controls (n = 2). The LOQ for the method was 0.4 µg test item/L. All recovery data meet the requirements of SANCO/3029/99 rev. 4 and are provided along with other validation data in the tables below. Well-labelled chromatograms are provided in the original study report.

Table A 54: Recovery results from method validation of mesotrione using the analytical method

Matrix	Analyte	Fortification level (ug/L)	Mean recovery (%)	RSD (%)	Comments
Water	Mesotrione	0.4 (n = 5)	93	3	-
		800 (n = 5)	94	2	-

Table A 55: Characteristics for the analytical method used for validation of mesotrione residues in water supplemented with modified Andrews solution

	Mesotrione
Specificity	Two mass transitions were monitored during each analysis. No interference observed in controls Concentration of mesotrione in controls (blanks) was < 30% LOQ.
Calibration (type, number of data points)	An eight-point curvilinear calibration curve was used for target analyte quantification and is presented in the study. The equation of the calibration curve is: y = y = -9.36e+004x^2 + 2.32e+006x + 3.74e+004 r = > 0.999
Calibration range	0.05 – 10 ng mesotrione/L
Limit of quantification Limit of detection	LOQ: 0.4 µg test item/L LOD: 0.120 µg test item/L

Conclusion
The method detailed above was fully validated according to the requirements of to SANCO/3029/99 rev. 4. This method is therefore acceptable for the determination of mesotrione in water supplemented with modified Andrews solution.

A 1.1.1.1.5 Analytical method GRM007.11A

A 1.1.1.1.5.1 Method validation

Comments of zRMS:	The analytical method GRM007.11A for the determination of mesotrione and its metabolites MNBA in maize has been demonstrated to be satisfactory in terms of specificity, linearity, precision and accuracy according to the guideline SANCO/3029/99 rev. 4, 11/07/2000. The limit of quantification (LOQ) of the analytical method was 0.01 mg test item/kg. The study has been accepted.

Reference:	KCP 5.1.2.6/10
Report	North. L., 2016 Mesotrione – Foliage Decline Study with A12739A on Maize in Northern France and the United Kingdom in 2015. Report No. S15-02057
Guideline(s):	SANCO/3029/99 revision 4 (11 Jul 2000)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

A. Materials
1. Standards

Reference item:	Mesotrione
CAS No.:	104206-82-8
Purity:	99.5% (wt/wt)
Lot/batch No.:	492970
Expiry date:	Feb 2016
Standard for calibration:	As above.

Reference item:	MNBA
CAS No.:	434935-69-0
Purity:	99.9% (wt/wt)
Lot/batch No.:	454319
Expiry date:	Feb 2016
Standard for calibration:	As above.

2. Test item

Name:	Mesotrione wet paste (ZA1296)
Active ingredient:	Mesotrione 99.3 (g/L)
Batch No.:	SAV5A15007
Expiry data:	March 2018

3. Test medium: Maize whole plant

B. Sample preparation and processing

Samples are extracted with acetonitrile:water (50:50 v/v) after addition of sodium chloride. Aliquots are diluted with ultra-pure water. The fortified samples were homogenised, diluted with acetonitrile by a factor of two and analysed and concentrated using an Oasis® HLB solid phase extraction (SPE) cartridge on which residues of mesotrione and MNBA are retained. Mesotrione and MNBA are eluted using a solution of methanol containing 2% formic acid. Samples are evaporated under a stream of dry air and dissolved in ultra-pure water:methanol (90:10 v/v). Final determination is by high performance liquid chromatography with tandem mass spectrometric detection (HPLC-MS-MS).

C. Analytical instrumentation and analysis

1. HPLC parameters

Instrumentation:	Agilent Series 1200
Column:	Synergi 4µ Polar RP 80A (50 × 20 mm)
Column temp.:	20 °C
Mobile phase:	A: 0.1% Formic acid in ultra pure water B: 0.1% Formic acid in acetonitrile

2. MS parameters

Instrumentation:	API 5500 Mass spectrometer	
Transitions monitored	1: m/z 338.1 → m/z 291.000	(Mesotrione)
	1: m/z 224.0 → m/z 141.900	(MNBA)

D. Calibration

Principle: Eight-point linear

Results and discussions

An HPLC-MS/MS method was used to determine concentrations of Mesotrione and MNBA. The analytical method in support of this ecotoxicological study is GRM007.11.A. The method was already evaluated by the authority and published in the RAR UK, 2015 ; therefore in the study itself, only procedural recoveries and calibration data are provided and reference to the original method validation is made : Watson G, Crook S (2013). « Mesotrione - Analytical Method (GRM007.11A) for the Determination of Residues of Mesotrione and 4-(Methylsulfonyl)-2-Nitrobenzoic Acid (MNBA) in Crop Matrices by LC-MS/MS.

Syngenta Method GRM007.11A ». The detector response was linear within the range 0.003 – 0.02 µg/mL for both active substance and metabolite . The LOQ for the method was 0.01 mg test item/kg. Well-labelled chromatograms are provided in the original study report.

Table A 56: Procedural recovery data for mesotrione and MNBA

Matrix	Analyte	Fortification level (mg test item/kg)	Mean recovery (%)	RSD (%)	Comments
Maize whole plant	Mesotrione	0.01 (n = 4)	81	11	-
		10 (n = 3)			-
		15 (n = 1)			-
Maize whole plant	MNBA	0.01 (n = 3)	78	11	
		10 (n = 2)			
		15 (n = 1)			

Table A 57: Characteristics for the analytical method used for validation of mesotrione and MNBA residues in Maize whole plant

	Mesotrione and MNBA
Calibration (type, number of data points)	A eight-point linear calibration curve was used for target analytes quantification and is presented in the study. The equation of the calibration curve is: Mesotrione : y = 360213682 x – 15714 r = 0.9961 MNBA : y = 40358946 x + 1139 r = 0.9999
Calibration range	0.003 – 0.0200 µg reference items/mL
Limit of quantification	LOQ: 0.01 mg test items/kg

Conclusion

The method GRM007.11.A was fully validated according to the requirements of to SANCO/3029/99 rev. 4 in the original study Watson G, Crook S (2013). Procedural recoveries are provided in the study summarised above and the original method is therefore acceptable for the determination of mesotrione in plant matrices.

A 2.2.1.6 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 2.2.2 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 5.2.1)

No new data have been submitted in the framework of this application.

A 2.2.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2.2)

No new data have been submitted in the framework of this application.

A 2.2.2.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2.3)

No new data have been submitted in the framework of this application.

A 2.2.2.4 Description of Methods for the Analysis of Soil (KCP 5.2.4)

No new or additional studies have been submitted

A 2.2.2.5 Description of Methods for the Analysis of Water (KCP 5.2.5)

No new or additional studies have been submitted

A 2.2.2.6 Description of Methods for the Analysis of Air (KCP 5.2.6)

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 nicosulfuron

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

A 2.3.1.1 Description of analytical methods for the determination of residues in support of environmental fate studies (KCP 5.1.2.1)

Additional studies have been conducted and a summary presented below:

A 2.3.1.1.1 DuPont 28685

Comments of zRMS:	DuPont Method 12059 was adapted to determine the concentration of nicosulfuron and its metabolites IN-37740, IN-V9367, IN-J0290, IN-HYY21, IN-GDC42 and IN-64859 in groundwater. The analytical method for the determination of nicosulfuron and its metabolites in groundwater has been demonstrated to be satisfactory in terms of specificity, linearity, precision and accuracy according to the guideline SANCO/3029/99 rev. 4. The mean recoveries were within 70-110%, with a RSD < 20% (for the second ion transition (confirmatory method) too). The limit of quantification for nicosulfuron (DPX-V9360) was 0.05 µg/L. The limit of quantification for the metabolites ASDM (IN-V9367), UCSN (IN-GDC42), AUSN (IN-HYY21), HMUD (IN-37740), ADMP (IN-J0290) and MU-466 (IN-64859) was 0.1 µg/L. The study is acceptable.

Reference:	KCP 5.1.2.1 (report available from data owner)
Report	Schneider M., Holzer S., 2016 Groundwater Monitoring for Nicosulfuron and Six Metabolites in Four Representative Regions in Germany , Report: 28685,
Guideline(s):	SANCO/825/00 rev.7 which is also complying with SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Groundwater samples were acidified with formic acid and were analyzed after injection by means of high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).
The groundwater of a deep well, which was not contaminated, was collected by SGS INSTITUT FRESENIUS GmbH as the substrate for validation and frozen storage stability experiments prior to the analytical phase.

HPLC-MS/MS Analysis parameters:

Instrument: model API 5500 Triple Quad – Applied Biosystems
Column: Zorbax®Eclipse XDB Phenyl, 150 mm x 4.6 mm, 3.5 µm
Solvent A: 0.1 mmol/L of formic acid in 0.1 mmol/L of ammonium formiate in water
Solvent B: Methanol ("Optigrade")

Time (min.)	%A	%B	Flow [µL/min]
0.0	95	5	800
4.0	50	50	800
4.1	35	65	800
7.5	5	95	800
12.0	5	95	800
12.5	95	5	1000
14.0	95	5	1000
14.1	95	5	800
15.0	95	5	800

Injection volume: 20µL
Column temperature: 40ºC
Interface: ESI
Polarity: Positive

Compound	Parent (m/z)	Fragment ions (m/z)	Remark
Nicosulfuron	411	213	Qualifier
	411	182	Quantifier
HMUD	397	213	Qualifier
	397	106	Quantifier
ASDM	230	78	Qualifier
	230	106	Quantifier
ADMP	156	57	Qualifier
	156	100	Quantifier
AUSN	315	213	Qualifier
	315	86	Quantifier
UCSN	316	106	Qualifier
	316	213	Quantifier
MU-466	216	135	Qualifier
	216	108	Quantifier

Discussion

Linearity: The calibration was performed using standards in the range of 0.02 – 0.8 ng/mL, for nicosulfuron, and in the range of 003 – 0.8 ng/mL for its metabolites, which is covering a concentration range from the LOQ to 10 x LOQ ± at least 20%. Typical calibration curve and coefficient of correlation are given in the tables below:

	Primary method		Confirmatory method	
Compounds	**Calibration curve**	**R^2**	**Calibration curve**	**R^2**
Nicosulfuron (DPX-V9360)	y = 282.6 + 2001284.46 x	0.99928	y = 161 + 5098174.87 x	0.99952
HMUD (IN-37740)	y = -19320 + 165433089.8 x	0.99780	y = -12905 + 115121735.5 x	0.99662
ASDM (IN-V9367)	y = -10120 + 77178169.34 x	0.99688	y = - 24217 + 122271844.3 x	0.99861

UCSN (IN-GDC42)	y = -233.2 + 5405892.79 x	0.99981	y = -589.6 + 7397323.33 x	0.99978
AUSN (IN-HYY21)	y = -49151 + 139771449.4 x	0.99654	y = -54923 + 231240558.2 x	0.99809
MU-466 (IN-64859)	y = 2365 + 29376772.81 x	0.99879	y = 334.3 + 25839460.21 x	0.99952
ADMP (IN-J0290)	y = -33505 + 245752488 x	0.99955	y = 12287 + 789419180.5 x	0.99911

Specificity: A highly specific detection system was used (MS/MS). The retention time of the analytes in solvent matched the retention time in the soil samples. No peak interferences occurred at the retention time of the analytes above 30% of the LOQ. The analytical method can be therefore regarded as highly specific for nicosulfuron, and its metabolites (HMUD, ASDM, UCSN, AUSN, MU-466 and ADMP).

Accuracy and Precision: The mean recoveries, standard deviations and relative standard deviations were within the SANCO requirements (mean recoveries within 70-110%, with a RSD < 20%).

Confirmatory method: The mean recoveries, standard deviations and relative standard deviations were within the SANCO requirements (mean recoveries within 70-110%, with a RSD < 20%) for the second ion transition (confirmatory method)

Summary of accuracy and precision results in groundwater (primary and confirmatory method)

	Fortification Recovery Summary							
	0.1 µg/L (n = 5)			**1.0 µg/L (n = 5)**			**Overall (n=10)**	
Analytes	**Mean %**	**%RSD**	**Range %**	**Mean %**	**%RSD**	**Range %**	**Mean %**	**%RSD**
HMUD (IN-37740)								
397/213	102	1.5	101-104	99	1.5	97-100	100	2.4
397/106	101	1.9	99-103	100	1.7	98-102	100	1.9
MU-466 (IN-64859)								
216/135	97	4.4	93-103	97	3.5	96-104	97	3.8
216/108	99	3.5	93-101	97	2.	95-99	98	3.1
UCSN (IN-GDC42)								
316/106	101	3.9	95-106	104	1.6	103-107	103	3.3
316/213	104	1.1	102-105	104	0.8	103-105	104	0.9
AUSN (IN-HYY21)								
315/213	106	1.7	104-109	96	2.0	94-98	101	5.6
315/86	104	1.2	102-105	99	2.0	95-101	101	3.1
ADMP (IN-J0290)								
156/57	99	1.1	98-100	97	1.7	95-99	98	1.6
156/100	96	0.9	95-97	100	1.9	97-101	98	2.4
ASDM (IN-V9367)								
230/78	103	2.6	100-106	99	1.4	97-100	101	3.0
230/106	104	0.4	104-105	97	2.2	96-101	101	4.0
Nicosulfuron (DPX-V9360)								
411/213	94	1.3	93-96	97	1.7	95-99	96	2.1
411/182	92	1.6	91-94	98	1.5	96-100	95	3.7

	Fortification Recovery Summary		
	0.05 µg/L (n = 5)		
Analytes	**Mean %**	**%RSD**	**Range %**
Nicosulfuron (DPX-V9360)			
411/213	97	4.9	92-104
411/182	97	4.9	98-109

Limit of Quantification (LOQ): The limit of quantification (LOQ) for nicosulfuron (DPX-V9360) was 0.05 µg/L since this was the lowest validated levels. The limit of quantification (LOQ) for the metabolites ASDM (IN-V9367), UCSN (IN-GDC42), AUSN (IN-HYY21), HMUD (IN-37740), ADMP (IN-J0290) and MU-466 (IN-64859) was 0.1 µg/L.

Conclusion:
The method was successfully validated following SANCO/3029/99 rev 4. and is suitable for the determination of residues of nicosulfuron, and its metabolites (HMUD, ASDM, UCSN, AUSN, MU-466 and ADMP).

A 2.3.1.1.1.1 Confirmatory method

Additional confirmatory analysis is not required as the primary method is a highly specific method (LC-MS), with an analysis using 2 transitions.

A 2.3.1.1.2 Analytical method DuPont 40798

A 2.3.1.1.2.1 Method validation

Comments of zRMS:	The analytical method for the determination of nicosulfuron and its 6 metabolites in groundwater has been demonstrated to be satisfactory in terms of specificity, linearity, precision and accuracy according to the guideline SANCO/3029/99 rev. 4. The mean recoveries were within 70-110%, with a RSD <20% (for the second ion transition (confirmatory method) too). The limit of quantification for nicosulfuron (DPX-V9360) was 0.05 µg/L. The limit of quantification for the metabolites ASDM (IN-V9367), UCSN (IN-GDC42), AUSN (IN-HYY21), HMUD (IN-37740), ADMP (IN-J0290) and MU-466 (IN-64859) was 0.1 µg/L. The study is acceptable.

Reference:	KCP 5.1.2.1 (report available from data owner)
Report	Ferrari F., 2016 Groundwater Monitoring for nicosulfuron and 6 Metabolites in Maize Growing Regions of Italy, Report: 40798,
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No

Materials and methods
Water samples, after filtration at 0.45 mm, were acidified adding 25 mL of a 4% formic acid solution in water for every mL of water sample. The samples were then analyzed using LC-MS/MS.
Groundwater samples were analysed at "LABCAM s.r.l. – Centro di Saggio" (Test Facility) using a verified analytical method.

HPLC-MS/MS Analysis parameters:

Instrument:	Liquid chromatograph with mass spectrometer triple quadrupole (LC-MS/MS) - Thermo Accela Pump &Autosampler + TSQ Quantum Access
Column:	Zorbax®Eclipse XDB Phenyl, 150 mm x 4.6 mm, 3.5 µm
Interface:	ESI
Polarity:	Positive

Compound	Parent (m/z)	Fragment ions (m/z)	Remark
Nicosulfuron	411	182	Qualifier
	411	106	Quantifier
HMUD	397	168	Qualifier
	397	106	Quantifier
ASDM	230	106	Qualifier
	230	213	Quantifier
ADMP	156	57	Qualifier

	156	124	Quantifier
AUSN	315	213	Qualifier
	315	106	Quantifier
UCSN	316	213	Qualifier
	316	106	Quantifier
MU-466	216	108	Qualifier
	216	135	Quantifier

Discussion

Linearity: The calibration was performed using standards in the range of 0.02 – 1.5 µg/L for nicosulfuron and of 0.03 – 1.5 µg/L for its metabolites, which is covering a concentration range from the LOQ to 10 x LOQ ± at least 20%. Typical calibration curve and coefficient of correlation are given in the tables below:

	Primary method		**Confirmatory method**	
Compounds	**Calibration curve**	**R^2**	**Calibration curve**	**R^2**
Nicosulfuron (DPX-V9360)	y = 2958935 x + 4228	1.000	y = 592215 x + 6570	1.000
HMUD (IN-37740)	y = 515201 x - 11999	1.000	y = 709388 x + 8291	1.000
ASDM (IN-V9367)	y = 822736 x + 14916	0.998	y = 2496868 x - 8883	0.999
UCSN (IN-GDC42)	y = 653584 x + 16969	0.997	y = 255551 x + 692	1.000
AUSN (IN-HYY21)	y = 2761819 x + 106622	0.994	y = 828731 x + 31049	0.996
MU-466 (IN-64859)	y = 134080 x + 1043	1.000	y = 325349 x + 1110	0.999
ADMP (IN-J0290)	y = 168741 x - 3173	1.000	y = 161390 x - 4737	1.000

Specificity: A highly specific detection system was used (MS/MS). The retention time of the analytes in solvent matched the retention time in the soil samples. No peak interferences occurred at the retention time of the analytes above 30% of the LOQ. The analytical method can be therefore regarded as highly specific for nicosulfuron, and its metabolites (HMUD, ASDM, UCSN, AUSN, MU-466 and ADMP).

Accuracy and Precision: The mean recoveries, standard deviations and relative standard deviations were within the SANCO requirements (mean recoveries within 70-110%, with a RSD < 20%).

Confirmatory method: The mean recoveries, standard deviations and relative standard deviations were within the SANCO requirements (mean recoveries within 70-110%, with a RSD < 20%) for the second ion transition (confirmatory method

Summary of accuracy and precision results in groundwater (primary and confirmatory method)

	Fortification Recovery Summary			
	0.1 µg/L (n = 5)		**1.0 µg/L (n = 5)**	
Analytes	**Mean %**	**%RSD**	**Mean %**	**%RSD**
HMUD (IN-37740)				
397/213	96	6	84	6
397/106	101	5	92	7
MU-466 (IN-64859)				
216/135	100	9	97	7
216/108	91	8	95	7
UCSN (IN-GDC42)				
316/106	84	5	104	2
316/213	98	6	87	5
AUSN (IN-HYY21)				
315/213	90	11	90	3
315/86	103	6	93	4
ADMP (IN-J0290)				
156/57	97	8	85	4
156/100	99	11	90	11

ASDM (IN-V9367)				
230/78	101	9	102	4
230/106	104	5	94	6
Spike level	**0.05 µg/L (n = 5)**		**0.5 µg/L (n = 5)**	
Nicosulfuron (DPX-V9360)				
411/213	107	7	109	4
411/182	85	17	110	4

Limit of Quantification (LOQ): The limit of quantification (LOQ) for nicosulfuron (DPX-V9360) was 0.05 µg/L since this was the lowest validated levels. The limit of quantification (LOQ) for the metabolites ASDM (IN-V9367), UCSN (IN-GDC42), AUSN (IN-HYY21), HMUD (IN-37740), ADMP (IN-J0290) and MU-466 (IN-64859) was 0.1 µg/L.

Conclusion:
The method was successfully validated following SANCO/3029/99 rev 4. and is suitable for the determination of residues of nicosulfuron, and its metabolites (HMUD, ASDM, UCSN, AUSN, MU-466 and ADMP).

A 2.3.1.1.2.2 Confirmatory method

Additional confirmatory analysis is not required as the primary method is a highly specific method (LC-MS), with an analysis using 2 transitions.

A 2.3.1.2 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 2.3.1.3 Description of analytical methods for the determination of residues in support of residues studies (KCP 5.1.2.5)

A 2.3.1.3.1 Method GRM074.01A

Comments of zRMS:	Analytical method GRM074.01A is suitable for the determination of nicosulfuron in plant matrices. The method has been validated on maize kernels (dry commodity) and maize whole plant (high water commodity). The limit of quantification (LOQ) of the method has been established at 0.01 mg/kg. The method validation data are reported in Battelle Report No. NC/15/022 (Andrews, G (2016), Syngenta Report No. TK0258007-REG) and a summary is included below.

Reference:	KCP 5.1.2.5/08
Report	Crook, S. & Andrews, G., 2016 Nicosulfuron - Analytical Method GRM074.01A for the Determination of Nicosulfuron in Plant Matrices Syngenta Report No. GRM074.01A; Syngenta File No. ASF628_11278
Guideline(s):	EC SANCO/3029/99 rev 4 (2000) EC SANCO/825/00 rev 8.1 (2010)
Deviations:	Yes; Method is not proposed for post authorisation control – Independent validation according to EC SANCO/825/00 rev 8.1 (2010) has not been conducted
GLP:	Yes
Acceptability:	Yes

Principle of the method
Specimen material is extracted by homogenisation with a mixture of acetonitrile and hydrochloric acid (HCl) followed by a second extraction by homogenisation with acetonitrile only. An aliquot is taken and evaporated to dryness. The sample is dissolved in sodium chloride solution and the pH adjusted to >10 with 1 M sodium hydroxide (NaOH) solution and partitioned with dichloromethane. The aqueous layer is retained and the sample acidified with 6 M HCl solution. The acidified sample is then partitioned with dichloromethane. Combined dichloromethane extracts are evaporated to dryness and reconstituted in acetonitrile/ultra-pure water (30/70 v/v) prior to final determination by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) monitoring for the primary transition (*m/z* 411 -182) and the confirmatory transition (*m/z* 411 - 213). The limit of quantification of the method is 0.01 mg/kg.

A 2.3.1.3.1.1 Method validation GRM074.01A

Comments of zRMS:	The analytical method, GRM074.01A was successfully validated according to SANCO/3029/99 rev. 4. for the determination of nicosulfuron in maize kernels (dry commodity) and maize whole plant (high water commodity) with an LOQ of 0.01 mg/kg. Acceptable mean recoveries between 70 and 110%, with relative standard deviations (RSDs) less than 20% were found for both the primary and confirmatory LC-MS/MS transitions for nicosulfuron (primary transition: m/z 411 → 182; confirmatory transition: m/z 411 → 213) in all matrices tested. Residues of nicosulfuron in all control and reagent blank samples were below 30% of the limit of quantification in all of the samples used in this validation. The study is acceptable.

Reference:	KCP 5.1.2.5/09
Report	Andrews G., 2016 Nicosulfuron and Dicamba – Residue Validation and Study on Maize in Northern France, Germany and Poland in 2015. Battelle UK Ltd, United Kingdom Syngenta Report No. TK0258007-REG Syngenta File No. A19658H_10060
Guideline(s):	Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/825/00 rev. 8.1, 16/11/2010). Commission of the European Communities. Guidance Document on Residue Analytical Methods (SANCO/3029/99 rev. 4, 11/07/2000). OECD Guidance Document on Pesticide Residue Analytical Methods ENV/JM/MONO(2007)17. Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Residue Chemistry Test Guidelines OPPTS 860.1340 Residue Analytical Method, EPA 712-C-96-174, August 1996.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
Analytical method GRM074.01 was validated in maize kernels and whole plant.

Results and discussions
Summaries of the results for nicosulfuron are presented in the following tables.

Table A 58: Recovery results from validation of GRM074.01 for nicosulfuron in maize kernels and whole plant: primary transition *m*/z 411-182

Matrix	Fortification Level (mg/kg)*	Recovery (%)	Number of Analysis (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Maize Kernels	0.01*	76, 83, 82, 83, 88, 82	6	82	4.6	76 - 88
	0.1	81, 78, 74, 73, 78, 78	6	77	3.8	73 - 81
	Overall		12	80	5.5	73 - 88
Maize Whole Plant	0.01*	91, 95, 95, 98, 70, 97	6	91	11.8	70 - 98
	0.01	101, 91, 80, 95, 73, 86	6	87	11.7	73 - 101
	Overall		12	89	11.4	70 - 101

*Limit of quantitation, defined by the lowest validated fortification level

Table A 59: Recovery results from validation of GRM074.01 for nicosulfuron in maize kernels and whole plant: confirmatory transition *m*/z 411-213

Matrix	Fortification Level (mg/kg)*	Recovery (%)	Number of Analysis (n)	Mean Recovery (%)	RSD (%)	Recovery Range (%)
Maize Kernels	0.01*	73, 80, 81, 82, 86, 79	6	80	5.5	73 - 86
	0.1	81, 77, 74, 73, 75, 77	6	76	3.6	73 - 81
	Overall		12	78	5.2	73 - 86
Maize Whole Plant	0.01*	90, 93, 94, 99, 71, 97	6	91	11.3	71 - 99
	0.1	96, 91, 80, 94, 71, 85	6	86	10.9	71 - 96
	Overall		12	88	10.9	71 - 99

*Limit of quantitation, defined by the lowest validated fortification level

Specificity

LC-MS/MS with two transitions is considered to be a highly specific detection technique and therefore according to EU guidance (***SANCO/825/00 rev.8.1, 16/11/2010***) no further confirmatory technique is required. The method includes two MS/MS transitions, both of which have been validated. No significant interferences arising from the crop matrices, the labware, reagents or solvents have been observed at the retention times of interest.

Linearity

The linearity of the LC-MS/MS detector was tested using matrix matched standard solutions (0.25 ng/ml to 25 ng/ml). Linearity was tested for both MS/MS transitions. Standards at eight different concentrations were injected and the signal area plotted against concentration for all calibration points. Straight lines with correlation coefficients greater than 0.99 were obtained for nicosulfuron.

Accuracy

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at ten times the LOQ (0.1 mg/kg). Acceptable mean recoveries of between 70% and 110% were found for both transitions on all matrices tested and therefore according to EU guidance (***SANCO 3029/99 rev.4 11/7/00***) demonstrate the method has satisfactory accuracy.

Repeatability

The relative standard deviations (RSDs) of nicosulfuron recoveries at each fortification level and overall for each crop tested during method validation were <20% and therefore according to the EU guidance (***SANCO 3029/99 rev.4 11/7/00***) demonstrate the method has satisfactory repeatability.

Limit of Quantification

The limit of quantification for nicosulfuron residues in crop matrices using method GRM074.01 was established at 0.01 mg/kg. No interfering peaks around the retention time of nicosulfuron were found in

any of the control samples at levels above 30% of the limit of quantification.

Limit of Detection
The limit of detections (LODs) were calculated to be 0.00004 and 0.00008 mg/kg for the primary transition, respectively, for maize kernel and maize whole plant 0.00005 and 0.0001 mg/kg for the confirmatory transition, respectively, for maize kernel and maize whole plant.

Matrix Extract
Significant matrix effects (suppression) were observed in the maize whole plant and no significant matrix effects were observed maize kernels, therefore matrix matched linearity standards were used for quantification.

Stability of Final Extracts
The stability of sample extracts fortified with nicosulfuron at the LOQ level was checked after a storage period of 8 to 9 days in a refrigerator at 3-8 ºC against freshly prepared calibration standards. The results proved that the nicosulfuron residues in the stored fortified samples were stable. The mean recovery values at the LOQ level were between 70 % and 110 %, with a RSD of ≤ 20 % when re-analysed.

Stability of Standard Solutions
The stability of the stored working standard solutions of nicosulfuron were checked after a storage period of 30 days in a refrigerator at 3-8 ºC against freshly prepared calibration standards. The results demonstrated that nicosulfuron residues in the stored working standard solutions were stable.

Conclusion
Analytical method GRM074.01 has been demonstrated to be a reliable and accurate procedure for the determination of nicosulfuron in crops to a limit of quantification of 0.01 mg/kg, using commercially available laboratory equipment and reagents.

A 2.3.1.3.1.2 Confirmatory method

LC-MS/MS with two transitions is considered to be a highly specific detection technique and no further confirmatory technique is required.

A 2.3.1.4 Description of analytical methods for the determination of residues in support of ecotoxicological studies (KCP 5.1.2.6)

Reference: KCP 5.1.2.6 (report available from data owner)
Report Obert-Rauser P. 2016
MU-466: Toxicity to the Duckweed Lemna gibba under Laboratory Conditions. Eurofins Agroscience Services. Report: S15-05478

Comments of zRMS:	**Summary** An analytical method for the determination of MU-466 was validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000 and is characterised as follows: Method principle: Direct analysis of test medium samples by HPLC-MS/MS. Specificity: MU-466 Linearity: The calibration function was second order within the range from 10 ng/mL to 150 ng/mL of MU-466 with r2 > 0.999 (for both transitions). Second order calibration was applied because it resulted in much lower deviations from all nominal concentrations than linear calibration and was therefore more accurate. Recovery: The recovery was determined by fortification of test medium with the test

item at the concentration levels given below:

Test item	Fortification level (mg/L)*	n	Mean recovery ± RSD (%) (Transition m/z 135.1)	Mean recovery ± RSD (%) (Transition m/z 108.1)
MU-466	0.09	5	105 ± 4	105 ± 3
	130	5	94 ±2	85 ± 5

RSD: relative standard deviation, *purity considered

The mean recovery per fortification level was within the guideline requirements (within 70-110%), for both transitions.

Repeatability: The relative standard deviation per fortification level was within the guideline requirements (≤ 20%), for both transitions.

LOQ: The limit of quantification was 0.09 mg/L of test item .

Blanks/LOD: The analyte was not detectable in the test medium used for recovery samples. The limit of detection (LOD) was defined as 30% of the limit of quantification (0.027 mg/L).

The analytical method was successfully validated according to SANCO/3029/99 rev. 4. for the determination of MU-466 in test medium with an LOQ of 0.09 mg/L of test item.
Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/3029/99 rev. 4, 11/07/2000 (70-110% mean recovery, ≤ 20% RSD).
The study is acceptable.

Reference: KCP 5.1.2.6 (report available from data owner)
Report Dengler D. (2009)
Assessment of Toxic Effects of HMUD on the Duckweed Lemna gibba in a Semi Static Test. Eurofins-GAB GmbH. Report: GAB S08-00827

Comments of zRMS:

Summary

An analytical method for the determination of HMUD was validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000 and is characterised as follows:

Method principle: Direct analysis of water samples by HPLC-MS/MS.

Specificity: HMUD parent compound

Linearity: The calibration function was linear within the range from 0.001 mg/L to 0.10 mg/L with r^2-> 0.999.

Accuracy/Recovery: The accuracy is given as the recovery of the test item from test medium:

Test item	Fortification level (mg/L)	HMUD nominal (mg/L)	n	Mean recovery ± RSD (%)
HMUD	0.01	5	5	99 ± 3
	4.0	5	5	103 ± 5

Repeatability: The relative standard deviation for each fortification level was 3% and within the guideline requirements (≤ 20%).

LOQ: The quantification limit was 0.01 mg/L of HMUD (lowest fortification level).

Blanks / LOD: The analyte was not detectable(< 30% of LOQ, i.e. 0.003 mg/L) in the test medium used for recovery samples.

The analytical method was successfully validated according to SANCO/3029/99 rev. 4. for the determination of HMUD in water samples with an LOQ of 0.01 mg/L of HMUD.

	Mean recoveries and relative standard deviations per fortification level fulfil the criteria of guideline SANCO/3029/99 rev. 4, 11/07/2000 (70-110 % mean recovery, ≤20 % RSD). The study is acceptable.

A 2.3.1.5 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 2.3.2 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 5.2.1)

A 2.3.2.1.1 Analytical method Steinhilper, 2008, 107 NIS

A 2.3.2.1.1.1 Method validation

Comments of zRMS:	It was demonstrated that the validation method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries of SANCO/825/00 rev. 8.1 and is therefore applicable to correctly determine residues of nicosulfuron in maize matrices (plant, grain and stover) with a limit of quantification (LOQ) of 0.01 mg/kg, using LC-MS/MS. The study is acceptable.

Reference:	KCP 5.2.1 (report available from data owner)
Report	Steinhilper, D. 2008 Validation of a Multiresidue method for the determination of Nicosulfuron in maize, Cheminova A/S. Unpublished Report No.: 107 NIS
Guideline(s):	SANCO/825/00 rev.7
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Notes:	The right to refer Regulatory Authorities to these data has been granted to the notifier by Cheminova A/S via a letter of access. This report has been submitted by Cheminova A/S previously to the UK CRD (November 2008)

Principle of the method

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:5mM ammonium acetate (1:9 v/v) ready for determination of Nicosulfuron by LC – MS/MS: quantifier and qualifier ion transitions.

Results and discussions

Summaries of the results for nicosulfuron are presented in the following tables.

Table A 60: Validation results – Determination of Nicosulfuron residues in maize matrices (mass transition 411 →182 m/z)

Sample matrix	Fortification level (mg/kg)	Range Recovery (%)	Mean Recovery (%)	RSD (%)	n
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

Table A 61: Validation results – Determination of Nicosulfuron residues in maize matrices (mass transition 411 → 213 m/z)

Sample matrix.	Fortification level (mg/kg)	Range Recovery (%)	Mean Recovery (%)	RSD (%)	n
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

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.

Linearity

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 (r^2) of 0.9997 for the primary and confirmatory mass transitions. Representative calibration lines are presented in the report.

Accuracy

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.

Repeatability

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.

Limit of Quantification

Acceptable accuracy and precision was obtained at 0.01 mg/kg for all maize matrices.

Comments of zRMS:	The analytical method (Steinhilper (2008), Report No.107 NIS) was independently and successfully validated for the determination of nicosulfuron in maize matrices (plant, grain and straw) with a limit of quantification (LOQ) of 0.01 mg/kg, using LC-MS/MS, in accordance to SANCO/825/00 rev. 8.1 requirements. The study is acceptable.

Reference:	KCP 5.2.1 (report available from data owner)
Report	Schwarz, T. 2008 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, Cheminova A/S. Unpublished Report No.: 119 NIS
Guideline(s):	SANCO/825/00 rev.7 SANCO/3029/99 rev. 4 ENV/JM/MONO(2007)17
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Notes:	The right to refer Regulatory Authorities to these data has been granted to the notifier by Cheminova A/S via a letter of access. This report has been submitted by Cheminova A/S previously to the UK CRD (November 2008)

Principle of the method

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

Summaries of the results for nicosulfuron are presented in the following tables.

Table A 62: Independent Laboratory Validation results – Determination of Nicosulfuron residues in maize matrices (mass transition 411 → 182 m/z)

Sample matrix.	Fortification level (mg/kg)	Range Recovery (%)	Mean Recovery (%)	RSD (%)	n
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

Table A 63: Independent Laboratory Validation results – Determination of Nicosulfuron residues in maize matrices (mass transition 411 → 213 m/z)

Sample matrix.	Fortification level (mg/kg)	Range Recovery (%)	Mean Recovery (%)	RSD (%)	n
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

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.

Linearity
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.

Accuracy
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.

Repeatability
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.

Limit of Quantification
Acceptable accuracy and precision was obtained at 0.01 mg/kg for all maize matrices.

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

Conclusion
The analytical method has been successfully and independently validated for post-registration monitoring for the determination of nicosulfuron residues in maize matrices with a LOQ of 0.01 mg/kg.

A 2.3.2.1.1.2 Confirmatory method

LC-MS/MS with two transitions is considered to be a highly specific detection technique and no further confirmatory technique is required.

A 2.3.2.1.1.3 Extraction efficiency

No new data are submitted as part of this submission.

A 2.3.2.1.2 Analytical method 107 NIS

A 2.3.2.1.2.1 Method validation

Comments of zRMS:	The analytical method was validated for the determination of nicosulfuron (DPX-V9360) in corn (grain, forage, silage, and fodder), cherry, lemon and soybean matrices at a limit of quantitation (LOQ) of 0.010 mg/kg. The overall average recovery for nicosulfuron from the different crop matrices were 75%-104% with relative standard deviations <15%. There were no detectable residues of nicosulfuron in control samples. Residues of nicosulfuron were confirmed based on detection and the relative ratios of the two MS/MS parent-to-daughter ion transitions monitored during sample analysis. Residues of nicosulfuron were also confirmed based on the acceptable linear calibration curves (i.e. R2 > 0.99) and acceptable average fortification recoveries per fortification level per matrix (i.e., 70–120% with RSD <20%) generated from each of the MS/MS parent-to-daughter ion transitions monitored during sample analysis (SANCO/825/00 rev. 8.1). The study is acceptable.

Reference:	KCP 5.2.1 (report available from data owner)
Report	Analytical Method for the Determination of Nicosulfuron and Rimsulfuron in Corn, Cherry, Lemon and Soybean Matrices using HPLC/ESI-MS/MS Cabusas, M.E., Pentz A. 2012, Report No.:DuPont- 11776, Revision No.2
Guideline(s):	SANCO/825/00 rev.8.1
Deviations:	No
GLP:	No
Acceptability:	Yes

Reference:	KCP 5.2.1 (report available from data owner)
Report	Validation report DuPont-11776 RV2: Extension of the Linearity Range for Nicosulfuron in Oily and Acidic Crop, McInerney K., 2016. Report No.: 100077587-03
Guideline(s):	SANCO/825/00 rev.8.1
Deviations:	No
GLP:	No
Acceptability:	Yes

The purpose of the study was to validate an analytical method for the determination of residues of nicosulfuron in representative watery, dry, oily and acidic crop matrices following the requirements of SANCO/825/00 rev 8.1. Only the results for nicosulfuron are summarised below:

Materials and methods:
Sample preparation: The samples were extracted with 0.1M potassium phosphate (pH 8)/methanol (8:2, v/v) solution using a Tekmar homogenizer. Following centrifugation, extract aliquots were diluted with 0.5% acetic acid (1:1), and purified by solid phase extraction on Oasis™ MAX cartridges. The analytes were eluted with 0.1% formic acid in acetonitrile after the cartridges were sequentially washed with water, methanol, ethyl acetate and acetonitrile. Eluates were evaporated to dryness at 30-35°C, reconstituted in

5mM ammonium formate/methanol (8:2, v:v) solution and filtered. The purified extracts were analysed by reverse-phase HPLC-MS/MS.

Instrument conditions:

- For corn grain, forage, silage and fodder validation:

Instrument: Agilent HP Series 1100 HPLC coupled to ThermoFinnigan TSQ 7000 MS (a triple quadrupole MS) with an electrospray ion source.
Column: Phenomenex Luna ® Phenyl-hexyl 3 µm, 50 mm x 4.6 mm
Temperature: 40 °C
Solvent system: A: 0.01% formic acid in 0.1mM ammonium formate (aq)
B: methanol

Gradient:

Time [min]	solvent A [%]	solvent B [%]	Flow rate [mL/min]
0.00	52	48	1.0
6.00	40	60	1.0
6.01	5	95	1.0
6.50	5	95	1.5
7.75	5	95	1.5
7.76	52	48	1.0
10.00	52	48	1.0

Detector: ThermoFinnigan TSQ 7000 MS (a triple quadrupole MS) with an electrospray ion source.
Detection mode: ESI, positive

Analyte	Parent ion (m/z)	Daughter ion (m/z)	Dwell (ms)	CE (V)	
Nicosulfuron	411.1	182.1	250	20	Primary
		213.1	250	20	Confirmatory

- For cherry and lemon validation:

Instrument: Agilent HP Series 1100 HPLC coupled to MDS SCIEX API 4000 MS (a triple quadrupole MS) with an electrospray ion source.
Column: Phenomenex Luna ® Phenyl-hexyl 3 µm, 50 mm x 4.6 mm
Temperature: 40 °C
Solvent system: A: 0.01% formic acid in 0.1mM ammonium formate (aq)
B: methanol

Gradient:

Time [min]	solvent A [%]	solvent B [%]	Flow rate [mL/min]
0.00	45	55	1.0
7.00	30	70	1.0
7.10	5	95	1.0
9.00	5	95	1.0
9.10	45	55	1.0
11.00	45	55	1.0

Detector: MDS SCIEX API 4000 MS (a triple quadrupole MS) with an electrospray ion source
Detection mode: ESI, positive

Analyte	Parent ion (m/z)	Daughter ion (m/z)	Dwell (ms)	CE (V)	
Nicosulfuron	411.0	182.0	250	25	Primary
		213.0	250	25	Confirmatory

- For soybean seed validation:

Instrument: Agilent Series 1290 Infinity coupled to MDS SCIEX API 5000 MS (a triple quadrupole MS) with an electrospray ion source.
Column: Phenomenex Luna ® Phenyl-hexyl 3 µm, 50 mm x 4.6 mm
Temperature: 40 °C
Solvent system: A: 0.01% formic acid in 0.1mM ammonium formate (aq)
B: methanol

Gradient:

Time [min]	solvent A [%]	solvent B [%]	Flow rate [mL/min]
0.00	35	65	1.0
2.50	22	78	1.0
2.60	5	95	1.5
3.60	5	95	1.5
3.61	35	65	1.0
5.00	35	65	1.0

Detector: MDS SCIEX API 5000 MS (a triple quadrupole MS)
Detection mode: ESI, positive

Analyte	Parent ion (m/z)	Daughter ion (m/z)	Dwell (ms)	CE (V)	
Nicosulfuron	411.0	182.0	100	40	Primary
		213.0	100	25	Confirmatory

Findings:
Specificity: All control samples showed no detectable residue of nicosulfuron. Specificity of the method is provided by the use of LC/MS/MS.

Linearity: The calibration was performed in the concentration range from 0.3 to 20 ng/mL (covering a range from 30% of the LOQ to at least 10xLOQ +20%) using a least square fit of a linear function. Typical calibration curves are presented below:

mass transition m/z	**Calibration curve**	**R^2**
411 / 182	Y = 126506000 x – 22731.88873	0.99939
411 / 213	Y = 63789600 x – 13491.74781	0.99946

Accuracy and precision: The total ion current (TIC) was used in residue calculations, except in the soybean validation. The summary of the recovery results is given in the table below. The results fall within the requirements of the SANCO/825/00 rev 8.1.

Table A 64: Summary of recovery results

Matrix	**Nicosulfuron in Corn, Cherry, Lemon and Soybean matrices[a]**					
	0.01 mg/kg (n=5)		**0.10 mg/kg (n=5)**		**Overall (n=10)**	
	Mean (± RSD)	**Range**	**Mean (± RSD)**	**Range**	**Mean (± RSD)**	**Range**
Corn grain	93 (± 7)	84-100	91 (± 4)	86-95	92 (± 5)	84-100
Corn forage	91 (± 10)	82-105	95 (± 7)	92-106	93 (± 8)	82-106
Corn silage	90 (± 6)	83-98	89 (± 2)	88-91	89 (± 4)	83-98
Corn fodder	104 (± 4)	98-110	104 (± 1)	103-106	104 (± 3)	98-110
Cherry	90 (± 3)	87-94	92 (± 3)	88-95	91 (± 3)	87-95
Lemon	86 (± 3)	83-89	86 (± 3)	83-88	86 (± 3)	83-89
Soybean seed [b]	78 (± 6)	71-84	72 (± 5)	68-77	75 (± 7)	68-84

[a] Quantification used the total ion current (TIC), except in the soybean analysis.
[b] The transition 411/182 (m/z) was used in the quantification

Confirmatory: the confirmatory method was based on detection and the relative ratios of the two MS/MS parent-to-daughter ion transitions monitored during the validation. Residues of nicosulfuron were also confirmed based on acceptable linear calibration curves and acceptable fortification recoveries per fortification level generated from the MS/MS ion transitions monitored during sample analysis.

- *Based on Detection and Ratio of Two MS/MS Ion transitions*

The ion ratios for the LOQ sample and the 10xLOQ sample fall within the upper and lower limits calculated. Based on the criteria outlined, the levels of nicosulfuron found in the above-fortified samples would be confirmed as a nicosulfuron residues

- *Based on fortification recoveries from Confirmatory Ion Data (SANCO/825/00 rev 8.1)*

Confirmatory data were generated from each of the two ion transitions that were monitored for nicosulfuron during method validation on cherry (watery crop), lemon (acidic crop), corn grain (dry crop) and soybean seed (oily crop). The summary of the recovery results from the two ion transition is given in the table below. The results fall within the requirements of the SANCO/825/00 rev 8.1

Table A 65: Summary of recovery results

Matrix	Mass transition (m/z)	Nicosulfuron			
		0.01 mg/kg (n=5)		0.10 mg/kg (n=5)	
		Mean (± RSD)	Range	Mean (± RSD)	Range
Cherry	411/182	92 (± 4.1)	88-97	91 (± 3.0)	87-94
	411/213 (Conf.)	88 (± 2.0)	86-90	92 (± 2.2)	89-93
Lemon	411/182	87 (± 1.9)	85-89	86 (± 3.4)	83-88
	411/213 (Conf.)	86 (± 2.8)	82-88	83 (± 2.5)	81-85
Corn grain	411/182	83 (± 7.3)	76-92	92 (± 3.4)	89-97
	411/213 (Conf.)	83 (± 7.3)	76-92	92 (± 3.4)	89-97
Soybean seed	411/182	78 (± 6.0)	71-84	72 (± 4.5)	68-77
	411/213 (Conf.)	76 (± 10.2)	66-85	72 (± 4.6)	70-77

Matrix effect: no significant matrix effect (<20%) on the instrument response was observed for nicosulfuron.

Limit of Detection (LOD) and Limit of Quantification (LOQ): A limit of quantification of 0.01 mg/kg was achieved for the determination of nicosulfuron in corn (grain, forage, silage and fodder), cherry and lemon. The estimated limit of detection (LOD) is approximately 1/3 of the LOQ (ca 0.003 mg/kg).

Stability of final extract: Standards and samples of nicosulfuron in 5.0 mM ammonium acetate/Methanol (8:2, v/v) are relatively stable under ambient conditions for at least 5 hours, or under ≤5°C, for at least 48 hours.

Conclusion: According to the analytical results obtained, the described method is considered to be appropriate for the quantification of nicosulfuron in the four crop groups: corn (grain, forage, silage and fodder), cherry, lemon and soybean matrices, following the latest guideline SANCO/825/00 rev 8.1. The data demonstrate that the analytical method for the determination of nicosulfuron in the four crop groups: corn (grain, forage, silage and fodder), cherry, lemon and soybean matrices) provides adequate specificity, accuracy, precision and linearity. A limit of quantitation of 0.01 mg/kg was validated.

A 1.1.1.1.6 Independent laboratory validation

Comments of zRMS:	The analytical method was successfully independently validated according to the requirements of the SANCO/825/00 rev 8.1. This analytical method is suitable as an enforcement method for the quantitation of nicosulfuron in corn grain and silage (watery and dry crop matrices) with LOQ of 0.010 mg/kg. . In SANCO/825/00 rev 8.1 it is stated that if the primary method is identical for all matrix groups, it is sufficient to perform the ILV for commodities of two of these groups, one of them with high water content. The overall average recoveries (±RSD) for nicosulfuron in 10 fortified samples of corn grain and silage were 96% (±7%) and 93% (±16%), respectively. There were no detectable residues in the control samples. Residues of nicosulfuron are confirmed based on detection and the relative ratios of the two MS/MS parent-to-daughter ion transitions monitored during sample analysis. The study is acceptable.

Reference: KCP 5.2.1 (report available from data owner)

Report	Ducat, N., Pigeon O., 2004 Independent Laboratory validation of DuPont-11776, "Analytical Enforcement Method for the Determination of Nicosulfuron in Corn Matrices using HPLC/ESI-MS/MS., Report No.: DuPont-12347
Guideline(s):	SANCO/825/00 rev.7
Deviations:	No
GLP:	Yes
Acceptability:	Yes

The purpose of the study was to independently validate an analytical method DuPont-11776 for the determination of residues of nicosulfuron in two representative matrices (watery and dry crop matrices) following the requirements of SANCO/825/00 rev 7. As the principle of the extraction is the same for the four crop groups, therefore an ILV on only two crop group is sufficient.

Materials and methods:

Sample preparation: The samples were extracted with 0.1M potassium phosphate (pH 8)/methanol (8:2, v/v) solution using a Tekmar homogenizer. Following centrifugation, extract aliquots were diluted with 0.5% acetic acid (1:1), and purified by solid phase extraction on Oasis™ MAX cartridges. The analytes were eluted with 0.1% formic acid in acetonitrile after the cartridges were sequentially washed with water, methanol, ethyl acetate and acetonitrile. Eluates were evaporated to dryness at 30-35°C, reconstituted in 5mM ammonium formate/methanol (8:2, v:v) solution and filtered. The purified extracts were analysed by reverse-phase HPLC-MS/MS

Instrument conditions:

Instrument:	ThermoFinnigan LCQDuo
Column:	Phenomenex Luna ® Phenyl-hexyl 3 µm, 100 mm x 3.0 mm
Temperature:	40 °C
Solvent system:	A: methanol B: 0.01% formic acid in 0.1mM ammonium formate (aq)

Gradient:

Time [min]	solvent A [%]	solvent B [%]	Flow rate [mL/min]
0	45	55	0.2
15	95	5	0.2
20	95	5	0.2
25	45	55	0.2
30	45	55	0.2

Injection volume:	20 µL
Detection mode:	ESI, positive

Analyte	Parent ion (m/z)	Daughter ion (m/z)	Dwell (ms)	CE (%)	
Nicosulfuron	411.0	182.0	200	30	Primary
		213.0	200	30	Confirmatory

Findings:

Specificity: No significant interference (above 30%) was detected in the control samples. Specificity of the method is provided by the use of LC/MS/MS.

Linearity: The calibration was performed in the concentration range from 0.5 to 20 ng/mL (covering a range from 50% of the LOQ to at least 10xLOQ +20%) using a least square fit of a linear function. Following SANCO/825/00 rev 8.1, the lowest standard must be at least 30% of the LOQ, in order to properly quantify any interference in the control samples. As the control samples were not detected (<LOD), this small deviation does not affect the validity of the study. Also, the appropriate range (0.3-20 ng/mL) is already re-validated within the original validation and it is not necessary to repeat it for the ILV too. The typical

calibration curve is y = 114176.9927 x – 15850.9630, with R^2 = 0.9993

Accuracy and precision: The summary of the recovery results is given in the table below. The results fall within the requirements of the SANCO/825/00 rev 8.1.

Table A 66: Summary of recovery results

Matrix	Nicosulfuron in Corn matrices					
	0.01 mg/kg (n=5)		0.10 mg/kg (n=5)		Overall (n=10)	
	Mean (± RSD)	Range	Mean (± RSD)	Range	Mean (± RSD)	Range
Corn grain	100 (± 8)	88-109	93 (± 3)	90-96	96 (± 7)	88-109
Corn silage	106 (± 7)	96-119	80 (± 8)	72-87	93 (± 16)	72-119

Confirmatory: Residues of nicosulfuron were confirmed based on detection and the relative ratios of the two MS/MS parent-to-daughter ion transitions monitored during sample analysis. This approach for the confirmatory method (relative ratio of two MS/MS transitions) is not the recommended way following the SANCO/825/00 rev.8.1, but it was acceptable following SANCO/825/00 rev.7, the guideline in force at the time the study was conducted. The two approaches (relative ratio of two MS/MS transitions and determination of recoveries on a second MS/MS transitions) have been validated in the primary validation (see CA 4.2-05), giving the same conclusion on the confirmatory. Therefore the fact to use one or the other approach in the ILV has no impact on the results for confirmatory method. The confirmatory method has demonstrated the selectivity of the primary method.

Limit of Quantification (LOQ): A limit of quantification of 0.01 mg/kg was achieved for the determination of nicosulfuron in corn (grain and silage).

Conclusion: The analytical method was successfully independently validated following the requirements of the SANCO/825/00 rev 8.1. It is concluded that the residue analytical method fulfils the reproducibility requirements as defined in the EC Guidance documents on residues analytical methods SANCO/825/00 rev 8.1 and is therefore, applicable as an enforcement method.

A 2.3.2.1.2.2 Extraction efficiency

No new data are submitted as part of this submission.

A 2.3.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2.2)

A 2.3.2.2.1 Analytical method S08-02037

A 2.3.2.2.1.1 Method validation

Comments of zRMS:	The analytical method was successfully validated for the determination of nicosulfuron (DPX-V9360) in animal tissues (milk, egg, muscle and liver) at a limit of quantitation (LOQ) of 0.010 mg/kg egg, muscle and liver and 0.01 g/L for milk according to the SANCO /825/00 rev. 8.1. Nicosulfuron was detected as follows: m/z 411.2 -> m/z 182.2 (primary method) m/z 411.2 -> m/z 106.1 (confirmatory method) The mean recovery for nicosulfuron from the different crop matrices were within the range of 70-110% with relative standard deviations <20%. There were no detectable residues of nicosulfuron in control samples. The study is acceptable. Remark: The mean recoveries and relative standard deviations for eggs and milk have been added in the Table A 67 below.

Reference:	5.2.2 (report available from data owner)
Report	Wolf, S., 2009 Development and Validation of a Residue Analytical Method for Nicosulfuron in Animal Tissues (Milk, Egg, Muscle and Liver). Report No.: 90011604
Guideline(s):	SANCO/3029/99 rev 4 and broadly complying with SANCO/825/00 rev.8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

An analytical method was developed for the determination of nicosulfuron in animal tissues (milk, egg, muscle and liver). The validation in muscle and liver is covering the data requirement for a validation on tissue. Only the results for nicosulfuron in muscle and liver are summarised below.

Materials and methods:
The method involves extraction of nicosulfuron with acetonitrile / acetic acid, followed by 1+9 dilution of the extract with water, followed by LC separation using MS/MS detection.
Cow tissues (muscle and liver) were obtained from a local butcher (Gunzenhauser Metzgerei AG, 4450 Sissach, Switzerland). The cow tissue (muscle and liver) were homogenised by the butcher using a meat mincer.

Instrument conditions:

Instrument:	High pressure gradient system consisting of two Shimadzu LC-10AD pumps and a Shimadzu SCL System Controller coupled to MDS SCIEX API 4000 LC-MS/MS
Column:	Luna C18 (2) 100A, 3 µm, 2.0 x 50 mm
Flow:	300 µL/min
Injection volume:	20 µL
Solvent system:	Solvent A: 95 vol. water + 5 vol. methanol + 0.1 vol formic acid; 5 mM ammonia formate (aq) Solvent B: 5 vol. water + 95 vol. methanol + 0.1 vol formic acid; 5 mM ammonia formate (aq)

Time [min]	solvent A [%]	solvent B [%]
0.00	70	30
3.00	20	80
3.10	70	30
4.50	70	30

Mass spectrometer:	MDS SCIEX API 4000
Ionization mode:	ESI
Scan Mode:	Multiple Reaction Monitoring (MRM)

Analyte	Transition	CE (eV)	Dwell Time (ms)	Method
Nicosulfuron	411.2 / 182.2	29	300	Primary
	411.2 / 106.1	48	300	Confirmatory

Findings:
Linearity: The calibration was performed using standards in the range of 0.05 - 5.0 ng/mL (corresponding to 0.06 – 2.5 ng/mL in the sample), covering the concentration range of 50% of the LOQ to 10xLOQ + 20%. Following SANCO/825/00 rev 8.1, the lowest standard must be at least 30% of the LOQ, in order to properly quantify any interference in the control samples. As the control samples were not detected (<LOD), this small deviation does not affect the validity of the study.

Compounds	Method	Typical calibration curve	R^2
Nicosulfuron	Primary	y = - 498 + 147669 x	0.9999
Nicosulfuron	Confirmatory	y = - 465 + 66886 x	0.9999

Specificity: The method allows the determination of nicosulfuron in tissues (muscle and liver). For analysis of nicosulfuron, the retention time in the specimen extracts matched the retention time in standard solution. No interferences at the retention time of nicosulfuron above 30% of the LOQ and above the LOD were observed in the untreated control samples (no nicosulfuron was detected). Therefore, the method is specific for nicosulfuron. Since in addition analysis were performed by MS/MS detection, the method is highly specific.

Accuracy and Precision: The following mean recoveries, standard deviations and relative standard deviations were found within the SANCO's requirements.

Table A 67: Summary of recovery results

Matrix	Fortification level	Primary method		Confirmatory method	
		Mean%	RSD% (n=5)	Mean%	RSD% (n=5)
Muscle	0.01 mg/kg	93	4	94	5
	0.10 mg/kg	81	4	81	3
Overall		87	8	87	9
Liver	0.01 mg/kg	91	8	95	6
	0.10 mg/kg	86	9	86	9
Overall		89	9	91	9
Egg	0.01 mg/kg	91	6	96	7
	0.10 mg/kg	88	11	88	9
Overall		90	8	92	9
Milk	0.01 mg/kg	92	1	89	2
	0.10 mg/kg	82	3	82	1
Overall		87	6	85	5

Matrix effect: The analysis of a spiked control extract shows that there is no significant matrix influence observed for the determination of nicosulfuron, in muscle and liver, using this LC-MS/MS method.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The limit of quantification was determined to be 0.01 mg/kg by successful recoveries at the concentration. The limit of detection was estimated from the lowest calibration standard concentration used (0.05 ng/mL). The LOD was 0.005 mg/kg for nicosulfuron in muscle and liver.)

Conclusion: The method for the determination of nicosulfuron in muscle and liver (tissues) was successfully validated at the limit of quantification of 0.01 mg/kg.

A 2.3.2.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2.3)

A 2.3.2.3.1 Analytical method 100077587-04

A 2.3.2.3.1.1 Method validation

Comments of zRMS:	The analytical method for the determination of nicosulfuron in mouse plasma was successfully validated by achieving a calibration with a coefficient of determination (R^2)> 0.990 and mean recoveries of fortified samples between 70% and 110% with a relative standard deviation < 20% for both transitions monitored (following SANCO/825/00 rev 8.1). The validated LOQ was 0.05 mg/L. The study is acceptable.

Reference: 5.2.3 (report available from data owner)

Report	xxxxxxxxxxxxxxx 2016a Method Validation for the Determination of Nicosulfuron in Mouse Plasma,. Report No.: 100077587-04
Guideline(s):	SANCO/825/00 rev.8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

An analytical method was developed for the determination of nicosulfuron in mouse plasma with a limit of quantification of 0.05 mg/L.

Materials and methods:
Pooled blank matrix (plasma of untreated mice) was supplied by Innovative Research obtained through whole blood donations from normal healthy mice to which the anticoagulant Lithium Heparin was added. The recommended storage temperature for the material is -20°C.
The frozen mouse plasma was allowed to thaw in a water bath, equilibrated to room temperature and centrifuged for 10 minutes at 2500 rpm. Acetonitrile was added to each cavity to be used in a Sirocco™ precipitation plate followed by an anliquot of the sample. The capped precipitation plate was shaken for 60 seconds on a shaker table then centrifuged for 10 minutes at 3700 rpm and 4oC. The plate was removed and an aliquot of 5mM ammonium formate solution was added to each of the cavities. The plate was resealed and shaken for an additional 5 minutes. The final extract was then aliquoted for analysis by liquid chromatography with tandem mass spectrometers (LC-MS/MS).

Instrument conditions:

Instrument:	ABSciex Qtrap 5500
Column:	Luna® C18 (2) 2.0 mm x 50 mm x 3.0 µm
Injection volume:	2 µL
Solvent system:	Solvent A: 5 mM ammonium formate (aq) / Methanol / formic acid (95:5:0.1) Solvent B: 5 mM ammonium formate (aq) / Methanol / formic acid (5:95:0.1

Time [min]	solvent A [%]	solvent B [%]
0.0	70	30
2.5	20	80
3.0	20	80
3.1	70	30
4.0	70	30

Flow:	0.4 mL/min
Ionization mode:	Turbo Ion Spray
Heater Gas Temp.:	450°C
Spray voltage:	4500V
Scan Mode:	Multiple Reaction Monitoring (MRM)
Polarity:	Positive

Analyte	Transition	CE (eV)	Method
Nicosulfuron	411 / 182	25	Primary
	411 / 106	45	Confirmatory

Findings:
Linearity: The calibration was performed using matrix-matched standards in the range of 15 - 60 ng/mL (corresponding to 0.015 – 0.06 mg/L in the sample), covering the concentration range of 30% of the LOQ to 10xLOQ + 20%.

Compounds	Method	Typical calibration curve	R^2
Nicosulfuron	Primary	Y = 11498900 x + 9913.81847	0.99507
Nicosulfuron	Confirmatory	y = 5606210 x + 2883.03176	0.99567

Specificity: The retention times of nicosulfuron signals in the specimen extracts match the retention time of the standard solution. Interferences were not observed. Conclusively the method is sufficiently specific for the determination of nicosulfuron in mouse plasma.
To demonstrate the method to be highly specific a different transition was monitored. As no nicosulfuron was recovered in the untreated control specimens (>30% LOQ) the specificity of the method is confirmed. The method is highly specific and appropriate for the determination of nicosulfuron in mouse plasma.

Accuracy and Precision: The mean recoveries, standard deviations and relative standard deviations were within the SANCO requirements.

Table A 68: Summary of recovery results

Matrix	Fortification level	Primary method		Confirmatory method	
		Mean%	RSD%	Mean%	RSD%
Mousse plasma	0.05 mg/L	104	1.6	103	1.3

Limit of Quantification (LOQ): The limit of quantification was determined to be 0.05 mg/L by successful recoveries at the concentration.

Conclusion: The method for the determination of nicosulfuron in mouse plasma was successfully validated at the limit of quantification of 0.05 mg/L.

A 2.3.2.4 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2.2)

No new or additional studies have been submitted.

A 2.3.2.5 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2.3)

No new or additional studies have been submitted.

A 2.3.2.6 Description of Methods for the Analysis of Soil (KCP 5.2.4)

No new or additional studies have been submitted.

A 2.3.2.7 Description of Methods for the Analysis of Water (KCP 5.2.5)

No new or additional studies have been submitted.

A 2.3.2.8 Description of Methods for the Analysis of Air (KCP 5.2.6)

No new or additional studies have been submitted.

A 2.3.2.9 Other Studies/ Information

No new or additional studies have been submitted.