European Commission

Draft Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

ISOFLUCYPRAM

Volume 3 – B.5 (AS)

Rapporteur Member State: United Kingdom
Co-Rapporteur Member State : France

Version History

When	What
March 2019	Initial DAR

Table of contents

B.5. METHODS OF ANALYSIS

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

B.5.1.1. Methods for the analysis of the active substance as manufactured

B.5.1.1.1. Active substance

Report:	KCA 4.1.1/01; Peiffer, C.; Uroic, K.; 2015; M-537692-01-1
Title:	BCS-CN88460 - Determination of technical grade active substance - HPLC - External standard
Report No.:	AM023714MP3
Document No.:	M-537692-01-1
Guideline(s):	Regulation (EC) 1107/2009 EU Directive 283/2013 Guideline OCSPP 830.1800
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Report:	KCA 4.1.1/02; Uroic, K.; Peiffer, C.; 2016; M-560314-01-1
Title:	Validation of AM023714MP3 - BCS-CN88460 - Determination of technical grade active substance - HPLC-external standard
Report No.:	VB1-AM023714MP3
Document No.:	M-560314-01-1
Guideline(s):	Regulation (EC) 1107/2009 EU Directive 283/2013 Guideline OCSPP 830.1800
Guideline deviation(s):	none
GLP/GEP:	**yes**

The content of Isoflucypram in technical grade active substance is quantified using analytical method AM023714MP3. The method was used to determine the content of isoflucypram in 5 batches of technical material produced at each of 2 sites (Dormagen and Muttenz) as reported in DAR Volume 4, C.1.2.3. The method was validated in study VB1-AM023714MP3. The method and its validation parameters and results are described below.

Principle of the method:
Samples of test item are dissolved in acetonitrile/methanol (75/25 v/v) and analysed by reverse phase HPLC-DAD (240 nm) using a Zorbax Eclipse plus C18, 50mm x 4.6mm x 1.8µm analytical column at 55°C and gradient elution with 0.1 %w/w aqueous phosphoric acid/acetonitrile mobile phase. Quantification was against external standards.

Specificity:
Specificity was demonstrated by retention time match with a reference standard and the absence of interfering peaks. Chromatograms of a solvent blank, lowest calibration standard and lowest fortification sample (expanded to an appropriate scale) were submitted to demonstrate this. The absence of interfering peaks from known impurities was demonstrated by the analysis of a mixed standard of impurities which showed that they did not elute at the same time as the active substance. In addition, UV spectra of reference standards, technical samples and spiked samples were examined and showed no spectral differences.

Linearity:
Linearity was demonstrated by the analysis (in duplicate) of five standards of increasing concentration. The range of standard concentrations used was 58.4 – 142% of the nominal concentration in the samples.
Equation of the line: $y = 0.1596x$
correlation coefficient: $r = 0.9999$

Note: as the concentration of active substance being determined falls in the middle of the calibration line and the correlation coefficient is very high (0.9999), the impact of forcing the calibration line through zero will be minimal and is therefore acceptable.

Accuracy:
Five synthetic samples, containing different ratios of reference standard and test item were prepared and analysed by the method described. The 'spike' concentrations ranged from 42 – 236% of the inherent concentrations.
Individual % recoveries: 99.7 @ 236% w/w (n=1)
99.2 @ 145 %w/w (n=1)
99.6 @ 100 %w/w (n=1)
100 @ 68 %w/w (n=1)
101 @ 42 %w/w (n=1)
% Recovery range: 99.2 – 101
Mean % recovery: 99.9

Precision:
Precision in the form of %RSD was calculated for the accuracy determinations described above.
%RSD: 0.56% (n=5)
Horwitz value for a nominal concentration of 98% is 1.34.

Assessment:
The method is acceptably validated in accordance with SANCO/3030/99 rev.4 and is suitable for the determination of isoflucypram in Isoflucypram technical material.

B.5.1.1.2. **Relevant impurities**

Report:	KCA 4.1.1/03; Frensemeier, L.; Peiffer, C.; 2017; M-608343-01-1
Title:	Isoflucypram (BCS-CN88460) - Determination of impurities in technical grade active substance - HPLC - external standard
Report No.:	M-608343-01-1
Document No.:	M-608343-01-1
Guideline(s):	Regulation (EC) 1107/2009 EU Directive 283/2013 Guideline OCSPP 830.1800
Guideline deviation(s):	None
GLP/GEP:	**no**

Report:	KCA 4.1.1/04; Frensemeier, L.; 2017; M-608465-02-1
Title:	Validation of HPLC-method AM029617MP1 - Isoflucypram (BCS-CN88460) - Determination of impurities in technical grade active substance - HPLC - external standard - Final report / 1. amendment
Report No.:	VB1-AM029617MP1
Document No.:	M-608465-02-1
Guideline(s):	Regulation (EC) 1107/2009 EU Directive 91/414/EEC EU Directive 283/2013 Guideline OCSPP 830.1800
Guideline deviation(s):	None
GLP/GEP:	yes

The method of analysis for the determination of BCS-CN45153 was reported in AM029617MP2 (Frensemeier, L.; Peiffer, C.; 2017) alongside the other organic impurities which are confidential. Information relevant to determination of impurity BCS-CN45153 only has been included in this section.

The method was validated in study VB1-AM029617MP1 (note : the validation report title refers to analytical method AM029617MP1 but the retention time of an impurity was described incorrectly in method AM029617MP1 therefore, a new version of the method was created (AM029617MP2) and the above referenced amendment to the validation report produced. This does not affect the results obtained during method validation VB1-AM029617MP1, which is valid for method AM029617MP2 and is described below). Validation parameters are described below and results are presented in Table B.5.1.1-1.

Principle of the method:
Samples of test item were dissolved in acetonitrile/0.1% aqueous phosphoric acid (70/30) and analysed by reverse phase HPLC-UV (210 nm) using a Zorbax Eclipse Plus C18, 4.6mm x 100mm x 1.8µm analytical column at 55ºC and gradient elution with acetonitrile/0.1% w/w aqueous phosphoric acid mobile phase. Quantification was against external standards.

Specificity:
Specificity was demonstrated by retention time match with a reference standard and the absence of interfering peaks in chromatograms. Chromatograms of a solvent blank, lowest calibration standard and lowest fortification sample (expanded to an appropriate scale) were submitted to demonstrate this. In addition MS and UV spectra of reference standards, technical samples and spiked samples were examined and showed no spectral differences.

Linearity:
Linearity was demonstrated by the analysis (in duplicate) of five standards of increasing concentration. The range of standard concentrations used in terms of %w/w in the sample, is reported in the summary table (Table B.5.1-1) as is the equation of the line and the correlation coefficient. The calibration was forced through zero, so the individual data points were requested from the applicant and the calibration lines were re-plotted without forcing the line through zero. No significant intercept was noted and the resulting correlation coefficient was >0.9999.

The demonstrated linear range is appropriate to the proposed specification limits for BCS-CN45153. It does not extend 20% below the concentration of the lowest fortification level, however, the calibration coefficients indicate that the lines are an excellent fit to the points and there is good sensitivity to the analytes, with very clear and visible peaks in the chromatograms of the lowest standards. Therefore the RMS considers that the data are acceptable and no further data are required.

Accuracy:
Five replicates at each of two fortification levels corresponding to approximately 0.5 and 5 g/kg were analysed by the method described. Standard addition method was used for the higher recovery level but because BCS-CN45153 was present in the test item above the LOQ prior to spiking, a synthetic recovery sample was used for the low level recovery.

Precision:
Precision in the form of %RSD was calculated for the accuracy determinations described above.

Stability:
BCS-CN45153 is stable in calibration and sample solutions for at least 120 hours when stored at room temperature.

Assessment:
The method is acceptably validated in accordance with SANCO/3030/99 rev.4 and is suitable for the determination the relevant impurity BCS-CY26497 in isoflucypram technical material at the level of 1 g/kg proposed in the technical specification.

Table B.5.1.1-1: Summary of validation data for relevant impurity BCS-CN45153 in active substance as manufactured.

Analyte	LOQ (%w/w)	Impurity level prior to fortification (% w/w)	Recovery fortification level (%w/w)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
BCS-CN45153 (relevant impurity)	0.05	<0.05 Synthetic sample was used	0.05	90.1 – 100 (93.9)	3.37 @ 0.05 %w/w (n=5) Modified Horwitz = 4.21	0.053 – 2.1 %w/w Y=0.4574 R^2 = 1.000 Specification limit: <0.1%w/w	Retention time match to reference standard. No significant interfering peaks observed. MS spectra and UV spectra of reference standards, technical samples and spiked samples were examined and showed no spectral differences.
		0.07	0.5	97.2 – 98.6 (98.1)	0.44 @ 0.57 %w/w (n=5) Modified Horwitz = 2.92		

B.5.1.2. Methods for risk assessment

B.5.1.2.1. Methods used in support of Environmental Fate studies

B.5.1.2.1.1. Soil and Sediment

Report:	KCA 4.1.2/01; Koch, V.; 2014; M-499794-01-1
Title:	Analytical method 01432 for the determination of BCS-CN88460 and the metabolite BCS-CY26497 in soil and sediment by HPLC-MS/MS
Report No.:	01432
Document No.:	M-499794-01-1
Guideline(s):	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 European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

The method was used to determine the content of isoflucypram and its carboxylic acid metabolite (BCS-CY26497) in soil and sediment samples from Environmental Fate studies. The method is validated in three different soils; Höfchen (silt loam), Laacher Hof (sandy loam) and Dollendorf (clay loam) and sediment (OECD 218/219). The method and validation parameters tested are summarised below and the results are presented in Tables B.5.1.2-1 and B.5.1.2-2.

Principle of the method

Soil/sediment samples were extracted with acetonitrile/water/acetic acid (40/10/0.3 v/v/v) in a microwave extractor. The resulting extract was spiked with internal standard solution of deuterated analytes, centrifuged to remove any fine particles, and then analysed by reverse phase HLPC-MS/MS using a YMC Ultra HT Hydrosphere C18, 30mm x 2mm x 2µm analytical column at 60 °C and gradient elution using a water (+1 ml/L formic acid)/acetonitrile (+ 1 ml/L formic acid) mobile phase. Two ion transitions (primary and confirmatory) were monitored for each analyte and each internal standard:

	Ion transitions	
	Primary	**Confirmatory**
Analyte		
BCS-CN88460	400.1→139.0	400.1→167.1
BCS-CY26497	430.1→177.0	430.1→412.1
Internal standard		
d5-BCS-CN88460	405.1→139.0	405.1→167.1
d5-BCS-CY26497	435.1→177.0	435.1→412.1

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) samples.

Linearity

Linearity was demonstrated by the analysis (in duplicate) of seven standards of increasing concentration. The range of standard concentrations used was 0.1 to 200 µg/L which is equivalent to 0.0002 to 0.40 mg/kg and

adequately encompasses the recovery concentrations of 0.001 mg/kg (LOQ) and 0.010 mg/kg (10xLOQ). The correlation coefficient is >0.99.

The impact of matrix effects was addressed by the use of deuterated internal standards.

Accuracy
Five control samples were fortified with reference standards at each of two levels and the samples analysed by the method described. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 110 %.

Precision
Precision in the form of %RSD was calculated for the accuracy determinations described above. Acceptable precision is demonstrated by <20%RSD.

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 0.001 mg/kg.

Confirmation
Residues were confirmed simultaneously to the primary detection by monitoring a second ion transition. Validation data as described above were submitted for the confirmatory method and the results are summarised in Table B.5.1.2-2.

Note that confirmatory methods are not required for methods used for risk assessment, but the submission of this additional data has been reported here for completeness.

Extraction efficiency
Not assessed for this method. The extraction procedure used in the soil degradation study reported in Section B.8.1.1.1.1. included triplicate extractions by shaking with acetonitrile/water (1:1 v/v), followed by two separate microwave extractions with different solvent/temperature combinations. This are summarise below:

Solvent	Volume	Minimum duration	Temperature	Extracts
ACN/H_2O 1/1 (*v/v*)	80 mL	30 min, shaking	ambient	3
ACN/H_2O 1/1 (*v/v*)	80 mL	10 min, stirring	microwave, 70°C	1
MeOH/H_2O 1/1 (*v/v*)	80 mL	10 min, stirring	microwave, 50°C	1

The majority of the radioactivity was extracted by shaking with acetonitrile/water (1:1 v/v) with at least 85% of the applied radioactivity being recovered in these extracts from all soils tested across all time points. There is no information relating to the proportion in each extract, but it can be assumed that the majority was extracted into the first extract, with smaller and smaller amounts being extracted by the two successive extractions.

The extraction procedure used in method 01432 involves a single microwave extraction with acetonitrile/water/acetic acid (40/10/0.3 v/v/v) therefore, based on the available information, it is not possible to assess the extraction efficiency of this method at this time. The applicant has been asked to comment. However, whilst it is scientifically valid to require this data (on the basis that residues can be incorporated into soil) , it is accepted that under Regulation (EU) No. 283/2013 there is no explicit requirement to do so and therefore the absence of this data is not regarded as a data gap.

Stability
Final soil/sediment extracts containing BCS-CN88460 and BCS-CY26497 were shown to be stable for at least 17 days when stored refrigerated in the dark.

Standard solutions of BCS-CN88460 were shown to be stable for at least 106 days when stored refrigerated in the dark.

Standard solutions of BCS-CY26497 were shown to be stable for at least 30 days when stored refrigerated in the dark.

Assessment

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram and BCS-CY26497 in soil and sediment samples at levels between 0.001 and 0.010 mg/kg.

The submission of the additional confirmatory data means that the method also complies with SANCO/825/00 rev.8.1 and is suitable for use as a monitoring method for soil and sediment at a lower limit of 0.001 mg/kg.

Table B.5.1.2-1 Summary of validation data for analytical method 01432 – **quantification** method for BCS-CN88460 and BCS-CY26497 residues in soil and sediment.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Soil (Höfchen) (silt loam)	BCS-CN88460 (*m/z* 400.1→139.0)	0.001	0.001 0.010	98-100 (99) 100 – 101 (100)	0.8 (5) 0.5 (5)	0.1 to 200 µg/L (0.0002 to 0.40 mg/kg) y = 1.1069x + 0.001605 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CY26497 (*m/z* 430.1→177.0)	0.001	0.001 0.010	94 – 100 (97) 98 – 101 (99)	2.7 (5) 1.4 (5)	0.1 to 200 µg/L (0.0002 to 0.40 mg/kg) y = 1.0304x + 0.0009279 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Soil (Laacher Hof) (sandy loam)	BCS-CN88460 (*m/z* 400.1→139.0)	10.001	0.001 0.010	96 – 99 (98) 100 – 102 (101)	1.3 (5) 1.1 (5)	0.1 to 200 µg/L (0.0002 to 0.40 mg/kg) y = 1.1069x + 0.001605 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CY26497 (*m/z* 430.1→177.0)	0.001	0.001 0.010	98 – 101 (99) 99 – 100 (100)	1.3 (5) 0.5 (5)	0.1 to 200 µg/L (0.0002 to 0.40 mg/kg) y = 1.0304x + 0.0009279 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Soil (Dollendorf) (clay loam)	BCS-CN88460 (*m/z* 400.1→139.0)	0.001	0.001 0.010	95 – 99 (97) 97 – 100 (98)	1.8 (5) 1.2 (5)	0.1 to 200 µg/L (0.0002 to 0.40 mg/kg) y = 1.1069x + 0.001605 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CY26497 (*m/z* 430.1→177.0)	0.001	0.001 0.010	95 - 101 (98) 95 – 102 (99)	2.2 (5) 2.8 (5)	0.1 to 200 µg/L (0.0002 to 0.40 mg/kg) y = 1.0304x + 0.0009279 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Sediment (OECD 218/219)	BCS-CN88460 (*m/z* 400.1→139.0)	0.001	0.001 0.010	97 – 100 (99) 97 – 100 (99)	1.2 (5) 1.2 (5)	0.1 to 200 µg/L (0.0002 to 0.40 mg/kg) y = 1.1069x + 0.001605 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CY26497 (*m/z* 430.1→177.0)	0.001	0.001 0.010	90 – 96 (94) 98 – 100 (98)	2.7 (5) 0.9 (5)	0.1 to 200 µg/L (0.0002 to 0.40 mg/kg) y = 1.0304x + 0.0009279 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Table B.5.1.2-2 Summary of validation data for analytical method 01432 - **confirmatory** method for BCS-CN88460 and BCS-CY26497 residues in soil and sediment.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Soil (Höfchen) (silt loam)	BCS-CN88460 (*m/z* 400.1→167.1)	0.001	0.001 0.010	98 – 99 (99) 101 – 104 (102)	0.6 (5) 1.2 (5)	0.1 to 200 µg/L (0.0002 to 0.40 mg/kg) y = 1.0929x +0.003210 r = 0.9996	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CY26497 (*m/z* 430.1→412.1)	0.001	0.001 0.010	86 – 96 (91) 99 – 104 (101)	4.4 (5) 1.9 (5)	0.1 to 200 µg/L (0.0002 to 0.40 mg/kg) Y = 0.9822x + 0.004511 R = 0.9996	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Soil (Laacher Hof) (sandy loam)	BCS-CN88460 (*m/z* 400.1→167.1)	0.001	0.001 0.010	97 – 100 (99) 99 – 103 (101)	1.4 (5) 1.5 (5)	0.1 to 200 µg/L (0.0002 to 0.40 mg/kg) y = 1.0929x +0.003210 r = 0.9996	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CY26497 (*m/z* 430.1→412.1)	0.001	0.001 0.010	89 – 94 (92) 97 – 103 (99)	2.3 (5) 2.4 (5)	0.1 to 200 µg/L (0.0002 to 0.40 mg/kg) Y = 0.9822x + 0.004511 R = 0.9996	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Soil (Dollendorf) (clay loam)	BCS-CN88460 (*m/z* 400.1→167.1)	0.001	0.001 0.010	99 – 101 (100) 99 – 101 (100)	0.9 (5) 0.8 (5)	00.1 to 200 µg/L (0.0002 to 0.40 mg/kg) y = 1.0929x +0.003210 r = 0.9996	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CY26497 (*m/z* 430.1→412.1)	0.001	0.001 0.010	83 – 96 (90) 95 – 98 (97)	5.5 (5) 1.3 (5)	0.1 to 200 µg/L (0.0002 to 0.40 mg/kg) Y = 0.9822x + 0.004511 R = 0.9996	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Sediment (OECD 218/219)	BCS-CN88460 (*m/z* 400.1→167.1)	0.001	0.001 0.010	93 – 101 (97) 100 – 101 (101)	3.1 (5) 0.5 (5)	0.1 to 200 µg/L (0.0002 to 0.40 mg/kg) y = 1.0929x +0.003210 r = 0.9996	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CY26497 (*m/z* 430.1→412.1)	0.001	0.001 0.010	82 – 98 (91) 99 -104 (101)	6.8 (5) 2.1 (5)	0.1 to 200 µg/L (0.0002 to 0.40 mg/kg) Y = 0.9822x + 0.004511 R = 0.9996	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

B.5.1.2.2. *Methods used in support of efficacy studies*

No analytical methods of analysis were used in support of efficacy studies.

B.5.1.2.3. *Methods used in support of toxicological studies*

B.5.1.2.3.1. Canine diet

Report:	KCA 4.1.2/02; Menettrier, P.; Vincent, M.; 2013; M-448255-01-1
Title:	BCS-CN88460 - Determination by high performance liquid chromatography analysis in canine diet
Report No.:	SA 12136
Document No.:	M-448255-01-1
Guideline(s):	O.E.C.D. Principles of Good Laboratory Practice, 1997 (January 26, 1998) and Article Annexe II à l'article D523-8 du Code de l'Environnement du 16 octobre 2007 (French GLP Legislation)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

The method was used to determine the content of isoflucypram in samples of canine diet from toxicological studies. The method and validation parameters tested are summarised below and the results are presented in Table B.5.1.2-3.

Principle of the method
Samples of canine diet were extracted twice by shaking with separate volumes of acetonitrile. The resulting extracts were centrifuged and combined, then analysed by reverse phase HLPC-UV (220 nm) using a Luna C18, 250mm x 4.6mm x 5µm analytical column and gradient elution using acetonitrile/water mobile phase. Quantification was by external standards.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample.

Linearity
Linearity was demonstrated by the analysis (in duplicate) of six standards of increasing concentration. The range of standard concentrations used was 1 to 25 mg/L, which is equivalent to 20 to 500 mg/kg and adequately encompasses the LOQ recovery concentration of 50 mg/kg. The other recovery sample extracts were diluted to be within the linear range.

The impact of matrix effects has not been specifically addressed. However, the excellent recovery data seen over three levels indicates that the method is performing accurately, and matrix effects are not indicated. Whilst it is possible that matrix enhancements could be offsetting lower recoveries to give an acceptable final value, the simplicity of the method (homogenisation of whole sample and analysis) provides little opportunity for loss of analyte and therefore it can be concluded that impact of any matrix effects is minimal.

Accuracy
Three control samples were fortified with reference standard at each of three levels and the samples analysed by the method described. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 110 %.

Precision
Precision in the form of %RSD was calculated for the accuracy determinations described above. Acceptable precision is demonstrated by <20%RSD.

LOQ

The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 50 mg/kg.

Extraction efficiency
Not assessed for this method as there are no incurred residues in the samples being analysed.

Stability
Stability of Isoflucypram in standards solutions and sample extracts has not been specifically addressed in this study but sufficient data are available throughout this Volume 3CA section B5 to demonstrate that stability is not an issue.

Assessment
SANCO/3029/99 rev. 4 suggests that accuracy and precision should be addressed by the analysis of 5 replicates at each of 2 levels. In this validation study, three replicates have been analysed at each of three levels. The available data indicate that the method is performing with a high degree of accuracy and precision compared to the acceptable guideline limits, and on that basis the presented data are satisfactory.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of canine diet at levels between 50 and 10000 mg/kg.

B.5.1.2.3.2. 0.5 % aqueous methylcellulose 400

Report:	KCA 4.1.2/04; Gomez, C.; Vincent, M.; 2012; M-432731-01-1
Title:	BCS-CN88460 determination by high performance liquid chromatography analysis in 0.5 percent aqueous methylcellulose 400
Report No.:	SA 12045
Document No.:	M-432731-01-1
Guideline(s):	O.E.C.D. Principles of Good Laboratory Practice, 1997 (January 26, 1998) and Article Annexe II à l'article D523-8 du Code de l'Environnement du 16 octobre 2007 (French GLP Legislation).
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

The method was used to determine the content of isoflucypram in samples of 0.5% aqueous methylcellulose 400 from toxicological studies. The method and validation parameters tested are summarised below and the results are presented in Table B.5.1.2-3.

Principle of the method
Aliquots of the aqueous suspension of methylcellulose were diluted with acetonitrile and analysed by reverse phase HLPC-UV (220 nm) using a Luna C18, 250mm x 4.6mm x 5µm analytical column and isocratic elution using acetonitrile/water (75:25 v/v) mobile phase. Quantification was by external standards.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample.

Linearity
Linearity was demonstrated by the analysis (in duplicate) of six standards of increasing concentration. The range of standard concentrations used was 1 to 25 mg/L, which is equivalent to approximately 50 to 1250 mg/L and adequately encompasses the LOQ recovery concentration of 100 mg/L. The other recovery sample extracts were diluted to be within the linear range.

The impact of matrix effects has not been specifically addressed. However, the excellent recovery data seen over three levels indicates that the method is performing accurately, and matrix effects are not indicated. Whilst it is possible that matrix enhancements could be offsetting lower recoveries to give an acceptable final value, the

simplicity of the method (homogenisation of whole sample and analysis) provides little opportunity for loss of analyte and therefore it can be concluded that impact of any matrix effects is minimal.

Accuracy
Three control samples were fortified with reference standard at each of three levels and the samples analysed by the method described. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 110 %.

Precision
Precision in the form of %RSD was calculated for the accuracy determinations described above. Acceptable precision is demonstrated by <20%RSD.

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 100 mg/L.

Extraction efficiency
Not assessed for this method as there are no incurred residues in the samples being analysed.

Stability
Stability of Isoflucypram in standards solutions and sample extracts has not been specifically addressed in this study but sufficient data are available throughout this section B5 to demonstrate that isoflucypram is stable in a range of standard solutions and sample extracts, therefore the RMS considers that further data are not required to address this.

Assessment
SANCO/3029/99 rev. 4 suggests that accuracy and precision should be addressed by the analysis of 5 replicates at each of 2 levels. In this validation study, three replicates have been analysed at each of three levels. The available data indicate that the method is performing with a high degree of accuracy and precision compared to the acceptable guideline limits, and on that basis the presented data are satisfactory.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of canine diet at levels between 100 and 200000 mg/L.

B.5.1.2.3.3. Ground rodent diet

BCS-CN88460 in ground rodent diet

Report:	KCA 4.1.2/06; Vincent, M.; Amir Tahmasseb, L.; 2012; M-435636-01-1
Title:	BCS-CN88460 - Determination by high performance liquid chromatography analysis in ground rodent diet
Report No.:	SA 12003
Document No.:	M-435636-01-1
Guideline(s):	OECD, 1997
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

The method was used to determine the content of isoflucypram in samples of ground rodent from toxicological studies. The method and validation parameters tested are summarised below and the results are presented in Table B.5.1.2-3.

Principle of the method
Samples of rodent diet were extracted twice by shaking with separate volumes of acetonitrile. The resulting extracts were centrifuged and combined, then analysed by reverse phase HLPC-UV (220 nm) using a Luna C18, 250mm x 4.6mm x 5µm analytical column and gradient elution using acetonitrile/water mobile phase. Quantification was by external standards.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample.

Linearity
Linearity was demonstrated by the analysis (in duplicate) of six standards of increasing concentration. The range of standard concentrations used was 1 to 25 mg/L, which is equivalent to approximately 20 to 500 mg/kg and adequately encompasses the LOQ recovery concentration of 50 mg/kg. The other recovery sample extracts were diluted to be within the linear range.

The impact of matrix effects has not been specifically addressed. However, the excellent recovery data seen over three levels indicates that the method is performing accurately, and matrix effects are not indicated. Whilst it is possible that matrix enhancements could be offsetting lower recoveries to give an acceptable final value, the simplicity of the method (homogenisation of whole sample and analysis) provides little opportunity for loss of analyte and therefore it can be concluded that impact of any matrix effects is minimal.

Accuracy
Three control samples were fortified with reference standard at each of three levels and the samples analysed by the method described. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 110 %.

Precision
Precision in the form of %RSD was calculated for the accuracy determinations described above. Acceptable precision is demonstrated by <20%RSD.

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 50 mg/kg.

Extraction efficiency
Not assessed for this method as there are no incurred residues in the samples being analysed.

Stability
Stability of Isoflucypram in standards solutions and sample extracts has not been specifically addressed in this study but sufficient data are available throughout this section B5 to demonstrate that isoflucypram is stable in a range of standard solutions and sample extracts, therefore the RMS considers that further data are not required to address this.

Assessment
SANCO/3029/99 rev. 4 suggests that accuracy and precision should be addressed by the analysis of 5 replicates at each of 2 levels. In this validation study, three replicates have been analysed at each of three levels. The available data indicate that the method is performing with a high degree of accuracy and precision compared to the acceptable guideline limits, and on that basis the presented data are satisfactory.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of ground rodent diet at levels between 50 and 20000 mg/kg.

BCS-CN45153 in ground rodent diet

Report:	KCA 4.1.2/24; Mathieu, C.; Vincent, M.; 2010; M-393802-01-1
Title:	BCS-CN45153 - Determination by high performance liquid chromatography analysis in ground rodent diet
Report No.:	SA 10145
Document No.:	M-393802-01-1
Guideline(s):	OECD, 1997
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

The method was used to determine the content of BCS-CN45153 in samples of ground rodent diet from toxicological studies. The method and validation parameters tested are summarised below and the results are presented in Table B.5.1.2-3.

Principle of the method
Samples of rodent diet were extracted twice by shaking with separate volumes of acetonitrile. The resulting extracts were centrifuged and combined, then analysed by reverse phase HLPC-UV (215 nm) using a Luna C18, 250mm x 4.6mm x 5µm analytical column and gradient elution using acetonitrile/water mobile phase. Quantification was by external standards.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample.

Linearity
Linearity was demonstrated by the analysis (in duplicate) of six standards of increasing concentration. The range of standard concentrations used was 0.25 to 5 mg/L, which is equivalent to approximately 5 to 100 mg/kg and adequately encompasses the LOQ recovery concentration of 10 mg/kg. The other recovery sample extracts were diluted to be within the linear range.

The impact of matrix effects has not been specifically addressed. However, the excellent recovery data seen over three levels indicates that the method is performing accurately, and matrix effects are not indicated. Whilst it is possible that matrix enhancements could be offsetting lower recoveries to give an acceptable final value, the simplicity of the method (homogenisation of whole sample and analysis) provides little opportunity for loss of analyte and therefore it can be concluded that impact of any matrix effects is minimal.

Accuracy
Three control samples were fortified with reference standard at each of three levels and the samples analysed by the method described. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 110 %.

Precision
Precision in the form of %RSD was calculated for the accuracy determinations described above. Acceptable precision is demonstrated by <20%RSD.

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 10 mg/kg.

Extraction efficiency
Not assessed for this method as there are no incurred residues in the samples being analysed.

Stability
Stability of BCS-CN45153 in standards solutions and sample extracts has not been specifically addressed in this study however in study VB1-AM029617MP1 reported in section B.5.1.1. above, it was shown to be stable in calibration and sample solutions for at least 120 hours at room temperature.

Assessment
SANCO/3029/99 rev. 4 suggests that accuracy and precision should be addressed by the analysis of 5 replicates at each of 2 levels. In this validation study, three replicates have been analysed at each of three levels. The available data indicate that the method is performing with a high degree of accuracy and precision compared to the acceptable guideline limits, and on that basis the presented data are satisfactory.

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of BCS-CN45153 in samples of ground rodent diet at levels between 10 and 25000 mg/kg.

Table B.5.1.2-3 Summary of validation data for the analytical methods used to determine concentration of BCS-CN88460 and BCS-CN45153 in oral dosing media used in toxicological studies.

Matrix	Analyte	LOQ (mg/kg)*	Recovery fortification level (mg/kg)*	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Canine diet	BCS-CN88460	50	50 1000 10000	93 – 96 (95) 95 – 97 (96) 98 – 100 (99)	1.6 (3) 1.0 (3) 1.0 (3)	1 to 25 mg/l (20 to 500 mg/kg) y = 28245x + 4516 r = 0.99912	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
0.5% aqueous methylcellulose	BCS-CN88460	100	100 10000 200000	95 - 96 (95) 94 - 98 (97) 99 – 100 (99)	0.6 (3) 2.4 (3) 0.6 (3)	1 to 25 mg/l (50 to 1250 mg/L) y = 32550x + 759 r = 0.99996	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Rodent diet	BCS-CN88460	50	50 1000 20000	99 – 100 (99) 104 – 109 (106) 97 – 99 (98)	0.6 (3) 2.7 (3) 1.0 (3)	1 to 25 mg/l (20 to 500 mg/kg) y = 32427x + 656 r = 0.99999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN45153	10	10 1000 25000	103 – 104 (103) 98 – 101 (99) 97 – 99 (98)	0.6 (3) 1.5 (3) 1.2 (3)	0.25 to 5 mg/l (5 to 100 mg/kg) y = 32047x - 392 r = 0.99999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

* units are mg/L for liquid samples

B.5.1.2.3.4. Plasma

Method No.: 01421

Report:	KCA 4.1.2/25; Desmaris, F.; 2014; M-499759-01-1
Title:	Analytical method no 01421 for the determination of BCS-CN88460 in rodent and dog plasma by HPLC-MS/MS
Report No.:	14-01
Document No.:	M-499759-01-1
Guideline(s):	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 • Guidance document on residue analytical methods, SANCO/3029/99 rev. 4.0 of July 11, 2000. European Commission, Directorate General Health and Consumer Protection US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

The method was used to determine the content of isoflucypram in samples of rodent plasma and dog plasma from toxicological studies. The method and validation parameters tested are summarised below and the results are presented in Table B.5.1.2-4.

Principle of the method
Samples of plasma were extracted by shaking on a vibratory mixer with acetonitrile/water (4:1 v/v). The resulting extract was centrifuged to give the raw extract which was then combined with internal standard solution of deuterated analyte and diluted with acetonitrile/water (1:4 v/v) to give the final extract. Analysis was performed by reverse phase HLPC-MS/MS using an Ascentis express C18, 50mm x 2.1mm x 2.7µm analytical column at 60 ^{o}C and gradient elution using water + 0.12 % formic acid + 10 mM ammonium formate and methanol/water (10/90 v/v) +10mM ammonium formate + 0.12% formic acid mobile phases. Two ion transitions were monitored (m/z 400.2 → 139 for quantification and m/z 400.2 → 177 for confirmation).

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample.

Linearity
Linearity was demonstrated by the analysis of eight standards of increasing concentration. The range of standard concentrations used was 0.025 to 10 µg/L, which is equivalent to 0.0025 to 1 mg/L in plasma samples and adequately encompasses the recovery concentrations of 0.01 mg/L (LOQ) and 0.1 mg/L (10 x LOQ). Where necessary, samples were diluted into the linear range.

The impact of matrix effects has been addressed by the use of deuterated internal standards

Accuracy
Five control samples were fortified with reference standard at each of three levels and the samples analysed by the method described. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 110 %.

Precision
Precision in the form of %RSD was calculated for the accuracy determinations described above. Acceptable precision is demonstrated by <20%RSD.

LOQ

The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 0.01 mg/L.

Confirmation
Residues were confirmed simultaneously to the primary detection by monitoring a second ion transition. Validation data as described above were submitted for the confirmatory method and the results are summarised in Table B.5.1.2-4.

Note that confirmatory methods are not required for methods used for risk assessment, but the submission of this additional data has been reported here for completeness.

Extraction efficiency
Not assessed in this study but the extraction efficiency of a similar method in several different animal products was assessed in Section B.5.1.2.5.3.

Stability
BCS-CN88460 was found to be stable:

- in stock solutions at 1000 mg/L in acetonitrile for 73 days, when stored at 4 °C ± 3 °C in the dark
- in solutions at 1 mg/L in acetonitrile for 27 days when stored at 4 °C ± 3 °C in the dark.
- in solutions at 1 mg/L and 10 µg/L in acetonitrile/Water, 20/80, v/v for 27 days when stored at 4 °C ± 3 °C in the dark.

BCS-CN88460 was found to be stable in raw and final extracts of rodent plasma and dog plasma for at least 54 hours and 51 hours respectively when stored at 4 °C ± 3 °C or 10 °C a.

Assessment
The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in rodent and dog plasma with a LOQ of 0.01 mg/L.

The confirmatory method is acceptably validated in accordance with SANCO/3029/99 rev.4 and SANCO/825/00 rev 8.1 for the determination of isoflucypram in rodent and dog plasma with a LOQ of 0.01 mg/L.

Table B.5.1.2-4 Summary of validation data for analytical method 01421 - determination of BCS-CN88460 in rodent and dog plasma.

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Rodent plasma	BCS-CN88460 (*m/z* 400.2→139)	0.01	0.01 0.10 1.00	100 – 102 (101) 101 – 103 (103) 98 – 107 (103)	0.8 (5) 0.9 (5) 3.2 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 1.06x + 0.00558 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (*m/z* 400.2→177)	0.01	0.01 0.10 1.00	98 – 102 (100) 100 – 102 (101) 96 – 104 (101)	1.4 (5) 0.8 (5) 3.1 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 0.592x + 0.00225 r = 1.0000	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Dog plasma	BCS-CN88460 (*m/z* 400.2→139)	0.01	0.01 0.10 1.00	98 – 101 (99) 100 – 102 (101) 103 – 109 (106)	1.1 (5) 1.6 (5) 2.3 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 1.06x + 0.00558 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (*m/z* 400.2→177)	0.01	0.01 0.10 1.00	94 – 99 (96) 97 – 100 (98) 101 – 106 (103)	2.0 (5) 1.2 (5) 1.9 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 0.592x + 0.00225 r = 1.0000	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Method no.: 01421/M001

Report:	KCA 4.1.2/26; Desmaris, F.; 2015; M-536997-01-1
Title:	Analytical method 01421/M001 for the determination of BCS-CN88460, BCS-CX99798 and BCS-CX99799 in rodent, rabbit and dog plasma by HPLC-MS/MS
Report No.:	01421/M001
Document No.:	M-536997-01-1
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and the Council of 21 October 2009 concerning the placing of plant protection products on the market Guidance document on residue analytical methods, SANCO/3029/99 rev. 4.0 of July 11, 2000. European Commission, Directorate General Health and Consumer Protection US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method of August 1996
Guideline deviation(s):	none
GLP/GEP:	**yes**

The method is a slight modification to method 01421 in that it includes the additional matrix, rabbit plasma, and determines the metabolites BCS-CX99798 and BCS-CX99799 in additional to the parent isoflucypram. It was used in support of toxicological studies. The method and validation parameters tested are summarised below and the results are presented in Table B.5.1.2-5 and B.5.1.2-6.

Principle of the method

Samples of plasma were extracted by shaking on a vibratory mixer with acetonitrile/water (4:1 v/v). The resulting extract was centrifuged to give the raw extract which was then combined with internal standard solution of deuterated analyte and diluted with acetonitrile/water (1:4 v/v) to give the final extract. Analysis was performed by reverse phase HLPC-MS/MS using an Ascentis express C18, 50mm x 2.1mm x 2.7µm analytical column at 60 ^{o}C and gradient elution using water + 0.12 % formic acid + 10 mM ammonium formate and methanol/water (10/90 v/v) +10mM ammonium formate + 0.12% formic acid mobile phases. Two ion transitions were monitored for each analyte as indicated below:

The following ion transitions were monitored for each matrix

Analyte	**1st MRM (quantitation)**	**2$_{nd}$ MRM (confirmation)**
BCS-CN88460	m/z 400.2 → 139	m/z 400.2 → 177
BCS-CX99798	m/z 220 → 163	m/z 220 → 87
BCS-CX99799	m/z 416 → 236	m/z 416 → 208

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample.

Linearity

Linearity was demonstrated by the analysis of eight standards of increasing concentration for each analyte. The range of standard concentrations used was 0.025 to 10 µg/L, which is equivalent to 0.0025 to 1 mg/L in plasma samples and adequately encompasses the recovery concentrations of 0.01 mg/L (LOQ) and 0.1 mg/L (10xLOQ). Where necessary, samples were diluted into the linear range.

The impact of matrix effects has been addressed by the use of deuterated internal standards

Accuracy

Five control samples were fortified with reference standard at each of three levels and the samples analysed by the method described. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 110 %.

Precision

Precision in the form of %RSD was calculated for the accuracy determinations described above. Acceptable precision is demonstrated by <20%RSD.

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 0.01 µg/L.

Confirmation
Residues were confirmed simultaneously to the primary detection by monitoring a second ion transition. Validation data as described above were submitted for the confirmatory method and the results are summarised in Table B.5.1.2-6.

Note that confirmatory methods are not required for methods used for risk assessment, but the submission of this additional data has been reported here for completeness.

Extraction efficiency
Not assessed in this study but the extraction efficiency of a similar method in several different animal products was assessed in Section B.5.1.2.5.3.

Stability
BCS-CN88460 and its metabolites BCS-CX99798, BCS-CX99799 were found to be stable:

- in stock solutions at 1000 mg/L in acetonitrile for at least 52 days, when stored at 4°C ± 3°C in the dark
- in solutions at 1 mg/L in acetonitrile for at least 52 days when stored at 4°C ± 3°C in the dark.

BCS-CN88460 and its metabolites BCS-CX99798, BCS-CX99799 were found to be stable in extracts of rodent, dog and rabbit plasma for at least 44, 46 and 49 hours respectively when stored at 4°C ± 3°C (raw extract) or 10°C (final extract).

Assessment
The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of BCS-CN88460, BCS-CX99798 and BCS-CX99799 in rodent, dog and rabbit plasma with a LOQ of 0.01 mg/L.

The confirmatory method is acceptably validated in accordance with SANCO/3029/99 rev.4 and SANCO/825/00 rev 8.1 for the determination of BCS-CN88460, BCS-CX99798 and BCS-CX99799 in rodent, dog and rabbit plasma with a LOQ of 0.01 µg/L.

Table B.5.1.2-5 Summary of validation data for analytical method 01421/M001 - **quantification** method for BCS-CN88460, BCS-CX99798 and BCS-CX99799 in rodent, dog and rabbit plasma.

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Rodent plasma	BCS-CN88460 (*m/z* 400.2→139)	0.01	0.01 0.10 1.00	105 – 110 (108) 107 – 110 (108) 94 – 99 (96)	2.0 (5) 1.3 (5) 2.3 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 1.05x + 0.00302 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CX99798 (*m/z* 220→163)	0.01	0.01 0.10 1.00	105 – 115 (109) 107 – 111 (108) 101 – 108 (105)	4.0 (5) 1.5 (5) 2.6 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 1.04x + 0.00873 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CX99799 (*m/z* 416→236)	0.01	0.01 0.10 1.00	107 – 121 (114) 109 – 113 (110) 103 – 108 (105)	4.4 (5) 1.6 (5) 2.0 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 2.27 - 0.00645 r = 0.9992	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
dog plasma	BCS-CN88460 (*m/z* 400.2→139)	0.01	0.01 0.10 1.00	107 – 111 (108) 107 – 109 (108) 99 – 102 (101)	1.8 (5) 0.8 (5) 1.1 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 1.05x + 0.00302 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CX99798 (*m/z* 220→163)	0.01	0.01 0.10 1.00	104 – 111 (108) 108 – 111 (110) 108 – 110 (109)	2.6 (5) 1.0 (5) 0.9 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 1.04x + 0.00873 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CX99799 (*m/z* 416→236)	0.01	0.01 0.10 1.00	113 – 118 (116) 108 – 112 (110) 106 – 109 (108)	1.8 (5) 1.4 (5) 1.1 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 2.27 - 0.00645 r = 0.9992	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Rabbit plasma	BCS-CN88460 (*m/z* 400.2→139)	0.01	0.01 0.10 1.00	106 – 110 (109) 108 – 111 (110) 90 – 99 (94)	1.5 (5) 1.4 (5) 3.5 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 1.05x + 0.00302 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CX99798 (*m/z* 220→163)	0.01	0.01 0.10 1.00	102 106 (103) 104 – 110 (107) 103 – 107 (105)	1.6 (5) 2.2 (5) 1.4 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 1.04x + 0.00873 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CX99799 (*m/z* 416→236)	0.01	0.01 0.10 1.00	108 – 120 (113) 105 – 111 (108) 101 – 109 (104)	4.0 (5) 2.5 (5) 2.9 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 2.27 - 0.00645 r = 0.9992	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Metabolites expressed as themselves, not as parent equivalents

Table B.5.1.2-6 Summary of validation data for analytical method 01421/M001 - **confirmatory** method for BCS-CN88460, BCS-CX99798 and BCS-CX99799 in rodent, dog and rabbit plasma.

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Rodent plasma	BCS-CN88460 (*m/z* 400.2→177)	0.01	0.01 0.10 1.00	103 – 112 (108) 107 – 110 (109) 94 – 100 (96)	3.1 (5) 1.2 (5) 2.7 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 0.667x + 0.00139 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CX99798 (*m/z* 220→87)	0.01	0.01 0.10 1.00	100 – 112 (108) 106 – 110 (109) 102 – 108 (105)	4.5 (5) 1.5 (5) 2.4 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 0.472x + 0.000939 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CX99799 (*m/z* 416→208)	0.01	0.01 0.10 1.00	106 – 114 (110) 110 – 111 (110) 103 – 110 (106)	2.9 (5) 0.5 (5) 2.8 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 1.13 + 0.00337 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
dog plasma	BCS-CN88460 (*m/z* 400.2→177)	0.01	0.01 0.10 1.00	107 – 110 (109) 107 – 109 (108) 100 – 102 (101)	1.0 (5) 0.7 (5) 0.8 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 0.667x + 0.00139 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CX99798 (*m/z* 220→87)	0.01	0.01 0.10 1.00	111 – 120 (116) 109 – 113 (111) 106 – 111 (109)	2.9 (5) 1.6 (5) 1.7 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 0.472x + 0.000939 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CX99799 (*m/z* 416→208)	0.01	0.01 0.10 1.00	102 – 113 (107) 110 – 114 (112) 105 – 111 (109)	4.2 (5) 1.8 (5) 2.5 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 1.13 + 0.00337 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Rabbit plasma	BCS-CN88460 (*m/z* 400.2→177)	0.01	0.01 0.10 1.00	104 – 110 (108) 106 – 110 (108) 90 – 98 (93)	2.1 (5) 1.5 (5) 3.3 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 0.667x + 0.00139 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CX99798 (*m/z* 220→87)	0.01	0.01 0.10 1.00	104 – 113 (109) 105 – 111 (108) 104 – 107 (106)	3.3 (5) 2.5 (5) 1.3 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 0.472x + 0.000939 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CX99799 (*m/z* 416→208)	0.01	0.01 0.10 1.00	105 – 118 (111) 107 – 111 (109) 100 – 105 (103)	4.4 (5) 1.5 (5) 2.3 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 1.13 + 0.00337 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Metabolites expressed as themselves, not as parent equivalents.

Method no.: 01549

Report:	KCA 4.1.2/27; Desmaris, F.; 2018; M-634664-01-1
Title:	Analytical method 01549 for the determination of BCS-CN88460 and its metabolites BCS-CX99798, BCS-CX99799, BCS-DC22055, BCS-DC20298, BCS-CY26497 and BCS-CY24813 in rodent plasma by HPLC-MS/MS
Report No.:	18-02
Document No.:	M-634664-01-1
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and 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[1] Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414, SANCO/3029/99 rev.4, July 2000 [2] OECD Guidance Document on Pesticide Residue Analytical Methods, Series on Testing and Assessment Document 72 and Series on Pesticides: Document 39, August 2007 (OECD Guideline, ENV/JM/MONO (2007) 17, Aug 13, 2007) [6]
Guideline deviation(s):	--
GLP/GEP:	**yes**

The analytical method 01549 was developed for the determination of BCS-CN88460 and its metabolites BCS-CX99798, BCS-CX99799, BCS-DC22055, BCS-DC20298, BCS-CY26497 and BCS-CY24813 in rodent plasma from toxicological studies. The method and validation parameters tested are summarised below and the results are presented in Table B.5.1.2-7.

Principle of the method

Samples of plasma were extracted by shaking on a vibratory mixer with acetonitrile/water (4:1 v/v). The resulting extract was centrifuged to give the raw extract which was then combined with internal standard solution of deuterated analytes and diluted with acetonitrile/water (1:4 v/v) to give the final extract. Analysis was performed by reverse phase HLPC-MS/MS using an Ascentis express C18, 100mm x 2.1mm x 2.7µm analytical column at 60 °C and gradient elution using water + 0.12 % formic acid + 10 mM ammonium formate and methanol/water (10/90 v/v) +10mM ammonium formate + 0.12% formic acid mobile phases. A single ion transition was monitored for each analyte as shown below:

Analyte	**Ion transition**
BCS-CN88460	*m/z* 400.2 → 139.0
BCS-CX99798	*m/z* 220.0 → 163.0
BCS-CX99799	*m/z* 416.0 → 236.0
BCS-DC22055	*m/z* 402.1 → 220.1
BCS-DC20298	*m/z* 416.2 → 398.0
BCS-CY26497	*m/z* 430.1 → 177.0
BCS-CY24813	*m/z* 416.2 → 234.1

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample.

Linearity

Linearity was demonstrated by the analysis of eight standards of increasing concentration for each analyte. The range of standard concentrations used was 0.025 to 10 µg/L, which is equivalent to 0.0025 to 1 mg/L in plasma samples and adequately encompasses the recovery concentrations of 0.01 mg/L (LOQ) and 0.1 mg/L (10xLOQ). Where necessary, samples were diluted into the linear range.

The impact of matrix effects has been addressed by the use of deuterated internal standards

Accuracy

Five control samples were fortified with reference standard at each of three levels and the samples analysed by the method described. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 110 %.

Precision
Precision in the form of %RSD was calculated for the accuracy determinations described above. Acceptable precision is demonstrated by <20%RSD.

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 0.01 mg/L.

Extraction efficiency
Not assessed in this study but the extraction efficiency of a similar in several different animal products was assessed in Section B.5.1.2.5.3

Stability
BCS-CN88460 and its metabolites BCS-CX99798, BCS-CX99799, BCS-DC22055, BCS-DC20298, BCSCY26497 and BCS-CY24813 were found to be stable:

- in rodent plasma when stored at -18°C or below for 416 hours,
- in the final extracts (subjected to LC-MS/MS analysis) when stored at approximately 10 °C for 53 hours.

Assessment
The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of BCS-CN88460, BCS-CX99798, BCS-CX99799, BCS-DC22055, BCS-DC20298, BCS-CY26497 and BCS-CY24813 in rodent plasma with a LOQ of 0.01 mg/L.

Table B.5.1.2-7 Summary of validation data for analytical method 01549 for the determination of BCS-CN88460, BCS-CX99798, BCS-CX99799, BCS-DC22055, BCS-DC20298, BCS-CY26497 and BCS-CY24813 in rodent plasma.

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Rodent plasma	BCS-CN88460 (*m/z* 400.2→139.0)	0.01	0.01 0.10	98 – 106 (102) 99 – 106 (102)	3.5 (5) 2.7 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 0.667x + 0.00139 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CX99798 (*m/z* 220.0→163.0)	0.01	0.01 0.10	93 – 101 (98) 96 – 105 (99)	3.0 (5) 3.9 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 0.472x + 0.000939 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CX99799 (*m/z* 416.0→236.0)	0.01	0.01 0.10	81 – 93 (88) 102 – 108 (105)	5.1 (5) 2.1 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 1.13 + 0.00337 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-DC22055 (*m/z* 402.1→220.1)	0.01	0.01 0.10	93 – 101 (98) 95 – 102 (98)	3.2 (5) 3.1 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 0.667x + 0.00139 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-DC20298 (*m/z* 416.2→398.0)	0.01	0.01 0.10	92 – 105 (101) 94 – 101 (97)	5.4 (5) 2.7 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 0.472x + 0.000939 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CY26497 (*m/z* 430.1→177.0)	0.01	0.01 0.10	91 – 101 (95) 98 – 104 (101)	4.6 (5) 2.8 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 1.13 + 0.00337 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CY24813 (*m/z* 416.2→234.1)	0.01	0.01 0.10	92 – 101 (97) 95 – 101 (98)	3.4 (5) 2.5 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/L) y = 0.667x + 0.00139 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Metabolites expressed as themselves not as parent equivalents.

B.5.1.2.4. *Methods used in support of operator, worker, resident and bystander studies.*

No analytical methods of analysis were used in support operator, worker, resident and bystander studies.

B.5.1.2.5. *Methods used in support of residues studies.*

B.5.1.2.5.1. **Plants**

Tomato, Orange, Wheat grain, Wheat straw, Rape seed, Bean seed (dry)

Report:	KCA 4.1.2/08; Uceda, L.; 2016; M-558986-01-1
Title:	Analytical method 01475 for the determination of residues of BCS-CN88460 and its metabolite BCS-CR60082 in/on plant by HPLC-MS/MS
Report No.:	**01475**
Document No.:	M-558986-01-1
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and 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 Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414, SANCO/3029/99 rev.4, July 2000 OECD Guidance Document on Pesticide Residue Analytical Methods, Series on Testing and Assessment Document 72 and Series on Pesticides: Document 39, August 2007 (OECD Guideline, ENV/JM/MONO (2007) 17, Aug 13, 2007) US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method of August 1996
Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCA 4.1.2/13; Traub, M.; 2018; M-609382-02-1
Title:	Amendment no.1 to final report - Extraction efficiency testing of the residue analytical method 01475 for the determination of residues of BCS-CN88460 in different wheat and soybean and oilseed rape RACs using incurred radioactive residues
Report No.:	S15-06246
Document No.:	M-609382-02-1
Guideline(s):	US EPA OCSPP Test Guideline No. 860.1340 OECD Series on Testing and Assessment No. 72 and Series on Pesticides No. 39 Guidance Document on Pesticide Residue Analytical Methods Commission Regulation (EU) No 283/2013 and 284/2013 in accordance with Regulation (EC) No 1107/2009
Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCA 4.1.2/14; Lamshoeft, M.; Doebbe, A.; 2017; M-598220-01-1
Title:	Extraction efficiency testing of the residue analytical method 01475 (data gathering method) for the determination of residues of BCS-CN88460 in the primary RAC tomato using incurred radioactive residues
Report No.:	EnSa-16-0204
Document No.:	M-598220-01-1
Guideline(s):	US EPA OCSPP Residue Chemistry Test Guideline OPPTS 860.1340: Residue Analytical Method Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009

Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCA 4.1.2/15; Lamshoeft, M.; Doebbe, A.; 2017; M-595703-01-1
Title:	Extraction efficiency testing of the residue analytical method 01475 (data gathering method) for the determination of residues of BCS-CN88460 and its metabolite BCS-CR60082 in succeeding RACs (turnip, Swiss chard, wheat) using incurred radioactive residues
Report No.:	EnSa-16-0179
Document No.:	M-595703-01-1
Guideline(s):	US EPA OCSPP Residue Chemistry Test Guideline OPPTS 860.1340: Residue Analytical Method Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009
Guideline deviation(s):	none
GLP/GEP:	**yes**

Method 01475 was used to determine the content of isoflucypram and its metabolite BCS-CR60082 in samples from residues trials. The method was validated in tomato, orange, wheat grain, wheat straw, rape seed and bean seed (dry). The method and validation parameters tested are summarised below and the results are presented in Tables B.5.1.2-8 and B.5.1.2-9.

Principle of the method

Samples were extracted twice by homogenisation (ultra turrax) with acetonitrile/water (8:2 v/v). The resulting extract was centrifuged, and the supernatants combined to give Extract A, which was filtered, diluted with water and spiked with internal standard solution of deuterated analytes to give the final extract. Final extracts were analysed by reverse phase HLPC-MS/MS using an Ascentis express C18, 50 mm x 2.1 mm x 2.7µm analytical column at 60 ºC and gradient elution using water/formic acid (1000/0.12 v/v) + 10 mM ammonium formate, water/methanol/formic acid (100/900/0.12 v/v/v) + 10 mM ammonium formate and water/methanol (500/500 V/v) mobile phases. Two ion transitions (primary and confirmatory) were monitored for each analyte and one for each internal standard.

Note 1: for dry crops (wheat grain, wheat straw, rape seed and bean seed), the extraction solvent was added as separate components; the water first, then after 20 minutes, the acetonitrile. The purpose of this was to soak the dry crops, thus assisting in the extraction efficiency. For wheat straw, the centrifugation step was replaced with a filtration through an empty SPE cartridge (with frit).

Note 2: Although not included in the validation, wheat green material (which may resemble a dry crop depending on the growth stage) was extracted by both methods and there was no significant difference between the two. Therefore, either method of extraction (with soaking or without) is applicable to the analysis of wheat/barley green material.

	Ion transitions	
	Primary	**Confirmatory**
Analyte		
BCS-CN88460	400.2→139	400.2→177
BCS-CR60082	234→177	234→157
Internal standard		
d5-BCS-CN88460	405.2→139	-
d5-BCS-CR60082	239→177	-

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) samples.

For the confirmatory method, lack of sensitivity meant it was not possible to rule out interferences in the control samples for orange and wheat straw >30% LOQ.

Linearity
Linearity was demonstrated by the analysis of eight standards of increasing concentration. The range of standard concentrations used was 0.01 to 2 µg/L which is equivalent to 0.002 to 0.40 mg/kg, and adequately encompasses the recovery concentrations of 0.01 (LOQ) and 0.10 mg/kg (10xLOQ). The correlation coefficient is >0.99.

The obtained sensitivity for BCS-CN88460 is higher than for BCS-CR60082. If only BCS-CN88460 is sought, the linearity range for BCS-CN88460 can be extended from 0.01 to 20 µg/L (corresponding to 0.002 mg/kg to 4 mg/kg), using a lower injection volume of 5 µL. The correlation coefficients for this lower range are 0.9999 for both MRM transitions.

The impact of matrix effects has been addressed by the use of deuterated internal standards.

Accuracy
Five control samples were fortified with reference standards at each of two levels and the samples analysed by the method described. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 110 %.

Precision
Precision in the form of %RSD was calculated for the accuracy determinations described above. Acceptable precision is demonstrated by <20%RSD.

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 0.01 mg/kg.

Confirmation
Note that confirmatory methods are not required for methods used for risk assessment, but the submission of this additional data has been reported here for completeness.

Residues were confirmed simultaneously to the primary detection by monitoring a second ion transition. Validation data as described above were submitted for the confirmatory method and the results are summarised in Table B.5.1.2-9.

Confirmatory data ae not available for BCS-CR60082 in rape seed and bean seed (dry) due to lack of sensitivity of the confirmatory method and a significant (>30%) interferent.

Confirmatory data are not available for BCS-CR60082 in orange and wheat straw at the LOQ due to lack of sensitivity of the confirmatory method.

Extraction efficiency
The efficiency of the extraction procedure used in analytical method 01475 was assessed for various crops by comparing the levels of incurred radioactive residues recovered by method 01475 and comparing them to the levels determined in plant metabolism and confined rotational crop metabolism studies. Extraction efficiencies were assessed for:

- BCS-CN88460 in tomato fruits from the tomato metabolism study performed with [pyrazole-4-^{14}C]BCS-CN88460
- BCS-CN88460 in wheat (hay, straw and grains), soybean (hay, forage, straw and seeds) and oilseed rape (intermediate harvest and seeds) from the respective metabolism studies performed with [pyrazole-4-^{14}C]BCS-CN88460
- BCS-CN88460 and its metabolite BCS-CR60082 in wheat (forage, hay, straw and grain), swiss chard and turnip leaves from the confined rotational crop metabolism study, also performed with [pyrazole-4-^{14}C]BCS-CN88460

The individual studies are described below and demonstrate that method 01475 (and consequently the monitoring method 01520) can adequately extract BCS-CN88460 from tomato, wheat (hay, straw and grains), soybean (hay,

forage, straw and seeds) and oilseed rape (intermediate harvest and seeds), which cover dry, high oil and high water crop groupings.

The method is also capable of efficiently extracting the metabolite, BCS-CR80062, from wheat (forage, hay, straw and grain), swiss chard and turnip leaves (dry and high water crop groups).

The extraction efficiency of method 01475 is not proven for high acid crops with respect to parent BCS-CN88460 or its metabolite BCS-CR80062.

M598220-01-1 (Lamshoeft, M.; Doebbe, A.; 2017)
In this study, stored, non-surface washed tomato fruits were homogenized and an aliquot was extracted using residue analytical method 01475. Extractable radioactivity accounted for 99.6% of the total radioactive residue (TRR), which was calculated by addition of radioactivity recovered in the extracts and that remaining in the post extraction solids. The major fraction in the extract was identified as parent compound BCS-CN88460 and represented 97.8% of the TRR.

A Solid Phase Extraction (SPE) clean-up/concentration step was applied to the samples following extraction and prior to HPLC analysis which is not included in method 01475. There were no losses of radioactivity during this step, therefore the impact of this additional step is negligible.

In the tomato metabolism study, 99.7% of the TRR was extracted from tomato fruits by surface washing with dichloromethane and repeat extractions (3 times) with acetonitrile/water (4:1 v/v). Analysis revealed that 96.7% TRR was made up of parent compound, BCS-CN88460.

Extraction efficiency was calculated by comparing the level of BCS-CN88460 determined by method 01475 with level determined in the tomato metabolism study. Due to the inhomogeneity of the tomato fruits, the absolute amounts (mg/kg) determined in the fruits after the different extractions varied distinctly, therefore the extraction efficiency calculation was based on %TRR rather than absolute residue level:

$$\text{Extraction Efficiency (\%)} = \frac{\text{97.8\% of TRR (residue analytical method)}}{\text{96.7\% of TRR (metabolism study)}} \times 100\% = 101.1\%$$

The extraction method in residue analytical method 01475 is therefore suitable for the extraction of BCS-CN88460 from tomato fruit and by extrapolation, from all high water crops.

M609382-02-1 (Traub, M.; 2018)
In this study, samples of wheat (hay, straw and grains), soybean (hay, forage, straw and seeds) and oilseed rape (intermediate harvest and seeds) were extracted using residue analytical method 01475. Dry crops (wheat – hay, straw and grain, soybean- hay, straw and seed, and oilseed rape seed) were soaked in the water part of the extraction solvent prior to addition of the acetonitrile and commencement of blending. This is consistent with method 01475 for dry crops.

A Solid Phase Extraction (SPE) clean-up/concentration step was applied to the samples following extraction and prior to HPLC analysis which is not included in method 01475. There were no losses of radioactivity during this step, therefore the impact of this additional step is negligible.

A summary of the extractability of the method and distribution of the radioactivity is presented in the table below along with a corresponding summary of data from the relevant metabolism studies.

Matrix	Distribution of radioactivity	Summary of Extraction				Efficiency of Extraction (%)
		Residue method 01475		Metabolism study		
		%TRR	mg/kg	%TRR	mg/kg	
Wheat hay	TRR	100	3.849	100	4.032	
	Extracted	89.2	3.350	95.8	3.864	
	- BCS-CN88460	40.4	1.517	50.0	2.016	81
	Unextracted	10.8	0.406	4.2	0.168	
Wheat straw	TRR	100	15.659	100	15.536	
	Extracted	86.3	13.511	98.7	15.330	
	- BCS-CN88460	61.8	9.674	64.0	9.933	97

	Unextracted	13.7	2.148	1.3	0.206	
Wheat grain	TRR	100	0.380	100	0.385	
	Extracted	94.4	0.358	93.6	0.360	
	- BCS-CN88460	86.0	0.327	92.0	0.354	93
	Unextracted	5.6	0.021	6.4	0.025	
Soybean forage	TRR	100	3.647	100	4.371	
	Extracted	92.7	3.380	97.2	4.248	
	- BCS-CN88460	20.0	0.731	18.7	0.819	107
	Unextracted	7.3	0.267	2.8	0.123	
Soybean hay	TRR	100	4.705	100	4.679	
	Extracted	85.3	4.013	94.3	4.413	
	- BCS-CN88460	9.0	0.425	10.4	0.487	87
	Unextracted	14.7	0.692	5.7	0.266	
Soybean straw	TRR	100	17.53	100	17.715	
	Extracted	93.6	16.413	96.6	17.110	
	- BCS-CN88460	73.5	12.895	64.5	11.424	114
	Unextracted	6.4	1.117	3.4	0.605	
Soybean seed	TRR	100	0.027	100	0.035	
	Extracted	83.8	0.023	87.7	0.031	
	- BCS-CN88460	83.8	0.023	76.6	0.027	109
	Unextracted	16.2	0.004	12.3	0.004	
Oilseed rape (intermediate harvest)	TRR	100	5.423	100	4.7581	
	Extracted	95.3	5.166	99.5	4.730	
	- BCS-CN88460	79.2	4.292	81.9	3.890	97
	Unextracted	4.7	0.257	0.5	0.022	
Oilseed rape seed	TRR	100	0.101	100	0.099	
	Extracted	70.5	0.071	93.3	0.093	
	- BCS-CN88460	70.5	0.071	71.0	0.070	99
	Unextracted	29.5	0.030	6.7	0.006	

The extraction method in residue analytical method 01475 is suitable for the extraction of BCS-CN88460 from the crops investgated which cover dry, high oil and high water crops. No extraction efficiency data is available for highly acidic crops, but this crop group is not relevant to the proposed use on wheat.

M595703-01-1 (Lamshoeft, M.; Doebbe, A.; 2017)

In this study, samples of wheat (forage, hay, straw and grain), swiss chard and turnip leaves from the confined rotational metabolism study were extracted using residue analytical method 01475. As is described in the method, the dry crops (wheat hay, wheat straw and wheat grain) had the extraction solvent added as separate components; the water first, then after 20 minutes, the acetonitrile. A summary of the extractability of the method and distribution of the radioactivity is presented in the table below along with a corresponding summary of data from the confined rotational crop metabolism study.

Matrix	Distribution of radioactivity	Summary of Extraction				Efficiency of Extraction (%)
		Residue analytical method		CRC metabolism study		
		%TRR	mg/kg	%TRR	mg/kg	
Wheat hay	TRR	100.0	0.201	100.0	0.187	
	Extracted	81.9	0.164	85.4	0.160	
	- BCS-CN88460	-	-	-	-	-
	- BCS-CR80062	2.5	0.0050	2.3	0.0043	109
	Unextracted	18.1	0.036	14.6	0.027	
Wheat straw	TRR	100.0	0.366	100.0	0.340	
	Extracted	71.0	0.260	83.7	0.284	
	- BCS-CN88460	-	-	-	-	-
	- BCS-CR80062	4.2	0.0153	5.8	0.0199	72
	Unextracted	29.0	0.106	16.3	0.055	
Wheat grain	TRR	100.0	0.014	100.0	0.016	
	Extracted	63.4	0.009	53.7	0.009	
	- BCS-CN88460	-	-	-	-	-
	- BCS-CR80062	-	-	-	-	-
	Unextracted	36.6	0.005	46.3	0.008	
Wheat forage	TRR	100.0	0.073	100	0.072	
	Extracted	92.2	0.067	91.9	0.066	

	- BCS-CN88460	-	-	-	-	-
	- BCS-CR80062	4.2	0.0031	4.3	0.0031	98
	Unextracted	7.8	0.006	8.1	0.006	
Swiss chard	TRR	100	0.026	100	0.026	
	Extracted	95.5	0.025	96.0	0.025	
	- BCS-CN88460	6.7	0.0018	4.6	0.0012	146
	- BCS-CR80062	17.0	0.0045	19.4	0.0051	88
	Unextracted	4.5	0.001	4.0	0.001	
Turnip leaves	TRR	100	0.019	100	0.018	
	Extracted	91.5	0.017	92.3	0.017	
	- BCS-CN88460	3.4	0.0006	4.8	0.0009	71
	- BCS-CR80062	17.6	0.0034	18.4	0.0033	96
	Unextracted	8.5	0.002	7.7	0.001	

The extraction efficiency of method 01475 with respect to the metabolite BCS-CR80062 has been demonstrated in wheat (forage, hay, straw and grain), swiss chard and turnip leaves.

With regards to the extraction efficiency of parent BCS-CN88460, only the swiss chard and turnip leaf samples contained any incurred residues, and even then, they were at such low levels that interpretation of the results was difficult. However, the previous two studies adequately demonstrate the extraction efficiency of the method for several crops covering dry, high oil and high water crop groups.

Stability

BCS-CN88460 and BCS-CR60082 were found to be stable in Extract A and final extracts for at least 105 hours in tomato, 149 hours in orange, 82 hours in wheat grain, 79 hours in wheat straw, 54 hours in rape seed and 77 hours in bean seed (dry) when stored at 4 °C ± 3 °C (Extract A) or 10 °C ± 3 °C (Final extract) in the dark.

Standard solutions of BCS-CN88460 and BCS-CR60082 at 1 mg/L and 1 g /L were shown to be stable for at least 106 days when stored refrigerated in the dark.

Assessment

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram and BCS-CR60082 residues in tomato, wheat grain, wheat straw, rape seed and bean seed (dry), with a LOQ of 0.01 mg/kg.

The confirmatory method is acceptably validated in accordance with SANCO/3029/99 rev.4 for isoflucypram in tomato, wheat grain, wheat straw, rape seed and bean seed (dry), with a LOQ of 0.01 mg/kg.

For BCS-CR60082, limitations with the sensitivity of the method in some matrices means that the confirmatory method is acceptably validated in tomato and wheat grain with a LOQ of 0.01 mg/kg, and in wheat straw with a LOQ of 0.1 mg/kg but is not validated in rape seed or bean seed (dry).

Regarding orange, and by extrapolation, high acid crops, acceptable recovery, precision, linearity and specificity data are available for the parent and the metabolite but the extraction efficiency of the method has not been demonstrated in this crop group. As a high acid crop is not proposed as the representative use for this evaluation, a data gap is not identified at this time but extraction efficiency of the method with respect to high acid crops will be required to support any future use on crops from this group.

Table B.5.1.2-8 Summary of validation data for analytical method 01475 - **quantification** method for BCS-CN88460 and BCS-CR60082 residues in tomato, orange, wheat grain, wheat straw, rape seed and bean seed.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Tomato	BCS-CN88460 (*m/z* 400.2→139)	0.01	0.01 0.1	87 – 95 (92) 89 – 96 (93)	3.9 (5) 2.9 (5)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 1.11x – 0.000277 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234→177)	0.01	0.01 0.1	80 – 87 (84) 86 – 92 (90)	3.6 (5) 2.8 (5)	0.01 to 1 µg/L (0.002 to 0.2 mg/kg) y = 0.0653x + 0.00869 r = 0.9994	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Orange	BCS-CN88460 (*m/z* 400.2→139)	0.01	0.01 0.1	96 – 99 (97) 99 – 101 (100)	1.6 (5) 0.9 (5)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 1.07x + 0.0105 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234→177)	0.01	0.01 0.1	71 – 75 (73) 86 – 91 (88)	2.3 (5) 2.3 (5)	0.01 to 1 µg/L (0.002 to 0.2 mg/kg) y = 0.621x + 0.0154 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Wheat grain	BCS-CN88460 (*m/z* 400.2→139)	0.01	0.01 0.1	90 – 100 (94) 91 - 96 (93)	3.9 (5) 1.9 (5)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 1.12x + 0.00857 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234→177)	0.01	0.01 0.1	83 -89 (85) 87 – 90 (88)	2.7 (5) 1.7 (5)	0.01 to 1 µg/L (0.002 to 0.2 mg/kg) y = 0.684x + 0.00583 r = 1.0000	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Wheat straw	BCS-CN88460 (*m/z* 400.2→139)	0.01	0.01 0.1	86 – 90 (89) 90 – 93 (92)	1.9 (5) 1.5 (5)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 1.12x + 0.0098 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234→177)	0.01	0.01 0.1	72 – 76 (75) 80 – 85 (83)	2.2 (5) 2.3 (5)	0.01 to 1 µg/L (0.002 to 0.2 mg/kg) y = 0.661x + 0.00796 r = 0.9995	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Rape seed	BCS-CN88460 (*m/z* 400.2→139)	0.01	0.01 0.1	94 – 97 (95) 97 – 103 (99)	1.2 (5) 2.4 (5)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 1.08x + 0.00928 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234→177)	0.01	0.01 0.1	102 – 119 (107) 100 – 108 (103)	6.3 (5) 3.0 (5)	0.01 to 1 µg/L (0.002 to 0.2 mg/kg) y = 0.603x + 0.0147 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Bean seed (dry)	BCS-CN88460 (*m/z* 400.2→139)	0.01	0.01 0.1	93 – 94 (94) 96 – 99 (97)	0.5 (5) 1.3 (5)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 1.08x + 0.0103 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234→177)	0.01	0.01 0.1	82 – 87 (86) 93 – 96 (94)	2.4 (5) 1.2 (5)	0.01 to 1 µg/L (0.002 to 0.2 mg/kg) y = 0.598x + 0.013 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Table B.5.1.2-9 Summary of validation data for analytical method 01475- **confirmatory** method for BCS-CN88460 and BCS-CR60082 residues in tomato, orange, wheat grain, wheat straw, rape seed and bean seed.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Tomato	BCS-CN88460 (*m/z* 400.2→177)	0.01	0.01 0.1	89 – 95 (93) 89 – 95 (93)	2.7 (5) 2.7 (5)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 0.876x – 0.0011 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234→157)	0.01	0.01 0.1	81 – 84 (82) 85 – 94 (90)	1.6 (5) 3.6 (5)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 0.0433x + 0.000568 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Orange	BCS-CN88460 (*m/z* 400.2→177)	0.01	0.01 0.1	96 – 101 (99) 101 – 102 (101)	2.2 (5) 0.4 (5)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 0.814x – 0.00781 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234→157)	0.01	0.01 0.1	- 82 – 92 (89)	- 4.3 (5)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 0.04x + 0.000988 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Wheat grain	BCS-CN88460 (*m/z* 400.2→177)	0.01	0.01 0.1	87 – 97 (91) 90 – 95 (93)	4.0 (5) 2.1 (5)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 0.864x + 0.00685 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234→157)	0.01	0.01 0.1	79 – 102 (89) 85 – 89 (87)	9.6 (5) 1.7 (5)	0.1 to 200 µg/L (0.2 to 400 µg/kg) Y = 0.0456x + 0.000213 R = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Wheat straw	BCS-CN88460 (*m/z* 400.2→177)	0.01	0.01 0.1	87 – 92 (89) 89 – 93 (91)	2.1 (5) 2.3 (5)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 0.852 x + 0.00728 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234→157)	0.01	0.01 0.1	- 76 – 83 (80)	- 3.5 (5)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 0.045x + 0.000204 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Rape seed	BCS-CN88460 (*m/z* 400.2→177)	0.01	0.01 0.1	90 – 96 (94) 98 -105 (101)	3.1 (5) 2.8 (5)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 0.823x + 0.00731 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234→157)	0.01	0.01 0.1	- -	- -	-	-
Bean seed (dry)	BCS-CN88460 (*m/z* 400.2→177)	0.01	0.01 0.1	94 – 98 (96) 97 – 100 (98)	2.1 (5) 1.4 (5)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 0.824x + 0.00671 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234→157)	0.01	0.01 0.1	- -	- -	-	

Barley (green material, grain and straw)

Report:	KCA 4.1.2/09; Schulte, G.; 2017; M-584388-02-1
Title:	Amendment no. 1 to final report - Determination of the residues of BCS-CN88460 in/on barley after spray application of BCS-CN88460 EC 050 in Portugal, southern France and Spain
Report No.:	15-2066
Document No.:	M-584388-02-1
Guideline(s):	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 OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Guideline deviation(s):	none
GLP/GEP:	**yes**

Method 01475 (as described above) was used to determine the content of isoflucypram in samples of barley green material, barley grain and barley straw in samples from four supervised residues trials reported in study number 15-2066.

Method 01475 has been acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of isoflucypram and BCS-CR60082 in five matrices representative of the major crop groupings; tomato (high water), orange (high acid), wheat grain (dry), wheat straw (no group), rape seed (high oil) and bean seed (dry).

In accordance with SANCO/3029/99 rev 4. it is acceptable to submit a reduced validation set for additional matrices if the method used is fully validated for a comparable matrix within the same crop group. Therefore, a reduced validation set has been submitted for barley green material (high water), barley grain (dry) and barley straw. The additional validation data, comprising one control and three replicate recovery determinations at two fortification levels, as well as example calibration graphs and chromatograms are summarised in Table B.5.1.2-10.

Procedural recoveries analysed alongside the study samples were in the range 80 – 104% across all matrices and fortification levels. Higher level recovery levels (up to 2 mg/kg) were performed in green material and straw to cover the levels determined in the trial samples since the highest level in the validation study was 0.1 mg/kg. Extracts were diluted into the linear range as appropriate.

The LOQ for isoflucypram in these additional matrices is 0.01 mg/kg. No residues above 30% of the LOQ were detected in the control samples.

Isoflucypram residues were found to be stable in all extract for at least 81 hours. This covers the duration of storage in the study.

Assessment

The method is acceptably validated in accordance with SANCO/3029/99 rev. 4 and supports the determination of isoflucypram residues in barley (green material, grain and straw) at the levels found in study 15-2066.

Wheat (green material, grain and straw)

Report:	KCA 4.1.2/10; Schulte, G.; 2017; M-584384-02-1
Title:	Amendment no. 1 to final report - Determination of the residues of BCS-CN88460 in/on wheat and durum after spray application of BCS-CN88460 EC 050 in Portugal, southern France and Spain
Report No.:	15-2069
Document No.:	M-584384-02-1
Guideline(s):	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 OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP 860.1500, Crop Field Trial
Guideline deviation(s):	yes, see report
GLP/GEP:	**yes**

Method 01475 (as described above) was used to determine the content of isoflucypram in samples of wheat green material, wheat grain and wheat straw in samples from the supervised residues trials reported in study number 15-2069.

Method 01475 has been acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of isoflucypram and BCS-CR60082 in five matrices representative of the major crop groupings; tomato (high water), orange (high acid), wheat grain (dry), wheat straw (no group), rape seed (high oil) and bean seed (dry).

Additional validation data have not been presented for wheat grain and straw as these were fully validated within the original method validation, but supplementary validation data have been presented for wheat green material (high water) as this matrix was not included in the original validation. This supplementary validation data consists of one control and three replicate recovery determinations at two fortification levels, as well as example calibration graphs and chromatograms are summarised in Table B.5.1.2-10. This is in accordance with SANCO/3029/99 rev 4, which states that a reduced validation set is acceptable for additional matrices if the method used is fully validated for a comparable matrix within the same crop group, in this case high water.

Procedural recoveries analysed alongside the study samples were in the range 89 - 107% across all matrices and fortification levels. Higher level recovery levels, up to 2 mg/kg and 2.5 mg/kg, were performed in straw and green material respectively to cover the levels determined in the trial samples since the highest level in the validation study was 0.1 mg/kg. Extracts were diluted into the linear range as appropriate.

The LOQ for isoflucypram in these additional matrices is 0.01 mg/kg. No residues above 30% of the LOQ were detected in the control samples.

Isoflucypram residues were found to be stable in all extract for at least 81 hours. This covers the duration of storage in the study.

The stability of isoflucypram in wheat grain and straw extracts was addressed in study 15-12 and found to be 82 and 79 hours respectively. In the current study (15-2069), isoflucypram was found to be stable in wheat green material extracts for at least 98 hours. These periods of storage cover the duration of storage in the study.

Assessment

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and supports the determination of isoflucypram residues in wheat (green material, grain and straw) at the levels found in study 15-2069.

Table B.5.1.2-10 Summary of supplementary validation data for analytical method 01475 for determination of BCS-CN88460 residues in barley and wheat

Matrix	Analyte	LOQ (µg/kg)	Recovery fortification level (µg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Barley green material	BCS-CN88460 (*m/z* 400.2→139)	0.01	0.01 0.1	81 – 91 (86) 80 – 93 (85)	5.9 (3) 8.5 (3)	0.01 to 2.5 µg/L (0.002 to 0.5 mg/kg) y = 1.9173x + 0.0057346 r = 0.9994	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Barley grain		0.01	0.01 0.1	94 – 98 (96) 100 – 101 (100)	2.2 (3) 0.6 (3)		Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Barley straw		0.01	0.01 0.1	97 – 121 (105) 89 – 101 (97)	12.9 (3) 6.9 (3)		Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Wheat green material		0.01	0.01 0.1	96 – 104 (99) 95 – 100 (97)	4.4 (3) 2.7 (3)	0.01 to 2.5 µg/L (0.002 to 0.5 mg/kg) y = 1.9910x + 0.0054586 r = 0.9978	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Following crops

Report:	KCA 4.1.2/11; Freitag, T.; Effertz, C.; 2017; M-605725-01-1
Title:	Determination of the residues of BCS-CN88460 in/on soil and the field rotational crops barley, carrot, turnip and lettuce after spray application of BCS-CN88460 EC 050 to bare soil in Germany, the Netherlands, southern France and Italy
Report No.:	15-2502
Document No.:	M-605725-01-1
Guideline(s):	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 OECD Guidelines for the Testing of Chemicals. Residues in Rotational Crops (Limited Field Studies). 504. 2007-01-08 OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) US EPA OCSPP Guideline No. 860.1900, Field Accumulation in Rotational Crops US EPA OCSPP Guideline No. 860.1500 on Crop Field Trial
Guideline deviation(s):	yes, see report
GLP/GEP:	**yes**

Barley, carrots, turnip and lettuce

Method 01475 (as described above) was used to determine the content of isoflucypram and BCS-CR60082 in samples of barley (green material, grain and straw), carrot (tuber and leaf), turnip (tuber and leaf), and lettuce (head) in samples from the rotational crop supervised residues trials reported in study number 15-2502.

Method 01475 has been acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of isoflucypram and BCS-CR60082 in five matrices representative of the major crop groupings; tomato (high water), orange (high acid), wheat grain (dry), wheat straw (no group), rape seed (high oil) and bean seed (dry).

In accordance with SANCO/3029/99 rev 4. it is acceptable to submit a reduced validation set for additional matrices if the method used is fully validated for a comparable matrix within the same crop group. Therefore, a reduced validation set has been submitted for all of the matrices analysed in this rotational crop study, with the exception of barley green material for which a full validation data set has been generated. The reason for generating a full validation data set for barley green material is that within the original validation study, the comparison between the extraction procedures for 'wet' and 'dry' crops showed no difference, but this was only assessed for the parent compound. Therefore, as comparable data were not available for the metabolite it was decided to take a precautionary approach and use the most efficient extraction procedure, thus a full validation data set was performed. The additional validation data are summarised in Table B.5.1.2-11.

Procedural recoveries analysed alongside the study samples were in the range 76 - 109% across all matrices and fortification levels.

Assessment

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and supports the determination of isoflucypram and BCS-CR60082 residues in barley (green material, grain and straw), carrot (tuber and leaf), turnip (tuber and leaf), and lettuce (head) in samples from the rotational crop supervised residues trials reported in study number 15-2502.

Soil

Method 01432 (as described in Section B.5.1.2.1.1.) was used to determine the content of isoflucypram and BCS-CY60082 in samples of soil from the rotational crop supervised residues trials reported in study number 15-2502.

Validation data in accordance with SANCO/3029/99 rev 4. were reported with Method Report No. 01432 (Koch, V.; 2014; M-499794-01-1) and evaluated under section B.5.1.2.1.1. therefore, further validation data are not required. Chromatograms of control samples were submitted to demonstrate lack of interferences in the soil samples under investigation.

Procedural recoveries analysed alongside the study samples were in the range 95-104% for Isoflucypram and 73 -108% for BCS-CN88460 carboxylic acid, at levels of 0.001 and 0.01 mg/kg.

Assessment

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and supports the determination of isoflucypram and BCS-CY26497 residues in soil samples from the rotational crop supervised residues trials reported in study number 15-2502.

Table B.5.1.2-11 Summary of supplementary validation data for analytical method 01475 for determination of BCS-CN88460 and BCS CR60082 residues in barley, carrot, turnip and lettuce.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Barley green material	BCS-CN88460 (*m/z* 400.2→139.0)	0.01	0.01 0.1	101 – 108 (104) 97 – 117 (105)	3.1 (5) 7.0 (5)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 2.098x + 0.00101 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234.0→177.0)	0.01	0.01 0.1	84 – 90 (87) 92 – 109 (99)	3.1 (5) 6.4 (5)	0.0058 to 0.58 µg/L (0.002 to 0.2 mg/kg) y = 2.22x + 0.00235 r = 0.9993	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Barley grain	BCS-CN88460 (*m/z* 400.2→139.0)	0.01	0.01 0.1	94 – 97 (95) 96 – 100 (98)	1.6 (3) 2.0 (3)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 2.098x + 0.00101 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234.0→177.0)	0.01	0.01 0.1	86 – 92 (90) 95 – 95 (95)	3.6 (3) 0.0 (3)	0.0058 to 0.58 µg/L (0.002 to 0.2 mg/kg) y = 2.22x + 0.00235 r = 0.9993	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Barley straw	BCS-CN88460 (*m/z* 400.2→139.0)	0.01	0.01 0.1	93 – 100 (96) 87 – 100 (95)	3.6 (3) 7.4 (3)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 2.098x + 0.00101 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234.0→177.0)	0.01	0.01 0.1	90 – 92 (91) 87 – 98 (94)	1.3 (3) 6.3 (3)	0.0058 to 0.58 µg/L (0.002 to 0.2 mg/kg) y = 2.22x + 0.00235 r = 0.9993	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Carrot leaf	BCS-CN88460 (*m/z* 400.2→139.0)	0.01	0.01 0.1	104 – 107 (106) 103 – 105 (104)	1.6 (3) 1.1 (3)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 2.098x + 0.00101 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234.0→177.0)	0.01	0.01 0.1	92 – 95 (94) 96 – 103 (101)	1.6 (3) 4.0 (3)	0.0058 to 0.58 µg/L (0.002 to 0.2 mg/kg) y = 2.22x + 0.00235 r = 0.9993	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Carrot root	BCS-CN88460 (*m/z* 400.2→139.0)	0.01	0.01 0.1	100 -100 (100) 103 – 107 (106)	0.0 (3) 2.2 (3)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 2.098x + 0.00101 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234.0→177.0)	0.01	0.01 0.1	92 – 94 (93) 99 – 101 (100)	1.2 (3) 1.2 (3)	0.0058 to 0.58 µg/L (0.002 to 0.2 mg/kg) y = 2.22x + 0.00235 r = 0.9993	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Turnip leaf	BCS-CN88460 (*m/z* 400.2→139.0)	0.01	0.01 0.1	99 – 104 (102) 99 – 109 (104)	2.6 (3) 4.8 (3)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 2.098x + 0.00101 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234.0→177.0)	0.01	0.01 0.1	93 – 94 (93) 91 – 98 (96)	0.6 (3) 4.2 (3)	0.0058 to 0.58 µg/L (0.002 to 0.2 mg/kg) y = 2.22x + 0.00235 r = 0.9993	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Turnip tuber	BCS-CN88460 (*m/z* 400.2→139.0)	0.01	0.01 0.1	89 – 98 (94) 103 – 105 (104)	5.0 (3) 1.1 (3)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 2.098x + 0.00101 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234.0→177.0)	0.01	0.01 0.1	79 – 89 (85) 93 – 96 (95)	6.5 (3) 1.6 (3)	0.0058 to 0.58 µg/L (0.002 to 0.2 mg/kg) y = 2.22x + 0.00235 r = 0.9993	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Lettuce	BCS-CN88460 (*m/z* 400.2→139.0)	0.01	0.01 0.1	96 – 102 (99) 98 – 104 (101)	3.1 (3) 3.0 (3)	0.01 to 2 µg/L (0.002 to 0.4 mg/kg) y = 2.098x + 0.00101 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CR60082 (*m/z* 234.0→177.0)	0.01	0.01 0.1	88 – 95 (91) 94 – 95 (94)	4.0 (3) 0.6 (3)	0.0058 to 0.58 µg/L (0.002 to 0.2 mg/kg) y = 2.22x + 0.00235 r = 0.9993	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Tomato, Orange, Wheat grain, Soybean seed and Canola seed

Report:	KCA 4.1.2/16; Miller, A.; 2017; M-606616-01-1
Title:	An analytical method for the determination of residues of BCS-CN88460 in/on plant matrices using LC/MS/MS
Report No.:	LN-002-P16-01
Document No.:	M-606616-01-1
Guideline(s):	US EPA OCSPP Guideline 860.1340 Residue Analytical Method OECD Guidance Document on Pesticide Residue Analytical Methods, Series on Testing and Assessment Document 72 and Series on Pesticides: Document 39, August 2007 (OECD Guideline, ENV/JM/MONO (2007) 17, Aug 13, 2007) Residue Chemistry Guidelines, Regulatory Directive 98-02, Section 3, Residue Analytical Method, June 1998
Guideline deviation(s):	not specified
GLP/GEP:	**no**

Report:	KCA 4.1.2/17; Miller, A.; Arthur, E. L.; 2017; M-606610-01-1
Title:	Validation of analytical method LN-002-P16-01, an analytical method for the determination of BCS-CN88460 in/on plant matrices using LC-MS/MS
Report No.:	RALN0017
Document No.:	M-606610-01-1
Guideline(s):	US EPA Test Guideline OCSPP 860.1340: Residue Analytical Method
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Method LN-002-P16-01 is an adaptation of Method 01475 and was developed as a pre-registration method for the determination of residues of Isoflucypram in/on various plants. The method was validated in a range of matrices: tomato, orange, wheat grain, soybean seed and canola seed. The method and validation parameters tested are summarised below and the results are presented in Tables B.5.1.2-12.

Principle of the method

Samples were soaked in extraction solvent (acetonitrile/water 4:1 v/v) for 20 minutes prior to being extracted twice by homogenisation (ultra turrax) for 2 minutes. The resulting extracts were filtered (using vacuum if required). The combined filtrates were spiked with an internal standard solution of deuterated analyte and then diluted with acetonitrile/water (1:4v/v) to give the final extract. Analysis was performed using by reverse phase HLPC-MS/MS using a Phenomenex Luna C18, 50 mm x 2 mm x 2.5 µm analytical column at 40 ºC and gradient elution using 0.1 % formic acid in water and 0.1 % formic acid in acetonitrile mobile phase. The ion transition 400.2→139.2 was monitored for Isoflucypram and 405.2→139.2 for the deuterated internal standard.

The RMS notes that the final volume is taken as the volume of extraction solvent and does not take into account any contribution from the water in the sample matrix (usual practice would be to make the combined extracts up to a final volume), however, in this case the sample size is only 2.5 g relative to an extraction volume of 50 ml so the error associated with any contribution of water from the matrix would not have a significant impact of the final volume. The higher variability and occasionally low individual recoveries seen in the validation data for tomato (high water containing crop) could be explained by this.

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) samples.

Linearity

Linearity was demonstrated by the analysis in duplicate of eight standards of increasing concentration. The range of standard concentrations used was 0.05 to 100 µg/L which is equivalent to 0.005 to 10 mg/kg, and adequately encompasses the recovery concentrations of 0.01 (LOQ) and 0.10 mg/kg (10xLOQ). The correlation coefficient is >0.99.

The impact of matrix effects has been addressed by the use of deuterated internal standards.

Accuracy
Control samples were fortified with reference standards at each of two levels (7 replicates at 0.01 mg/kg and five replicates at 0.10 mg/kg) and the samples analysed by the method described. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 110 %.

Precision
Precision in the form of %RSD was calculated for the accuracy determinations described above. Acceptable precision is demonstrated by <20%RSD.

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 0.01 mg/kg.

Extraction efficiency
The extraction efficiency of this method was compared with that for method 01475 by conducting parallel extractions of barley straw samples from residues trials containing incurred residue of isoflucypram using both methods and then analysing them by the LC-MS/MS described here for method LN-002-P16-01. Barley straw samples were used as these are dry samples and are generally expected to be the most difficult to extract incurred residues from. One untreated control sample and two recovery samples (fortified at 0.01 mg/kg) were also analysed alongside the samples.

Results of the comparative analyses are presented below and indicate that both methods of extraction release similar residue levels from the samples:

Treated Sample ID	Method	Sample ID	Extraction Date	Analysis Date	BCS CN88460 (ppm)
C0601-16DA-C002-A	LN-002-P16-01	17BSLN004	06/05/17	06/07/17	0.2495
		17BSLN005	06/05/17	06/07/17	0.2793
		17BSLN006	06/05/17	06/07/17	0.2669
				Average:	**0.2652**
	01475	17BSLN007	06/05/17	06/07/17	0.2587
		17BSLN008	06/05/17	06/07/17	0.2659
		17BSLN009	06/05/17	06/07/17	0.2585
				Average:	**0.2610**

The concurrent recoveries were 89 and 81% indicating no unacceptable analytical losses during the analysis.

Stability
The storage period for the standard solutions and sample extracts in this study is covered by the stability data presented in the validation report for method 01475 (Uceda, L.; 2016; M-558986-01-1).

Assessment
The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram residues in tomato, orange*, wheat grain, soybean seed and canola seed, with a LOQ of 0.01 mg/kg.

* extraction efficiency from oranges (high acid crop) was not proven for method 01475 and is therefore not proven here.

Table B.5.1.2-12 Summary of validation data for analytical method LN-002-P16-01 for the determination of BCS-CN88460 residues in tomato, orange, wheat grain, soybean seed and canola seed.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Orange	BCS-CN88460 (*m/z* 400.2→139.2)	0.01	0.01 0.1	84 – 89 (86) 91 – 99 (95)	2.3 (7) 3.6 (5)	0.05 to 100 µg/L (0.005 to 10 mg/kg) y = 0.564x + 0.00508 r = 0.9996	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Wheat grain		0.01	0.01 0.1	86 – 93 (89) 93 – 98 (96)	2.8 (7) 1.9 (5)	0.05 to 100 µg/L (0.005 to 10 mg/kg) y = 0.563x + 0.00531 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Soybean seed		0.01	0.01 0.1	87 – 90 (88) 89 – 94 (92)	1.3 (7) 2.1 (5)	0.05 to 100 µg/L (0.005 to 10 mg/kg) y = 0.578x + 0.00215 r = 0.9992	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Canola seed		0.01	0.01 0.1	81 – 87 (84) 89 – 97 (93)	2.3 (7) 3.5 (5)	0.05 to 100 µg/L (0.005 to 10 mg/kg) y = 0.555x + 0.00631 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Tomato		0.01	0.01 0.10	72 – 97 (82) 65 – 90 (81)	9.3 (7) 13.1 (5)	0.05 to 100 µg/L (0.005 to 10 mg/kg) y = 0.57x + 0.00505 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

B.5.1.2.5.2. Processed Products

Report:	KCA 4.1.2/18; Harbin, A. M.; 2017; M-600505-02-1
Title:	BCS-CN88460: Magnitude of residues in/on wheat processed fractions following treatment with BCS-CN88460 EC50
Report No.:	RALNN137
Document No.:	M-600505-02-1
Guideline(s):	PMRA DACO 7.4.5, Processed Food/Feed US EPA OCSPP 860.1520, Processed Food/Feed OECD Guidance document on magnitude of pesticide residues in processed commodities, ENV/JM/MONO(2008)23). OECD Guideline for the Testing of Chemicals, Magnitude of the Pesticide Residues in Processed Commodities (TG 508 published in October 2008)
Guideline deviation(s):	None
GLP/GEP:	**yes**

Method LN-002-P16-01 (as described above) was used to determine the content of isoflucypram in wheat processed products of bran, white flour, whole meal flour, germ, middlings, shorts, pasta (fresh), pasta (dry), pasta (cooked), pasta (dried and cooked), gluten, starch, aspirated grain fractions, cooking water, white bread, and whole meal bread made from wheat grain harvested from two supervised residues trials reported in study number RALNN137.

Method LN-002-P17-01 has been acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of isoflucypram in five matrices representative of the major crop groupings; tomato (high water), orange (high acid), wheat grain (dry), soybean seed (high oil) and canola seed (high oil).

In accordance with SANCO/3029/99 rev 4. it is acceptable to submit a reduced validation set for additional matrices if the method used is fully validated for a comparable matrix within the same crop group. Therefore, a reduced validation set has been submitted for all of the matrices analysed in this processing study. The additional validation data are summarised in Table B.5.1.2-13.

Inherent levels in the control sample exceeded 30% of the LOQ for the matrices wheat pasta (max. 34% of the LOQ), gluten (max. 31% of the LOQ) and wheat aspirated grain (max. 63% of the LOQ). The recoveries were corrected by subtracting the measured amount in the control samples (or the average of the control samples) from the measured amount of the corresponding fortified control (recovery sample). For wheat pasta and gluten, the inherent levels were only slightly higher than the value specified in SANCO 3029/99 rev. 4 and are therefore considered to be acceptable. For the wheat aspirated grain control samples, the level in the control sample was 0.063 mg/kg but an average processing factor of 130X was determined for this matrix so the higher level in this control is not unexpected. As corrected recoveries at 0.01 and 2.5 mg/kg were acceptable and the levels of isoflucypram in the wheat aspirated grain trial samples were 0.92 and 2.4 mg/kg, the RMS considers that this higher value in the control samples is acceptable and no further data are required.

It appears from the report that the validation samples were analysed concurrently with the trial samples, therefore acting as procedural recoveries also.

All sample extracts were analysed within five days of extraction. Acceptable recoveries measured concurrently with each set of samples ensured the integrity of the sample extracts during the period of time between extraction and analysis.

Assessment

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and supports the determination of isoflucypram residues in wheat processed fraction from the supervised residues trials reported in study number RALNN137.

Table B.5.1.2-13 Summary of supplementary validation data for analytical method LN-002-P16-01 for the determination BCS-CN88460 residues in wheat processed products.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Bran	BCS-CN88460 (*m/z* 400.2→139.2)	0.01	0.01 0.1	94 – 103 (98) 93 – 95 (94)	4.7 (3) 1.1 (3)	0.01 to 10µg/L (0.001 to 1 mg/kg) y = 0.536x - 0.000566 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Flour		0.01	0.01 0.1	99 - 101 (100) 95 – 100 (97)	1.0 (3) 2.6 (3)	0.01 to 10µg/L (0.001 to 1 mg/kg) y = 0.525x + 0.00035 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Whole meal		0.01	0.01 0.1	91 – 99 (95) 89 – 95 (93)	4.2 (3) 3.5 (3)	0.01 to 10µg/L (0.001 to 1 mg/kg) y = 0.523x - 0.000202 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Germ		0.01	0.01 0.1	95 – 99 (98) 88 – 101 (95)	2.4 (3) 7.0 (3)	0.01 to 10µg/L (0.001 to 1 mg/kg) y = 0.516x + 0.00176 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Middlings		0.01	0.01 0.10	99 – 101 (100) 98 – 102 (100)	1.2 (3) 2.0 (3)	0.01 to 10µg/L (0.001 to 1 mg/kg) y = 0.516x + 0.00174 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Shorts		0.01	0.01 0.1	93 – 118 (108) 99 – 102 (101)	12.4 (3) 1.5 (3)	0.01 to 10µg/L (0.001 to 1 mg/kg) y = 0.524x + 0.000248 r = 1.000	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Pasta (fresh)		0.01	0.01 0.1	80 – 95 (87) 83 – 95 (89)	8.7 (3) 6.8 (3)	0.01 to 10µg/L (0.001 to 1 mg/kg) y = 0.520x + 0.00182 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Pasta (dry)		0.01	0.01 0.1	83 – 94 (89) 87 – 93 (91)	6.2 (3) 3.5 (3)	0.01 to 10µg/L (0.001 to 1 mg/kg) y = 0.522x + 0.000120 r = 1.000	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Pasta (cooked)	BCS-CN88460 (*m/z* 400.2→139.2)	0.01	0.01 0.1	91 – 120 (105) 97 – 99 (98)	13.9 (3) 1.0 (3)	0.01 to 10µg/L (0.001 to 1 mg/kg) y = 0.515x + 0.00142 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Pasta (dried and cooked)		0.01	0.01 0.1	94 – 99 (96) 97 – 101 (100)	2.6 (3) 2.3 (3)	0.01 to 10µg/L (0.001 to 1 mg/kg) y = 0.508x + 0.00275 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Gluten		0.01	0.01 0.1	95 – 110 (100) 97 – 101 (99)	8.7 (3) 2.1 (3)	0.01 to 10µg/L (0.001 to 1 mg/kg) y = 0.523x + 0.00127 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Starch		0.01	0.01 0.1	98 – 117 (105) 100 – 101 (101)	9.9 (3) 0.6 (3)	0.01 to 10µg/L (0.001 to 1 mg/kg) y = 0.495x + 0.00384 r = 0.9996	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Aspirated grain fractions		0.01	0.01 2.5	90 – 100 (95) 100 – 106 (104)	5.3 (3) 3.1 (3)	0.01 to 10µg/L (0.001 to 1 mg/kg) y = 0.462x + 0.00675 r = 0.9973	Retention time match to reference standard. Peaks representing 63% of LOQ were observed in the control sample. Refer to method discussion for further comment.
Cooking water		0.01	0.01 0.1	92 – 95 (94) 95 – 99 (97)	1.6 (3) 2.0 (3)	0.01 to 10µg/L (0.001 to 1 mg/kg) y = 0.509x + 0.00307 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
White bread		0.01	0.01 0.1	94 – 109 (101) 93 – 96 (95)	7.6 (3) 1.8 (3)	0.01 to 10µg/L (0.001 to 1 mg/kg) y = 0.540x - 0.000527 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Wholemeal bread		0.01	0.01 0.1	92 – 97 (95) 91 – 95 (92)	3.0 (3) 2.5 (3)	0.01 to 10µg/L (0.001 to 1 mg/kg) y = 0.531x - 0.000266 r = 1.000	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

B.5.1.2.5.3. Animal Products

Report:	KCA 4.1.2/19; Glaubitz, J.; Kuppels, U.; Eickstaedt, D.; 2017; M-599206-01-1
Title:	Residue analytical method 01511 for the determination of residues of BCS-CN88460 and its metabolites BCS-DC20298, BCS-CY26497, BCS-CY24813, BCS-DC22055 and BCS-CX99799 in/on animal tissues, milk and eggs by HPLC-MS/MS
Report No.:	P603166029
Document No.:	M-599206-01-1
Guideline(s):	European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 Guidance document on residue analytical methods; SANCO/825/00 rev. 8.1, European Commission, Directorate General Health and Consumer Protection, 2010-11-16 US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method OECD (2007). Guidance Document on Pesticide Residue Analytical Methods. Environment, Health and Safety Publications. Series on Testing and Assessment No. 72 and Series on Pesticides No. 39
Guideline deviation(s):	None
GLP/GEP:	**yes**

Report:	KCA 4.1.2/20; Diot, R.; Heinemann, D.; 2017; M-605551-01-1
Title:	Request for waiver of the requirement for radiovalidation of the analytical method for the determination of BCS-CN88460 residues in animal matrices
Report No.:	M-605551-01-1
Document No.:	M-605551-01-1
Guideline(s):	US EPA: OPPTS 860.1340, Residue Analytical Method Canada PMRA: DACO 7.2.2 Residue Analytical Method European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (Part A, Section 4) and Annex III (Part A, Section 5) of Commission Directive 91/414, SANCO/3029/99 Guidance Document on residue analytical methods; SANCO/825/00 rev. 8.1, European Commission, Directorate General Health and Consumer Protection, 2010-11-16 OECD (2007). Guidance Document on Pesticide Residue Analytical Methods. Environment, Health and Safety Publications. Series on Testing and Assessment No. 72 and Series on Pesticides No. 39.
Guideline deviation(s):	--
GLP/GEP:	**no**

The method 01511 was developed and validated for the determination of free residues of Isoflucypram and its metabolites BCS-DC20298, BCS-CY26497, BCSCY24813, BCS-DC22055 and BCS-CX99799 in/on animal tissues, milk and eggs and for the determination of free and conjugated residues of BCS-DC20298 and BCS-CY24813 in cow liver and kidney, as well as for the determination of free and conjugated (glucuronic acid conjugates) residues of BCS-DC22055 in hen liver by HPLC-MS/MS. The method and validation parameters tested are summarised below and the results are presented in Tables B.5.1.2-14 – B.5.1.2.25.

Principle of the method

Samples of milk, muscle, kidney and eggs were extracted once by homogenisation (ultra turrax) with acetonitrile/water (4:1 v/v). Samples of fat and liver were extracted twice by the same process. The resulting extracts were combined where necessary, spiked with internal standard solution of deuterated analytes and made

up to a fixed volume with acetonitrile/water (4:1 v/v) to give Extract A. These extracts were then diluted with water to give the Final Extracts, which were analysed by reverse phase HPLC-MS/MS using a YMC Triat C18 ExRS plus, 100 mm x 2.1mm x 2.7µm analytical column at 60 ºC and gradient elution using water (+ 120 µL/L formic acid) and acetonitrile (+ 120 µL/L formic acid) mobile phase. Two ion transitions were monitored for each analyte and one for each internal standard.
For cow kidney, cow liver and hen liver, Extract A's were not diluted as described above but were subjected to a hydrolysis step as follows. Extracts were evaporated to dryness and then reconstituted in water. Aliquots of pH 5 buffer solution and B-glucuronidase/arylsulfatase solution were added, and the extracts were incubated at 37ºC for 20 hours (cow kidney and liver and 96 hours (hen liver). Following incubation, the samples were cleaned up on Oasis HLB SPE cartridges. The eluates from the SPE cartridges were evaporated to dryness and reconstituted in acetonitrile/water (1:4 v/v) prior to analysis by the reverse phase HPLC-MS/MS method described above.

The following ion transitions were monitored for each matrix

Analyte	**1st MRM (quantitation)**	**2nd MRM (confirmation)**
BCS-CN88460	m/z 400.1 → 167.1	400.1 → 139.1
BCS-DC20298	m/z 416.1 → 177.0	416.1 → 398.0
BCS-CY26497	m/z 430.1 → 177.0	430.1 → 412.1
BCS-CY24813	m/z 416.2* → 234.1	416.2 → 177.0
BCS-DC22055	m/z 402.1 → 220.1	402.1 → 58.1
BCS-CX99799	m/z 416.1 → 236.2	416.1 → 208.2

* Due to fluctuation in the instrument during the measurement on this study there was a slight shift observed from 416.1 to 416.2 *m/z* during the course of the study.

The RMS notes that the deuterated internal standard was added to the cow liver, cow kidney and hen liver samples prior to the hydrolysis and clean up steps. Losses after his point will therefore not be indicated by the recovery samples as they will have been corrected for internally by the presence of the internal standard. There is no specific guidance on this point in SANCO/3029/99 rev.4 but the OECD Guidance Document on Pesticide Residue Analytical Methods (series on pesticides 39) states the following:

An internal standard is a chemical added in known quantity, at a specified stage in analysis, to facilitate determination of the identity and/or quantity of the analyte. The use of an internal standard method is only acceptable under certain circumstances, usually when added to the final extract (prior to quantitation). If used in this manner, the internal standard should show behaviour similar to that of the analyte(s) of interest. It should not degrade and not be prone to matrix effects. However, the use of an internal standard throughout the entire procedure to correct for recoveries is not acceptable unless data are available on numerous samples of each matrix to show that the analyte and internal standard behave very similarly in each step (extraction, clean-up, etc.). An example of an internal standard method that is fully acceptable is the use of stable isotopes (e.g. 2H, 13C) for facilitating quantitation by mass spectrometry.

Based on this, the use of a deuterated standard is considered to be fully acceptable and the RMS considers that no further data are required.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) samples.

Linearity
Linearity was demonstrated by the triplicate analysis of seven standards of increasing concentration. The range of standard concentrations used was 0.025 to 5 µg/L (corresponding to 0.0025 to 0.5 mg/kg for Isoflucypram and 0.025 µg/L to 10 µg/L (corresponding to 0.0025 mg/kg to 1.0 mg/kg) for its metabolites BCS-DC20298, BCS-CY26497, BCS-CY24813, BCS-DC22055 and BCS-CX99799, expressed as parent equivalents. These ranges adequately encompass the recovery concentrations of 0.01 mg/kg (0.005 for milk) and 0.10 mg/kg (0.05 for milk). The correlation coefficients were all >0.99.

The impact of matrix effects has been addressed by the use of deuterated internal standards.

Accuracy
Five control samples were fortified with each analyte in each matrix at each of two levels; 0.01 mg/kg and 0.10 mg/kg in tissues and eggs; 0.005 mg/kg and 0.05 mg/kg in milk. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 110 %.

Mean recovery values for BCS-DC20298 in hen egg at 0.1 mg/kg, cow milk at 0.05 mg/kg and cow liver at 0.01 mg/kg were all 111% as were recoveries of BCS-CX99799 in cow kidney at 0.01 mg/kg. These values fall just outside of the acceptable mean recovery criterion according to SANCO/3029/99 rev.4 of 70-110%. However, that criterion does not take into account the residue level being sought whereas the OECD Guidance on Pesticide Residue Analytical Methods (series on pesticides 39) and, indeed, SANCO 825/00 rev 8.1 guidance for post registration methods, both tie acceptable mean recovery values to the level of analyte being sought and at the levels of 0.01, 0.05 and 0.1 mg/kg the acceptable maximum mean recovery value is 120%. On that basis, the RMS considers that the mean recoveries of 111% seen for the quantification method are acceptable and that no further data are required.

Precision
Precision in the form of %RSD was calculated for the accuracy determinations described above. Acceptable precision is demonstrated by <20%RSD.

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 0.01 mg/kg in tissues and eggs and 0.005 mg/kg in milk.

Confirmation
Note that confirmatory methods are not required for methods used for risk assessment, but the submission of this additional data has been reported here for completeness.

Residues were confirmed simultaneously to the primary detection by monitoring a second ion transition. Validation data as described above were submitted for the confirmatory method and the results are summarised in Table B.5.1.2-20 to B.5.1.2-25.

Mean recovery values for some analytes in some matrices were greater than maximum value permitted in accordance with SANCO/3029/99 rev.4. (see below):

Analyte	Matrix	Fortification level (mg/kg)	Measured recovery level
BCS-CN88460	Cow milk	0.05	112
BCS-DC20298	Hen egg	0.01	111
	Cow muscle	0.01	131
	Cow kidney	0.10	131
BCS-CX99799	Hen liver	0.01	111
	Cow fat	0.01	111
	Cow kidney	0.01	118

However, confirmatory methods are not required for residues methods designed for the generation of pre-registration data, to which SANCO/3030/99 rev.4 applies. Confirmatory methods are required for post registration control and monitoring purposes, and this is covered by SANCO/825/00 rev 8.1 which ties the acceptable mean recovery values to the level of analyte being sought. According to this guidance document, the acceptable maximum mean recovery at levels of 0.01, 0.05 and 0.1 mg/kg is 120%. On that basis, the mean recoveries of 111%, 112% and 118% seen for this confirmation method are acceptable. The higher mean recoveries of 131% for BCS-DC20298 fall outside of this criterion, and individual recovery data for this analyte in cow muscle and cow kidney hydrolyse samples are as high as 145% and 153% respectively. These data indicate that the method is not performing satisfactorily for these analyte/matrix combinations and the confirmatory method must be regarded as qualitative only in these cases.

The precision data for BCS-DC20298 in cow muscle at 0.1 mg/kg are also outside the acceptable criterion laid down in SANCO/3029/99 rev.4, SANCO 825/00 rev.8.1 and also OECD Guidance on Pesticide Residue

Analytical Methods (series on pesticides 39). The high precision arises from a spread of recoveries between 78 and 137%. These data are not acceptable and the confirmatory method for this analyte/matrix combination must be regarded as qualitative only.

Confirmatory data are not available for BCS-DC20298 in the cow liver hydrolyse and cow kidney hydrolyse samples at the LOQ (0.01 mg/kg) due to lack of sensitivity of the confirmatory method.

Extraction efficiency

The applicant submitted a waiver for the radiovalidation of isoflucypram residues in animal matrices on the basis that the extraction procedure in analytical method 01511 is comparable to the one used in the lactating ruminant and laying hen metabolism studies. In those studies, it was shown that:

The first extraction step with ACN/water (8/2; v/v) released:
≥ 85.7% TRR in muscle
≥ 85.6% TRR in eggs
≥ 95.8% TRR in milk
≥ 88.6% TRR in kidney

The two first extraction steps with ACN/water (8/2; v/v) released
≥ 89.1% TRR in fat
≥ 82.7% TRR in liver.

This is acceptable.

Enzymatic hydrolysis yield

The yield of the enzymatic hydrolysis was tested during the livestock metabolism studies referenced in Volume 3CA, Section 7 Point 7.2.2 and Point 7.2.3 and showed that, overall, hydrolysis yields were good (>87%).

Stability

Standard solutions were shown to be stable (<20% change when compared to freshly prepared samples) over a period of 181 days. The active substance and all metabolites were found to be stable in all extracts, including from the hydrolysis experiments, from all matrices for a period of at least 21 days. This covers the duration of storage of standards and extracts in the study.

Assessment

The primary method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of:

- free residues of Isoflucypram and its metabolites BCS-DC20298, BCS-CY26497, BCSCY24813, BCS-DC22055 and BCS-CX99799 in animal tissues and eggs with a LOQ of 0.01 mg/kg and milk with a LOQ of 0.005 mg/kg.
- free and conjugated residues of BCS-DC20298 and BCS-CY24813 in cow liver and kidney with a LOQ of 0.01 mg/kg.
- free and conjugated (glucuronic acid conjugates) residues of BCS-DC22055 in hen liver with a LOQ of 0.01 mg/kg.

The confirmatory method is acceptably validated in accordance with SANCO/825/rev.8.1 and is suitable for the determination of:

- free residues of Isoflucypram and its metabolites BCS-CY26497, BCSCY24813, BCS-DC22055 and BCS-CX99799 in animal tissues and eggs with a LOQ of 0.01 mg/kg and milk with a LOQ of 0.005 mg/kg.
- free and conjugated residues of BCS-CY24813 in cow liver and kidney with a LOQ of 0.01 mg/kg.
- free and conjugated (glucuronic acid conjugates) residues of BCS-DC22055 in hen liver with a LOQ of 0.01 mg/kg.

Note: due to issues with high variability and high recoveries for BCS-DC20298 in some matrices, the confirmatory method is only considered to be valid as a quantitative method for the determination of free residues of BCS-DC20298 in hen eggs, hen liver, cow fat and cow liver with a LOQ of 0.01 mg/g, and cow milk with a LOQ of 0.0.05 mg/kg.

For the determination of free residues of BCS-DC20298 in cow muscle and cow kidney, the method can be regarded as qualitative only.

There is no confirmatory method available for the determination of conjugated residues of BCS-DC20298 in cow liver or cow kidney.

Table B.5.1.2-14 Summary of validation data for analytical method 01511 - **quantification** method for **BCS-CN88460** residues in animal products

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Hen egg	BCS-CN88460 m/z 400.1→167.1	0.01	0.01 0.1	95 – 101 (98) 94 – 107 (103)	2.6 (5) 5.1 (5)	0.025 to 5.0 µg/L (0.0025 to 0.50 mg/kg) y = 0.7028x + 0.00266 r = 0.99935	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Hen liver		0.01	0.01 0.1	94 – 100 (98) 99 – 104 (101)	2.6 (5) 1.9 (5)		
Cow milk		0.005	0.005 0.05	98 – 105 (102) 107 – 110 (108)	2.5 (7) 1.1 (5)		
Cow muscle		0.01	0.01 0.1	94 – 101 (97) 100 – 104 (102)	3.1 (5) 1.6 (5)		
Cow fat		0.01	0.01 0.1	98 – 100 (99) 102 – 105 (104)	1.1 (5) 1.5 (5)		
Cow liver		0.01	0.01 0.10	84 – 104 (97) 95 – 105 (101)	8.2 (5) 3.6 (5)		
Cow kidney		0.01	0.01 0.10	95 – 99 (97) 105 – 109 (107)	1.7 (5) 1.4(5)		

Table B.5.1.2-15 Summary of validation data for analytical method 01511 - **quantification** method for **BCS-DC20298** residues in animal products

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Hen egg	BCS-DC20298 m/z 416.1→177.0	0.01	0.01 0.1	91 – 117 (102) 102 – 118 (111)	9.2 (5) 5.5 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/kg) y = 0.9010x + 0.000874 r = 0.9979	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Hen liver		0.01	0.01 0.1	76 – 109 (92) 97 – 112 (104)	12.9 (5) 6.2 (5)		
Cow milk		0.005	0.005 0.05	84 – 118 (98) 101 – 120 (111)	13.3 (7) 8.2 (5)		
Cow muscle		0.01	0.01 0.1	96 – 117 (104) 96 – 116 (104)	8.4 (5) 7.9 (5)		
Cow fat		0.01	0.01 0.1	79 – 102 (93) 98 – 113 (105)	10.1 (5) 5.3 (5)		
Cow liver		0.01	0.01 0.10	74 – 111 (91) 84 – 106 (94)	14.6 (5) 8.7 (5)		
Cow liver hydrolyse		0.01	0.01 0.10	100 – 129 (113) 96 – 108 (100)	10.8 (5) 4.8 (5)		
Cow kidney		0.01	0.01 0.10	84 – 114 (94) 89 – 117 (98)	12.4 (5) 11.9 (5)		
Cow kidney hydrolyse		0.01	0.01 0.10	90 – 113 (99) 104 -119 (109)	10.5 (5) 5.4 (5)		

Table B.5.1.2-16 Summary of validation data for analytical method 01511 - **quantification** method for **BCS-CY26497** residues in animal products

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Hen egg	BCS-CY26497 m/z 430.1→177.0	0.01	0.01 0.1	92 – 106 (101) 99 – 110 (104)	5.3 (5) 3.8 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/kg) y = 1.0285x + 0.00230 r = 0.99895	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Hen liver		0.01	0.01 0.1	96 – 102 (98) 93 – 103 (97)	2.6 (5) 3.9 (5)		
Cow milk		0.005	0.005 0.05	94 -116 (106) 102 – 120 (110)	7.3 (7) 6.0 (5)		
Cow muscle		0.01	0.01 0.1	86 – 97 (92) 100 – 105 (102)	4.5 (5) 1.9 (5)		
Cow fat		0.01	0.01 0.1	98 – 106 (103) 99 – 112 (105)	3.3 (5) 5.9 (5)		
Cow liver		0.01	0.01 0.10	84 – 111 (97) 81 -100 (94)	10.0 (5) 8.1 (5)		
Cow kidney		0.01	0.01 0.10	102 – 116 (108) 105 – 112 (109)	5.2 (5) 3.1 (5)		

Table B.5.1.2-17 Summary of validation data for analytical method 01511 - **quantification** method for **BCS-CY24813** residues in animal products

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Hen egg	BCS-CY24813 m/z 416.2→234.1	0.01	0.01 0.1	101 – 111 (105) 97 – 108 (102)	4.4 (5) 4.5 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/kg) y = 1.1423x + 0.000920 r = 0.99932	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Hen liver		0.01	0.01 0.1	95 – 102 (99) 95 – 99 (97)	3.1 (5) 1.6 (5)		
Cow milk		0.005	0.005 0.05	95 – 109 (104) 98 – 114 (104)	4.7 (7) 6.1 (5)		
Cow muscle		0.01	0.01 0.1	94 – 105 (97) 94 – 99 (97)	4.5 (5) 1.9 (5)		
Cow fat		0.01	0.01 0.1	95 – 108 (102) 94 – 103 (100)	5.5 (5) 3.4 (5)		
Cow liver		0.01	0.01 0.10	85 – 104 (97) 89 -97 (95)	7.7 (5) 3.7 (5)		
Cow liver hydrolyse		0.01	0.01 0.10	75 (102 (91) 88 – 97 (93)	11.1 (5) 3.9 (5)		
Cow kidney		0.01	0.01 0.10	94 – 106 (98) 103 – 107 (105)	4.6 (5) 1.7 (5)		
Cow kidney hydrolyse		0.01	0.01 0.10	96 – 106 (100) 102 – 110 (105)	3.9 (5) 3.0 (5)		

Table B.5.1.2-18 Summary of validation data for analytical method 01511 - **quantification** method for **BCS-DC22055** residues in animal products

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Hen egg	BCS-DC22055 m/z 402.1→220.1	0.01	0.01 0.1	89 – 109 (99) 88 – 107 (98)	9.5 (5) 7.3 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/kg) y = 1.0380x - 0.000450 r = 0.99919	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Hen liver		0.01	0.01 0.1	92 – 106 (98) 97 – 102 (100)	5.8 (5) 2.2 (5)		
Hen liver hydrolyse		0.01	0.01 0.1	88 – 116 (99) 95 – 109 (101)	11.5 (5) 5.1 (5)		
Cow milk		0.005	0.005 0.05	98 – 107 (102) 94 – 108 (102)	3.1 (7) 5.2 (5)		
Cow muscle		0.01	0.01 0.1	93 – 100 (95) 87 – 95 (92)	2.9 (5) 3.4 (5)		
Cow fat		0.01	0.01 0.1	93 – 100 (96) 93 – 103 (98)	2.8 (5) 4.5 (5)		
Cow liver		0.01	0.01 0.10	75 – 102 (88) 86 – 94 (90)	12.2 (5) 3.8 (5)		
Cow kidney		0.01	0.01 0.10	98 – 108 (102) 97 – 107 (102)	4.0 (5) 3.9 (5)		

Table B.5.1.2-19 Summary of validation data for analytical method 01511 - **quantification** method for **BCS-CX99799** residues in animal products

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Hen egg	BCS-CX99799 m/z 416.1→236.2	0.01	0.01 0.1	85 – 91 (88) 89 – 110 (96)	2.5 (5) 8.4 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/kg) y = 2.3512x - 0.02965 r = 0.99920	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Hen liver		0.01	0.01 0.1	82 – 107 (96) 95 – 109 (101)	10.5 (5) 5.1 (5)		
Cow milk		0.005	0.005 0.05	88 – 104 (96) 99 – 112 (105)	6.1 (7) 4.8 (5)		
Cow muscle		0.01	0.01 0.1	70 – 95 (87) 94 – 101 (97)	11.8 (5) 2.8 (5)		
Cow fat		0.01	0.01 0.1	105 – 111 (108) 97 – 105 (100)	2.2 (5) 3.6 (5)		
Cow liver		0.01	0.01 0.10	83 – 100 (91) 89 – 95 (91)	8.3 (5) 2.9 (5)		
Cow kidney		0.01	0.01 0.10	104 – 117 (111) 101 -109 (104)	5.1 (5) 3.2 (5)		

Table B.5.1.2-20 Summary of validation data for analytical method 01511 - **confirmatory** method for **BCS-CN88460** residues in animal products

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Hen egg	BCS-CN88460 m/z 400.1→139.1	0.01	0.01 0.1	95 – 103 (97) 100 – 111 (107)	3.4 (5) 4.0 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/kg) y = 0.92939x + 0.00919 r = 0.99878	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Hen liver		0.01	0.01 0.1	92 – 101 (97) 102 – 107 (105)	3.3 (5) 2.1 (5)		
Cow milk		0.005	0.005 0.05	99 – 105 (101) 110 – 114 (112)	2.0 (7) 1.5 (5)		
Cow muscle		0.01	0.01 0.1	94 – 98 (96) 104 – 109 (106)	1.9 (5) 1.9 (5)		
Cow fat		0.01	0.01 0.1	99 – 102 (101) 101 – 107 (104)	1.3 (5) 2.4 (5)		
Cow liver		0.01	0.01 0.10	84 – 104 (96) 96 – 105 (102)	8.7 (5) 3.6 (5)		
Cow kidney		0.01	0.01 0.10	97 - 103 (99) 107 – 112 (110)	2.6 (5) 1.8 (5)		

Table B.5.1.2-21 Summary of validation data for analytical method 01511 - **confirmatory** method for **BCS-DC20298** residues in animal products

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Hen egg	BCS-DC20298 m/z 416.1→398.0	0.01	0.01 0.1	90 – 131 (111) 92 – 133 (106)	13.7 (5) 16.3 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/kg) y = 2.11361x - 0.04436 r = 0.99454	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Hen liver		0.01	0.01 0.1	80 – 100 (93) 86 – 137 (107)	9.1 (5) 18.2 (5)		
Cow milk		0.005	0.005 0.05	87 – 131 (107) 84 – 124 (101)	15.1 (7) 16.7 (5)		
Cow muscle		0.01	0.01 0.1	112 – 145 (131) 78 – 137 (106)	9.1 (5) 21.7 (5)		
Cow fat		0.01	0.01 0.1	78 – 94 (87) 90 – 117 (106)	7.3 (5) 10.0 (5)		
Cow liver		0.01	0.01 0.10	78 – 110 (89) 85 – 108 (93)	13.5 (5) 9.4 (5)		
Cow liver hydrolyse		0.01	0.01 0.10	- 87 – 114 (103)	- 9.9 (5)		
Cow kidney		0.01	0.01 0.10	74 – 108 (97) 98 – 119 (108)	14.2 (5) 8.1 (5)		
Cow kidney hydrolyse		0.01	0.01 0.10	- 91 – 153 (131)	- 18.8 (5)		

Table B.5.1.2-22 Summary of validation data for analytical method 01511 - **confirmatory** method for **BCS-CY26497** residues in animal products

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Hen egg	BCS-CY26497 m/z 430.1→412.1	0.01	0.01 0.1	94 – 105 (98) 92 – 105 (98)	4.7 (5) 5.7 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/kg) y = 0.35861x - 0.000610 r = 0.99918	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Hen liver		0.01	0.01 0.1	89 – 110 (101) 90 – 110 (99)	8.3 (5) 7.4 (5)		
Cow milk		0.005	0.005 0.05	88 – 118 (101) 100 – 118 (105)	9.7 (7) 7.2 (5)		
Cow muscle		0.01	0.01 0.1	85 – 108 (96) 95 – 98 (96)	9.9 (5) 1.2 (5)		
Cow fat		0.01	0.01 0.1	86 – 110 (97) 93 – 108 (100)	9.4 (5) 5.5 (5)		
Cow liver		0.01	0.01 0.10	87 – 113 (102) 86 – 93 (89)	9.5 (5) 2.8 (5)		
Cow kidney		0.01	0.01 0.10	90 – 113 (100) 101 – 108 (104)	10.0 (5) 2.6 (5)		

Table B.5.1.2-23 Summary of validation data for analytical method 01511 - **confirmatory** method for **BCS-CY24813** residues in animal products

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Hen egg	BCS-CY24813 m/z 416.2→177.0	0.01	0.01 0.1	96 – 105 (101) 104 – 112 (107)	3.9 (5) 3.1 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/kg) y = 1.55370x + 0.00591 r = 0.99889	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Hen liver		0.01	0.01 0.1	92 – 100 (94) 99 – 108 (102)	3.6 (5) 3.6 (5)		
Cow milk		0.005	0.005 0.05	102 – 110 (104) 102 – 114 (108)	2.9 (7) 4.1 (5)		
Cow muscle		0.01	0.01 0.1	96 – 105 (100) 96 – 104 (101)	3.4 (5) 3.8 (5)		
Cow fat		0.01	0.01 0.1	90-107 (97) 93 – 103 (97)	6.8 (5) 4.0 (5)		
Cow liver		0.01	0.01 0.10	84 – 99 (92) 88 – 101 (94)	7.2 (5) 5.1 (5)		
Cow liver hydrolyse		0.01	0.01 0.10	72 – 97 (89) 92 – 96 (95)	11.0 (5) 1.8 (5)		
Cow kidney		0.01	0.01 0.10	94 – 105 (100) 106 – 112 (110)	4.5 (5) 2.3 (5)		
Cow kidney hydrolyse		0.01	0.01 0.10	96 – 112 (105) 104 – 112 (109)	6.6 (5) 2.9 (5)		

Table B.5.1.2-24 Summary of validation data for analytical method 01511 - **confirmatory** method for **BCS-DC22055** residues in animal products

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Hen egg	BCS-DC22055 m/z 402.1→58.1	0.01	0.01 0.1	94 – 110 (101) 87 – 102 (96)	6.5 (5) 6.5 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/kg) y = 0.55419x - 0.00651 r = 0.99829	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Hen liver		0.01	0.01 0.1	98 – 112 (105) 97 – 107 (101)	6.3 (5) 3.7 (5)		
Hen liver hydrolyse		0.01	0.01 0.1	92 – 108 (98) 101 – 113 (105)	6.8 (5) 4.7 (5)		
Cow milk		0.005	0.005 0.05	74 – 102 (93) 90 – 101 (97)	10.6 (7) 4.5 (5)		
Cow muscle		0.01	0.01 0.1	91 – 109 (99) 83 – 97 (91)	7.3 (5) 6.6 (5)		
Cow fat		0.01	0.01 0.1	87 – 109 (98) 95 – 106 (101)	8.6 (5) 5.0 (5)		
Cow liver		0.01	0.01 0.10	89 – 102 (95) 84 – 102 (90)	7.1 (5) 7.6 (5)		
Cow kidney		0.01	0.01 0.10	97 – 106 (103) 94 – 108 (103)	3.7 (5) 5.4 (5)		

Table B.5.1.2-25 Summary of validation data for analytical method 01511 – **confirmatory** method for **BCS-CX99799** residues in animal products

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Hen egg	BCS-CX99799 m/z 416.1→208.2	0.01	0.01 0.1	85 – 99 (92) 91 – 114 (97)	5.5 (5) 10.1 (5)	0.025 to 10 µg/L (0.0025 to 1.0 mg/kg) y = 1.12130x - 0.00885 r = 0.99918	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Hen liver		0.01	0.01 0.1	98 – 121 (111) 96 – 114 (102)	7.5 (5) 7.2 (5)		
Cow milk		0.005	0.005 0.05	86 – 108 (93) 105 – 114 (109)	8.9 (5) 3.1 (5)		
Cow muscle		0.01	0.01 0.1	89 – 109 (99) 93 – 104 (99)	7.7 (5) 4.1 (5)		
Cow fat		0.01	0.01 0.1	100 – 120 (111) 95 – 110 (103)	6.6 (5) 6.6 (5)		
Cow liver		0.01	0.01 0.10	74 – 112 (97) 90 – 100 (95)	14.3 (5) 4.4 (5)		
Cow kidney		0.01	0.01 0.10	114 – 120 (118) 93 – 111 (102)	1.9 (5) 6.5 (5)		

B.5.1.2.6. *Methods used in support of ecotoxicological studies*

B.5.1.2.6.1. Test water

Method no.: 01388

Report:	KCA 4.1.2/21; Krebber, R.; Sandau, C.; 2014; M-479643-01-1
Title:	Method 01388 for the determination of BCS-CN88460 in test water by HPLC-MS/MS
Report No.:	MR-13/075
Document No.:	M-479643-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

The method was used to determine the content of isoflucypram in samples of test water, used in ecotoxicological studies. The method and its validation are summarised below.

Principle of the method
Samples of test water were analysed directly by reverse phase HLPC-MS/MS (MRM 401 → 139) using an Ascentis Express C18, 50mm x 2.1mm x 2.7µm analytical column at 80°C and gradient elution using water/formic acid (1000/0.12 v/v) + 10 mM ammonium formate, water/methanol/formic acid (100/900/0.12 v/v/v) and water/methanol (500/500 V/v) mobile phases. Quantification was by external standards.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample.

Linearity
Linearity was demonstrated by the analysis (in duplicate) of six standards of increasing concentration. The range of standard concentrations used was 0.05 µg/L to 10.0 µg/L, which is directly equivalent to the concentrations in the samples. The equation of the calibration line was $y = 1.29 \times 10^5 x + 553$ and the correlation coefficient was 0.9999.

The impact of matrix effects has not been specifically addressed but matrix matched standards were used during the analysis.

Accuracy
Not relevant as the samples were analysed directly.

Precision
As no recovery determinations were performed, the precision (repeatability) of the method was determined as by repeat injections of a calibration standard. Calibration standards at levels of 0.05 and 0.5 µg/L were injected 10 times each and the RSD calculated to be 0.2% at each level.

LOQ
The LOQ is defined by the lowest concentration at which acceptable recovery and precision data have been generated. However as recovery determinations are not relevant to this direct analysis method, the LOQ is taken as the lowest level at which acceptable precision data are available; 0.05 µg/L.

Extraction efficiency
Not applicable as no extraction is performed.

Stability
Samples were analysed directly so stability of sample extracts is not relevant. The stability of the matrix matched standards was not assessed in this study but the stability of BCS-CN88460 in several different sample extracts has been adequately demonstrated throughout this section B5, so the RMS considers that no further data on stability in test water are required.

Assessment
The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.05 µg/L.

Method no.: 01388/M001

Report:	KCA 4.1.2/22; Krebber, R.; Leppelt, L.; 2014; M-502733-01-1
Title:	Modification 001 of method 01388 for the determination of BCS-CN88460 in test water by HPLC-MS/MS
Report No.:	MR-14/160
Document No.:	M-502733-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

This method is a slight modification of method 01388 described above. The modifications include the addition of acetonitrile to the samples prior to analysis and the use of a second ion transition as confirmation. The method was used to determine the content of isoflucypram in samples of test water used in ecotoxicological studies. The method and its validation are summarised below.

Principle of the method
Samples of test water were combined with acetonitrile in the ratio 4:1 and analysed directly by reverse phase HLPC-MS/MS (MRMs 400 → 139 and 400 → 177) using an Ascentis Express C18, 50mm x 2.1mm x 2.7µm analytical column at 80ºC and gradient elution using water/formic acid (1000/0.12 v/v) + 10 mM ammonium formate, water/methanol/formic acid (100/900/0.12 v/v/v) and water/methanol (500/500 V/v) mobile phases. Quantification was by external standards.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample.

Linearity
Linearity was demonstrated by the analysis (in duplicate) of seven standards of increasing concentration. The range of standard concentrations used was 0.015 µg/L to 10.0 µg/L, which is equivalent to 0.0188 to 12.5 µg/L in the samples. The equation of the calibration lines and correlation coefficients were as follows:

Ion transition	Equation of line	Correlation coefficient
400 → 139	$y = 1.4932 \times 10^{6}x + 9862$	0.99938
400 → 177	$y = 6.326 \times 10^{5}x + 1517$	0.99995

The impact of matrix effects has not been specifically addressed but matrix matched standards were used during the analysis.

Accuracy
Not relevant as the samples were analysed directly.

Precision
As no recovery determinations were performed, the precision (repeatability) of the method was determined as by repeat injections of a calibration standard. Calibration standards at levels of 0.05 and 0.5 µg/L (equivalent to sample concentrations of 0.0625 and 0.625 µg/L) were injected 10 times each and the RSDs calculated to be 2.2 and 0.8% respectively for the quantification ion and 1.3 and 0.7% for the confirmatory ion.

LOQ
The LOQ is defined by the lowest concentration at which acceptable recovery and precision data have been generated. However as recovery determinations are not relevant to this direct analysis method, the LOQ is taken as the lowest level at which acceptable precision data are available; 0.0625 µg/L.

Extraction efficiency

Not applicable as no extraction is performed.

Stability
Samples were analysed directly so stability of sample extracts is not relevant. The stability of the matrix matched standards was not assessed in this study but the stability of BCS-CN88460 in several different sample extracts has been adequately demonstrated throughout this section B5, so the RMS considers that no further data on stability in test water are required.

Assessment
The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.0625 µg/L.

The submission of the additional confirmatory data means that the method also complies with SANCO/825/00 rev.8.1 and is suitable for use as a monitoring method for surface water at a lower limit of 0.0625 µg/L.

Method no.: 01476

Report:	KCA 4.1.2/23; Krebber, R.; Leppelt, L.; 2016; M-552469-01-1
Title:	Analytical method 01476 for the determination of BCS-CN88460-carboxylic-acid (BCS-CY26497) in test water from aquatic toxicity tests by HPLC-UV
Report No.:	P 604 157029
Document No.:	M-552469-01-1
Guideline(s):	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 Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1 of November 16, 2010 European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, July 11, 2000
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

The method was used to determine the content of BCS-CY26497 (BCS-CN88460-carboxylic acid) in samples of test water from ecotoxicological studies. The method and its validation are summarised below.

Principle of the method
Samples of test water were combined with acetonitrile in the ratio 4:1 and analysed directly by reverse phase HLPC-UV using an Ascentis Express C18, 50mm x 2.1mm x 2.7µm analytical column at 40ºC and gradient elution using water (adjusted to pH 3 with orthophosphoric acid) and acetonitrile mobile phases. Quantification was by external standards.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample.

Linearity
Linearity was demonstrated by the analysis (in duplicate) of seven standards of increasing concentration. The range of standard concentrations used was 0.03 mg/L to 10.0 mg/L, which is equivalent to 0.0375 to 12.5 mg/L in the samples. The equation of the calibration line was $y = 342x - 0.295$ ($r = 1.0000$).

The impact of matrix effects has not been specifically addressed but matrix matched standards were used during the analysis.

Accuracy
Not relevant as the samples were analysed directly.

Precision

As no recovery determinations were performed, the precision (repeatability) of the method was determined as by repeat injections of a calibration standard. Calibration standards at levels of 0.10 and 1.0 mg/L (equivalent to sample concentrations of 0.125 and 1.25 mg/L) were injected 10 times each and the RSDs calculated to be 0.6 and 0.1% respectively.

LOQ
The LOQ is defined by the lowest concentration at which acceptable recovery and precision data have been generated. However as recovery determinations are not relevant to this direct analysis method, the LOQ is taken as the lowest level at which acceptable precision data are available; 0.125 mg/L.

Extraction efficiency
Not applicable as no extraction is performed.

Stability
Samples were analysed directly so stability of sample extracts is not relevant. The stability of the matrix matched standards was not assessed in this study but the stability of BCS-CN88460 in several different sample extracts has been adequately demonstrated throughout this section B5, so the RMS considers that no further data on stability in test water are required.

Assessment
The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of BCS-CN88460-carboxylic acid in samples of test water at a LOQ of 0.125 mg/L.

Method used in Study EBLNN023

Report:	KCA 8.2.1/03; [redacted] 2015; M-537137-01-1
Title:	Acute toxicity of BCS-CN88460 technical to the sheepshead minnow (Cyprinodon variegatus) under static conditions
Report No.:	EBLNN023
Document No.:	M-537137-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP 850.1075
Guideline deviation(s):	none
GLP/GEP:	**yes**

Principle of the method
Samples were diluted (x25) with acetonitrile/water (1:4 v/v) containing 0.1% acetic acid. Further dilutions were made as necessary to obtain a final concentration of approximately 0.2 µg/L. Extracts were analysed by reverse phase HLPC-MS/MS (MRM 400 → 139) using a Synergi MAX-RP, 75mm x 2.0mm x 4µm analytical column at 40oC and gradient elution using acetonitrile and 0.1% acetic acid mobile phases. Quantification was by external standards.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of an untreated control.

Linearity
Linearity was demonstrated by the analysis of seven standards of increasing concentration. The range of standard concentrations used was 0.04 µg/L to 4 µg/L, which is equivalent to 1 to 100 µg/L in the test water samples. The equation of the calibration line was y = 57011x + 23543 and the correlation coefficient was 0.9995.

The impact of matrix effects has not been specifically addressed.

Accuracy and precision
Five control samples were fortified with reference standard at 5 and 1200 µg/L and the samples analysed by the method described. The higher recovery level samples were diluted into the demonstrated linear range. Recovery

values were calculated as a percentage of measured concentration relative to fortified concentration and precision was determined based on the %RSD of these values. Data are presented below and are acceptable:

Fortification level (µg/L)	% Recovery range (mean)	%RSD (n)
5	104 – 111 (107)	2.6 (5)
1200	94 – 100 (97)	3.2 (5)

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 5 µg/L.

Extraction efficiency
Not applicable as no extraction is performed.

Stability
The stability of BCS-CN88460 in standard and sample extracts was not assessed in this study but the stability of BCS-CN88460 in several different sample extracts has been adequately demonstrated throughout section B5, so the RMS considers that no further data on stability in test water are required.

Assessment
The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 5 µg/L.

Method used in Study 044SRLS15C20 (Report No. EBLNN360)

Report:	KCA 8.2.2.1/02; [redacted] 2016; M-575119-01-1
Title:	Early life stage toxicity of BCS-CN88460 technical to the sheepshead minnow (Cyprinodon variegatus) under flow-through conditions
Report No.:	044SRLS15C20
Document No.:	M-575119-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP 850.1400
Guideline deviation(s):	None
GLP/GEP:	**Yes**

Principle of the method
Samples were spiked with internal standard of deuterated analyte and then diluted (x2) with acetonitrile/water (1:4 v/v) containing 0.1% acetic acid. Further dilutions were made as necessary to obtain a final concentration of approximately 0.2 µg/L. Extracts were analysed by reverse phase HLPC-MS/MS (MRM 400 → 139) using a Synergi MAX-RP, 75mm x 2.0mm x 4µm analytical column at 40ºC and gradient elution using acetonitrile and 0.1% acetic acid mobile phases. Quantification was by external standards.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of an untreated control.

Linearity
Linearity was demonstrated by the analysis of seven standards of increasing concentration. The range of standard concentrations used was 0.0625 µg/L to 10 µg/L, which is equivalent to 0.125 to 20 µg/L in the test water samples. The equation of the calibration line was y = 0.50575x + 0.0050175 and the correlation coefficient was 0.9999.

The impact of matrix effects was addressed by the use of deuterated internal standards.

Accuracy and precision
Five control samples were fortified with reference standard at 0.25 and 75.0 µg/L and the samples analysed by the method described. The higher recovery level samples were diluted into the demonstrated linear range. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration and precision was determined based on the %RSD of these values. Data are presented below and are acceptable:

Fortification level (µg/L)	% Recovery range (mean)	%RSD (n)
0.25	88 – 101 (97)	5.4 (5)
75	102-106 (103)	1.6 (5)

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 0.25 µg/L.

Extraction efficiency
Not applicable as no extraction is performed.

Stability
The stability of BCS-CN88460 in standard and sample extracts was not assessed in this study but the stability of BCS-CN88460 in several different sample extracts has been adequately demonstrated throughout section B5, so the RMS considers that no further data on stability in test water are required.

Assessment
The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.25 µg/L.

Method used in Studies 149A-257B, 149A-256, 149A-258

Report:	KCA 8.2.4.2/01; Brougher, D. S.; Siddiqui, A. I.; Gallagher, S. P.; 2016; M-547041-01-1
Title:	BCS-CN88460: A 96-hour static-renewal acute toxicity test with the saltwater mysid (Americamysis bahia)
Report No.:	149A-257B
Document No.:	M-547041-01-1
Guideline(s):	US EPA OCSPP 850.1035
Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCA 8.2.5.2/01; Milligan, A. L.; Siddiqui, A. I.; Gallagher, S. P.; Krueger, H. O.; 2016; M-567966-01-1
Title:	BCS-CN88460: A flow-through life-cycle toxicity test with the saltwater mysid (Americamysis bahia)
Report No.:	149A-256
Document No.:	M-567966-01-1
Guideline(s):	US EPA OCSPP 850.1350
Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCA 8.2.5.2/03; Brougher, D. S.; Siddiqui, A. I.; Gallagher, S. P.; 2016; M-547035-01-1
Title:	BCS-CN88460: A 96-hour shell deposition test with the eastern oyster (Crassostrea virginica)
Report No.:	149A-258
Document No.:	M-547035-01-1
Guideline(s):	U.S. EPA OPPTS Number 850.1025
Guideline deviation(s):	none
GLP/GEP:	**yes**

Principle of the method
Samples were diluted (4:1) with methanol (dilution factor 1.25). Further dilutions with methanol/test water (1:4 v/v) were performed as necessary to ensure that samples extracts were within the linear range. Extracts were

analysed by reverse phase HLPC-UV (220 nm) using a YMC-PACK ODS-AM, 150mm x 4.6mm x 3µm analytical column at 40ºC and gradient elution using acetonitrile and 0.1% phosphoric acid mobile phases. Quantification was by external standards.

Note: despite the three studies claiming to use the same analytical method, the retention time of BCS-CN88460 in study 149A-256 is 2 minutes longer than in studies 149A-257B and 148A-258

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of an untreated control.

Linearity
Linearity was demonstrated by the analysis of five standards of increasing concentration. The range of standard concentrations used in the individual studies was:

149A-257B: 0.0250 µg/L to 0.125 mg/L, which is equivalent to 0.0313 to 0.156 µg/L in the test water samples. The equation of the calibration line was y = 312x + 0.277 and the correlation coefficient was 0.99997.

149-256: 0.01 µg/L to 0.12 mg/L, which is equivalent to 0.0125 to 0.15 µg/L in the test water samples. The equation of the calibration line was y = 0.301x - 0.146 and the correlation coefficient was 0.9999.

149A-257B: 0.0250 µg/L to 0.125 mg/L, which is equivalent to 0.0313 to 0.156 µg/L in the test water samples. The equation of the calibration line was y = 307x + 0.055 and the correlation coefficient was 0.9997.

The impact of matrix effects has not been specifically addressed.

Accuracy and precision
At each sampling occasion, three separate control samples were fortified with reference standard at three different concentrations and the samples analysed by the method described. The higher recovery level samples were diluted into the demonstrated linear range. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration and precision was determined based on the %RSD of these values. Data are presented below and are acceptable:

Fortification level (mg/L)	% Recovery range (mean)	%RSD (n)
149A-257B		
0.05	98 – 106 (102)	3.9 (3)
0.50	96 – 100 (98)	2.3 (3)
1.0	98 – 99 (99)	0.9 (3)
149A-256		
0.02	101 – 108 (106)	1.9 (9)
0.10	101 – 107 (104)	1.6 (9)
0.375	101 – 108 (104)	2.1 (8)
149A-256		
0.05	100 – 102 (101)	1.2 (3)
0.50	97 – 99 (98)	0.9 (3)
1.0	98 – 101 (99)	1.4 (3)

SANCO/3029/99 rev.4 recommends that five replicates at each of two fortification levels are generated to demonstrate accuracy and precision. In this case, data have been generated for three replicates over three levels. In view of the excellent recovery and precision values presented, the RMS considers that the available data demonstrate that the method is sufficiently accurate and precise and that the requirements of SANCO 3029/99 rev.4 have therefore been met.

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 0.05mg/L for studies 149A-257B and 149A-258, and 0.02 mg/L for study 149A-256. The applicant claims lower LOQs based on lowest calibration standard. These lower values are not validated for quantification. to be 0.0313, based on lowest analytical standard

Extraction efficiency
Not applicable as no extraction is performed.

Stability
The stability of BCS-CN88460 in standard and sample extracts was not assessed in this study but the stability of BCS-CN88460 in several different sample extracts has been adequately demonstrated throughout section B5, so the RMS considers that no further data on stability in test water are required.

Assessment
The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test (salt) water at a LOQ of 0.05 mg/L. A lower LOQ was validated for study 149A-256, but the retention time in this study is 2 minutes different to that in the other two studies for a seemingly identical method and on that basis, it would appear that the analytical conditions were not identical and therefore it is not appropriate to read across this lower LOQ to the other two studies.

Method used in Studies 149P-111, 149P-113, 149P-112A

Report:	KCA 8.2.6.2/01; Arnie, J. R.; Siddiqui, A. I.; Porch, J. R.; Martin, K. H.; 2017; M-605074-01-1
Title:	BCS-CN88460: A 96-hour toxicity test with the cyanobacteria (Anabaena flos-aquae)
Report No.:	149P-111
Document No.:	M-605074-01-1
Guideline(s):	OECD 201 EU Directive 92/69/EEC, Method C.3. U.S. EPA OCSPP Number 850.4550
Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCA 8.2.6.2/02; Arnie, J. R.; Siddiqui, A. I.; Porch, J. R.; Martin, K. H.; 2017; M-604811-01-1
Title:	BCS-CN88460: A 96-hour toxicity test with the marine diatom (*Skeletonema costatum)*
Report No.:	149P-113
Document No.:	M-604811-01-1
Guideline(s):	OECD 201 EU Directive 92/69/EEC, Method C.3. U.S. EPA OCSPP Number 850.4500
Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCA 8.2.6.2/03; Arnie, J. R.; Siddiqui, A. I.; Porch, J. R.; Martin, K. H.; 2017; M-604809-01-1
Title:	BCS-CN88460: A 96-hour toxicity test with the freshwater diatom (Navicula pelliculosa)
Report No.:	149P-112A
Document No.:	M-604809-01-1
Guideline(s):	OECD 201 EU Directive 92/69/EEC, Method C.3. U.S. EPA OCSPP Number 850.4500
Guideline deviation(s):	none
GLP/GEP:	**yes**

Principle of the method

Samples were initially diluted with methanol/water (50:50 v/v), then further diluted as necessary with freshwater AAP medium/methanol/water (50:25:25 v/v/v) to ensure that samples extracts were within the linear range. Extracts were analysed by reverse phase HLPC-MS/MS using a Thermo betasil C-18, 50mm x 2.1mm x 5µm analytical column at 40ºC and gradient elution using 0.1% formic acid in water and 0.1% formic acid in acetonitrile mobile phases. Quantification was by external standards.

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of an untreated control.

Linearity

Linearity was demonstrated by the analysis of five standards of increasing concentration. The range of standard concentrations used in the individual studies was:

149P-111: 0.0005 mg/L to 0.009 mg/L, the equivalent concentration in test water was not stated and could not be calculated because the standard dilution factor was not reported. The equation of the calibration line was y = 76314100x + 2931 and the correlation coefficient was 0.9999.

149P-113: 0.0005 mg/L to 0.009 mg/L, the equivalent concentration in test water was not stated and could not be calculated because the standard dilution factor was not reported. The equation of the calibration line was y = 39105400x + 191 and the correlation coefficient was 0.9992.

149P-112A: 0.0005 mg/L to 0.009 mg/L, the equivalent concentration in test water was not stated and could not be calculated because the standard dilution factor was not reported. The equation of the calibration line was y = 58065000x + 3945 and the correlation coefficient was 0.9969.

The impact of matrix effects has not been specifically addressed.

Accuracy and precision

At each sampling occasion, three separate control samples were fortified with reference standard at 0.0024, 1.0 and 10.0 mg/L and the samples analysed by the method described. Where necessary samples were diluted into the demonstrated linear range. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration and precision was determined based on the %RSD of these values. Data are presented below and are acceptable:

Fortification level (mg/L)	% Recovery range (mean)	%RSD (n)
149P-111		
0.0024	99 – 106 (103)	3.8 (3)
1.0	99 – 103 (101)	2.3 (3)
10.0	91 – 100 (96)	4.7 (3)
149P-113		
0.0024	96 – 108 (101)	6.1 (3)
1.0	96 – 100 (98)	1.7 (3)
10.0	90 – 95 (92)	3.4 (3)
149P-112A		
0.0024	102 – 107 (104)	2.8 (3)
1.0	92 – 103 (98)	5.6 (3)
10.0	91 – 96 (94)	2.7 (3)
overall		
0.0024	96 – 108 (103)	4.0 (9)
1.0	92 – 103 (99)	3.5 (9)
10.0	90 – 100 (94)	3.7 (9)

SANCO/3029/99 rev.4 recommends that five replicates at each of two fortification levels are generated to demonstrate accuracy and precision. In this case, data have been generated for three replicates over three levels. In view of the excellent recovery and precision values presented, the RMS considers that the available data demonstrate that the method is sufficiently accurate and precise and that the requirements of SANCO 3029/99 rev.4 have therefore been met.

In addition, combining the individual data sets results in nine replicates at each of three levels and the overall mean recovery and %RSD are within acceptable limits.

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 0.0024 mg/L.

Extraction efficiency
Not applicable as no extraction is performed.

Stability
The stability of BCS-CN88460 in standard and sample extracts was not assessed in this study but the stability of BCS-CN88460 in several different sample extracts has been adequately demonstrated throughout section B5, so the RMS considers that no further data on stability in test water are required.

Assessment
The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of test water at a LOQ of 0.0024 mg/L.

B.5.1.2.6.2. Sediment and Water

Method used in study 13798.6105

Report:	KCA 8.2.5.4/01; Bradley, M. J.; 2017; M-596883-01-1
Title:	Life-cycle toxicity test exposing midges (*Chironomus dilutus*) to BCS-CN88460 technical applied to sediment under static-renewal conditions following EPA test methods
Report No.:	13798.6405
Document No.:	M-596883-01-1
Guideline(s):	US EPA Test Method 100.5 OCSPP 850.1760 (In Preparation)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Principle of the method
Sediment samples were extracted by successive shaking with a range of solvent solutions. The extracts were separated from the solid residues by centrifugation and subsequently combined and made up to a fixed volume with acetonitrile/water/formic acid (50:50:0.1v/v/v). The extraction solvents used were:

- 0.1% Formic acid in acetonitrile
- Acetonitrile/water/formic acid (80:20:0.1 v/v/v)
- Acetonitrile/water/formic acid (50:50:0.1 v/v/v)

Further dilutions (x2500) were made with acetonitrile/water/formic acid (80:20:0.1 v/v/v) to get the concentrations of the final extracts within the calibrated linear range. Where necessary, higher dilution factors were used.

Water samples were initially diluted (x10) with acetonitrile/water (80:20 v/v) to give a final composition 18:10:72 v/v/v of acetonitrile/test water/purified water. Further dilutions with 18:10:72 v/v/v of acetonitrile/test water/purified water were made as necessary.

Extracts were analysed by HPLC-MS/MS using an XBridge C18, 50mm x 2.1mm x 2.1 µm analytical column at 40ºC and 0.1% formic acid in acetonitrile and 0.1% formic acid in water mobile phases. Quantification was by external standards.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample.

Linearity

Linearity was demonstrated by the analysis (in duplicate) of six standards of increasing concentration. The range of standard concentrations used was 0.005 to 0.10 µg/L, which is equivalent to approximately 0.25 to 5.0 mg/kg in sediment and 0.05 to 1.0 µg/L in aqueous samples. These ranges adequately cover the lowest fortification levels of 3 mg/kg and 0.10 µg/L respectively. The other recovery sample extracts were diluted to be within the linear range. A quadratic calibration line was presented in the study however based on a replotting of the individual data points, the data display acceptable linearity over the range tested and the use of the quadratic equation is therefore not of concern. The equation of the linear calibration graph is y = 3475922x + 335 and the correlation coefficient is 0.9947.

The impact of matrix effects has not been specifically addressed.

Accuracy and precision

Five control samples were fortified with reference standard at each of two levels for the sediment analysis (3 and 100 mg/kg). For the aqueous samples, three replicates were fortified at each of three levels (0.10, 10 and 1000 µg/L). all fortified samples were analysed by the method described. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration and precision in the form of %RSD was calculated from these values. The results are presented below. Acceptable mean recovery levels are within the range 70 to 110 %. Acceptable precision is demonstrated by <20%RSD.

Fortification level	% Recovery range (mean)	%RSD (n)
Sediment (mg/kg)		
3	96 – 104 (101)	3.2 (5)
100	102 – 111 (105)	3.3 (5)
Water (µg/L)		
0.1	97 – 111 (104)	6.8 (3)
10	116 – 117 (116)	0.48 (3)
1000	105 – 118 (110)	6.0 (3)

LOQ

The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 3 mg/kg for sediment and 0.10 µg/L for water.

Extraction efficiency

Not assessed for sediment but as the test systems were artificial it is unlikely tha tthere will be any incurred residues in the sediment. No extraction was involved in the water analysis.

Stability

The stability of BCS-CN88460 in standard and sample extracts was not assessed in this study but the stability of BCS-CN88460 in several different sample extracts has been adequately demonstrated throughout section B5, so the RMS considers that no further data on stability in test water are required.

Assessment

For the sediment analysis, the method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of sediment at levels between 3 and 100 mg/kg.

For the water samples, the mean recovery was outside the acceptance criteria stated in SANCO/3029/99 rev.4, however, that criteria does not take into account the residue level being sought whereas the OECD Guidance on Pesticide Residue Analytical Methods (series on pesticides 39) and, indeed, SANCO 825/00 rev 8.1 guidance for post registration methods, both tie acceptable mean recovery values to the level of analyte being sought and at the levels of 10 µg/kg (comparable to 10 µg/L) the acceptable maximum mean recovery value is 120%. On that basis, the RMS considers that the mean recoveries of 111% seen for the quantification method are acceptable. Additionally, for the water validation, recovery/precision data were generated in triplicate over three levels rather than for five replicates over two levels as recommended in SANCO/3029/99 rev.4. As the available data are acceptable (albeit by referencing alternative quideline criteria), a case can be made that the available data demonstrate that the method is sufficiently accurate and precise and on that basis the method can be regarded as fit for purpose.

The requirements of SANCO/3029/99 rev.4 have not been met for the water method but the RMS considers that the method is sufficiently accurate and precise as to be considered fit for purpose.

B.5.1.2.6.3. Sugar solution

Method used in study 93851136

Report:	KCA 8.3.1.2/01; Gossmann, A.; 2015; M-540173-01-1
Title:	Chronic oral toxicity test of BCS-CN88460 SC 200 (200 G/L) on the honey bee (Apis mellifera L.) in the laboratory
Report No.:	93851136
Document No.:	M-540173-01-1
Guideline(s):	OECD 213 (1998) and CEB No. 230 with current recommendations of the ring test group (2014)
Guideline deviation(s):	none
GLP/GEP:	**yes**

The method used to analyse the sugar solutions containing BCS-CN88460 in this study is based on Method 01432, which is reported in section B5 and was developed to determine levels of BCS-CN88460 in soil and sediment.

Method 01432 was not used exactly as written and validated:
- there was no extraction, the samples were analysed after dilution only.
- the HPLC conditions were different – different column, mobile phase and gradient
- the MRM transitions were different.

Therefore the original validation of method 01432 is not application to the analysis in this study and the additional validation data generated in this study have therefore been evaluated and are reported below.

Principle of the method

Sugar solutions were diluted with acetonitrile/water (50:50 v/v) and analysed by reverse phase HPLC-MS/MS using a Phenomenex Luna, 50 mm x 2.0 mm x 2.5 µm analytical column at 60ºC and acetonitrile/water (1:9 v/v) + 0.1 ml/L formic acid, and acetonitrile + 0.1 ml/L formic acid mobile phases. Quantification was by internal standards of deuterated analytes. MRM transition were m/z 401→139 and m/z 401→177.

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample.

Linearity

Linearity was demonstrated by the analysis (in duplicate) of six standards of increasing concentration. The range of standard concentrations used was 0.05 to 5.0 µg/L, which is equivalent to 0.0025 to 0.25 mg/kg in the sugar solutions and adequately encompasses the recovery concentrations of 0.01 and 0.10 mg/kg. The other recovery samples at 5000 mg/kg and the study samples were diluted to be within the linear range. The equation of the linear calibration graph is $y = 30.11201x + 0.00454$ and the correlation coefficient is 0.99997.

The impact of matrix effects has been addressed by the use of deuterated internal standards.

Accuracy and precision
Five control samples were fortified with reference standard at each of two levels; 0.01 and 0.10 mg/kg, and two at 5000 mg/kg. The samples were analysed by the method described. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration and precision in the form of %RSD was calculated from these values. The results are presented below. Acceptable mean recovery levels are within the range 70 to 110 %. Acceptable precision is demonstrated by <20%RSD.

Fortification level (mg/kg)	% Recovery range (mean)	%RSD (n)
0.01	95 – 102 (99)	2.8 (5)
0.10	96 – 101 (99)	2.6 (5)
5000	99, 94	- (2)

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 0.01 mg/kg.

Extraction efficiency
Not relevant as no extraction was performed.

Stability
The stability of BCS-CN88460 in standard and sample extracts was not assessed in this study but the stability of BCS-CN88460 in several different sample extracts has been adequately demonstrated throughout section B5, so the RMS considers that no further data on stability in sugar solution are required.

Assessment
The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of sugar solution at levels between 0.01 and 5000 mg/kg.

B.5.1.2.6.4. Larval diet

Method used in study S16-00461

Report:	KCA 8.3.1.3/01; Oberrauch, S.; 2017; M-587515-01-1
Title:	BCS-CN88460 - Honey bee (Apis mellifera L.) larval toxicity test (repeated exposure)
Report No.:	S16-00461
Document No.:	M-587515-01-1
Guideline(s):	Regulation (EC) No 1107/2009 (2009) Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.SUPP OECD Draft Guidance Document on Honey bee (Apis mellifera) Larval Toxicity Test, Repeated Exposure (Version dated 20 July 2015)
Guideline deviation(s):	None with impact on the study outcome
GLP/GEP:	**yes**

The method used to analyse the larval diet samples containing BCS-CN88460 in this study is based on Method 01475, which is reported in section B5 and was developed to determine levels of BCS-CN88460 in plant matrices but was also adapted to determine levels of BCS-CN88460 in pollen, nectar and spray solution (B.5.1.2.6.5).

Method 01475 was not used exactly as written and validated:
- there was no extraction, the samples were analysed after dilution only.
- the HPLC mobile phase conditions were slightly different – different column, mobile phase and gradient
-

Therefore the original validation of method 01475 is not applicable to the analysis in this study and the additional validation data generated in this study have therefore been evaluated and are reported below this table.

Principle of the method
Diet solutions were spiked with internal standard solution then diluted with acetonitrile/water (50:50 v/v) and analysed by reverse phase HPLC-MS/MS using a SUPELCO Ascentis Express, 50 mm x 2.0 mm x 2.5 µm

analytical column at 60ºC and water/methanol (9:1 v/v) + 0.12 ml/L formic acid + 10 mmol ammonium formate, and water/methanol (1:9 v/v) + 0.12 ml/L formic acid + 10 mmol ammonium formate mobile phases. Quantification was by internal standards of deuterated analytes. MRM transition m/z 400→177.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample.

Linearity
Linearity was demonstrated by the analysis (in duplicate) of six standards of increasing concentration. The range of standard concentrations used was 0.05 to 5.0 µg/L, which is equivalent to 0.0025 to 0.25 mg/kg in the sugar solutions and adequately encompasses the recovery concentrations of 0.01 and 0.10 mg/kg. The other recovery samples at 5000 mg/kg and the study samples were diluted to be within the linear range. The equation of the linear calibration graph is y = 30.11201x + 0.00454 and the correlation coefficient is 0.99997.

The impact of matrix effects has been addressed by the use of deuterated internal standards.

Accuracy and precision
Three control samples were fortified with reference standard at each of two levels; 0.01 and 0.10 mg/kg, and two at 500 mg/kg. The samples were analysed by the method described. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration and precision in the form of %RSD was calculated from these values. The results are presented below. Acceptable mean recovery levels are within the range 70 to 110 %. Acceptable precision is demonstrated by <20%RSD.

Fortification level (mg/kg)	% Recovery (mean)	%RSD (n)
0.01	99, 103, 107 (103)	3.9 (3)
0.10	92, 94, 96 (94)	2.1 (3)
5000	91, 93 (92)	- (2)

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 0.01 mg/kg.

Extraction efficiency
Not relevant there is no extraction.

Stability
The stability of BCS-CN88460 in standard and sample extracts was not assessed in this study but the stability of BCS-CN88460 in several different sample extracts has been adequately demonstrated throughout section B5, so the RMS considers that no further data on stability in diet are required.

Assessment
Full validation data in accordance with SANCO/3029/99 rev.4 have not been presented for this method. But due to the simplicity of the method (dilution followed by analysis), its similarity to other versions of the same method that have been validated in a range of matrices and the fact that it was used to confirm nominal concentrations in larval diet rather than determine unknown residues, it is considered by the RMS that its use in this study is acceptable.

Method used in study 044SRLS14C01 (Report No. EBLNN001)

Report:	KCA 8.1.1.3/02; [redacted] 2018; M-611590-01-1
Title:	Toxicity of BCS-CN88460 technical in the reproduction of the northern bobwhite quail (Colinus virginianus)
Report No.:	044SRLS14C01
Document No.:	M-611590-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009

	OCSPP 850.2300 OECD Guideline 206
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Principle of the method
Samples were extracted by shaking with two separate aliquots of acetonitrile. The extracts were combined and made up to a fixed volume with acetonitrile then an aliquot was diluted (D x 625) with acetonitrile/water (1:4 v/v) + 0.1% acetic acid. Extracts were spiked with an internal standard solution of deuterated analyte prior to analysis by reverse phase HPLC-MS/MS (MRM 400 → 139) using a Kinetix XB-C18, 100mm x 2.1mm x 2.6µm analytical column at ambient temperature and gradient elution using acetonitrile and 0.1% aqueous acetic acid mobile phases. Quantification was by external standards. Where necessary sample extracts were further diluted into the linear range.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of an untreated control sample

Linearity
Linearity was demonstrated by the analysis of seven standards of increasing concentration. The range of standard concentrations used was 0.1 µg/L to 4.0 µg/L, which is equivalent to 2.5 to 100 mg/kg in the diet samples. The equation of a typical calibration line was y = 0.01968x - 0.003728 and the correlation coefficient was 0.9995.

The impact of matrix effects has not been specifically addressed.

Accuracy and precision
Five control samples were fortified with reference standard at 5 and 1200 mg/kg and the samples analysed by the method described. The higher recovery level samples were diluted into the demonstrated linear range. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration and precision was determined based on the %RSD of these values. Data are presented below and are acceptable:

Fortification level (mg/kg)	% Recovery range (mean)	%RSD (n)
5	98 – 103 (100)	1.9 (5)
1200	93 – 97 (95)	2.0 (5)

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 5 mg/kg.

Extraction efficiency
Not provided but there are no incurred residues as diet formulations are prepared by spiking.

Assessment
The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of isoflucypram in samples of avian diet with an LOQ of 5 mg/kg.

B.5.1.2.6.5. Nectar, Pollen and Spray Solution Samples

Report:	KCP 10.3.1.5/03; Schmitzer, S.; 2017; M-606834-01-1
Title:	Isoflucypram EC 50 G: Toxicity testing on honey bees (Apis mellifera L.) under semi-field conditions in Germany - Tunnel test
Report No.:	122701037
Document No.:	M-606834-01-1
Guideline(s):	OEPP/EPPO No. 170 (4)(2010) Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP Not Applicable

Guideline deviation(s):	None
GLP/GEP:	**yes**

Method 01475, as reported in section B.5.1.2.5.1 was used as the basis for the method for the determination of isoflucypram residues in nectar and pollen taken from samples of Honey Bees from this study. Due to the very small sample sizes, adaptations were made to method 01475 including the use of reduced sample sizes and extraction volumes and replacing the blending for shaking. In addition, different analytical conditions were used. Details of the adapted method are presented below along with a summary of the supplementary validation data that was generated to support it.

Principle of the method

Samples of pollen were extracted twice with acetonitrile/water (4:1 v/v) using a Precellys homogeniser. Extracts were combined, spiked with internal standard solution of deuterated analyte and then made up to volume prior to analysis by HPLC-MS/MS as described below.

Samples of nectar and spray solution were dissolved in acetonitrile:water (4:1 v/v) using an overhead shaker, then spiked with internal standard solution of deuterated analyte and made up to volume prior to analysis by reverse phase HPLC-MS/MS using a Phenomenex luna C18, 50mm x 2mm x 2.5µm analytical column at 60ºC and gradient elution using water (+0.1 ml/L acetic acid) and methanol (+0.1 ml/L acetic acid) mobile phases. Quantification was by external standards and the ion transition m/z 400.1→139.1 was monitored.

The impact of matrix effects was addressed by the use of deuterated internal standards.

Supplementary validation data, comprising one control and three replicate recovery determinations at each of two fortification levels (0.01 and 0.10 mg/kg), as well as an example calibration graph and representative chromatograms are summarised in Table B.5.1.2-26.

Procedural recovery determinations analysed alongside the study samples were submitted at levels of 1 mg/kg in all sample matrices and also at 2 mg/kg in spray solution. The levels detected in the trials samples, however, were up to 15.1 mg/kg in pollen and 184 mg/kg in spray solution. Levels of isoflucypram in the nectar samples were found at a maximum level of 0.237 mg/kg.

The LOQ for isoflucypram in these additional matrices is 0.01 mg/kg. No residues above 30% of the LOQ were detected in the control samples.

Assessment

A reduced validation data set of only three replicate determinations at each of 2 recovery levels has been submitted. In accordance with SANCO/3029/99 rev 4. it is acceptable to submit a reduced validation set for additional matrices if the method used is fully validated for a comparable matrix within the same crop group and Method 01475 has been acceptably validated for the determination of isoflucypram in five matrices representative of the major crop groupings; tomato (high water), orange (high acid), wheat grain (dry), wheat straw (no group), rape seed (high oil) and bean seed (dry). However, the current method is not exactly the same as Method 01475 due to differences in the sample extraction procedure and the analytical conditions and also the validated levels for these additional matrices do not cover the levels determined in the samples.

Justification for acceptability of the method validation and reliance on the results of the honey bee analysis is provided by additional validation data within a separate honey bee study; Vallon, A., 2017 (study number EBLN008) as described below. In that study, the same procedure as described here was used to analyse samples of nectar, pollen and spray solution and, by combining the validation data generated in both studies, the validation requirements of SANCO/3029/99 rev.4 are met.

Additionally, the procedural recoveries performed alongside the study samples in Vallon, A., 2017 were at much higher concentrations (up to 50 mg/kg in pollen and 200 mg/kg in spray solution samples). Acceptable recoveries were reported at both levels thereby demonstrating acceptable performance of the method at these higher levels, which cover the concentrations detected in the study samples.

In summary, using the validation data generated in this study in combination with that generated in Study EBLN0008 below, the validation requirements of SANCO/3029/99 rev.4 are met and this modified version of

method 01475 is valid for the determination of isoflucypram in samples of nectar, pollen and spray solution at the levels seen in both studies.

Report:	KCP 10.3.1.5/04; Vallon, A.; 2017; M-607771-01-1
Title:	Assessment of side-effects of isoflucypram EC 50 G on the honeybee (Apis mellifera L.) in a semi-field study after application in flowering Phacelia tanacetifolia in Spain 2017
Report No.:	EBLN0008
Document No.:	M-607771-01-1
Guideline(s):	OEPP/EPPO Guideline No. 170(4), 2010; EU Guideline 7029/VI/95 rev. 5 Regulation (EC) No 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP Not Applicable
Guideline deviation(s):	None
GLP/GEP:	**yes**

Method 01475, as reported in section B.1.5.2.5.1 was used as the basis for the method for the determination of isoflucypram residues in nectar and pollen taken from samples of Honey Bees from this study. Due to the very small sample sizes, adaptations were made to method 01475 including the use of reduced sample sizes and extraction volumes and replacing the blending for shaking. In addition, different analytical conditions were used. Details of the adapted method are presented below along with a summary of the supplementary validation data that was generated to support it.

Principle of the method

Samples of pollen were extracted twice with acetonitrile/water (4:1 v/v) using a Precellys homogeniser. Extracts were combined, spiked with internal standard solution of deuterated analyte and then made up to volume prior to analysis by HPLC-MS/MS as described below.

Samples of nectar and spray solution were dissolved in acetonitrile:water (4:1 v/v) using an overhead shaker, then spiked with internal standard solution of deuterated analyte and made up to volume prior to analysis by reverse phase HPLC-MS/MS using a Phenomenex luna C18, 50mm x 2mm x 2.5µm analytical column at 60ºC and gradient elution using water (+0.1 ml/L acetic acid) and methanol (+0.1 ml/L acetic acid) mobile phases. Quantification was by external standards and the ion transition m/z 400.1→139.1 was monitored.

Supplementary validation data, comprising one control and three replicate recovery determinations at two fortification levels (0.01 and 0.10 mg/kg), as well as an example calibration graph and representative chromatograms are summarised in Table B.5.1.2-27.

Procedural recovery determinations analysed alongside the study samples were submitted at levels up to 1 mg/kg in nectar, 50 mg/kg in pollen and 200 mg/kg in spray solution samples. Achieved recoveries were between 70 to 106% (n=17). The levels detected in the trials samples were covered by these higher recovery levels.

The LOQ for isoflucypram in these additional matrices is 0.01 mg/kg. No residues above 30% of the LOQ were detected in the control samples.

Assessment

Using the validation and procedural recovery data generated in this study in combination with that generated in Study 122701037 above, the validation requirements of SANCO/3029/99 rev.4 are met and this modified version of method 01475 is valid for the determination of isoflucypram in samples of nectar, pollen and spray solution at the levels seen in both studies.

Table B.5.1.2-26 Summary of supplementary validation data for analytical method 01475 - quantification method for BCS-CN88460 residues in nectar, pollen and spray solution (Schmitzer, S., 2017)

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Nectar	BCS-CN88460	0.01	0.01 0.1	95 – 107 (101) 89 – 94 (91)	6.0 (3) 2.8 (3)	0.050 to 50 µg/L (0.002 to 2.0 mg/kg) y = 1.1132x - 0.035516 r = 0.9986	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Pollen		0.01	0.01 0.1	86 – 98 (91) 90 – 104 (99)	6.7 (3) 8.1 (3)		
Spray solution		0.01	0.01 0.10	94 – 105 (99) 103 – 111 (106)	5.6 (3) 3.9 (3)		

Table B.5.1.2-27 Summary of supplementary validation data for analytical method 01475 - quantification method for BCS-CN88460 residues in nectar, pollen and spray solution (Vallon, A., 2017)).

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Nectar	BCS-CN88460	0.01	0.01 0.1	97 – 102 (99) 89 – 95 (92)	2.5 (3) 3.3 (3)	0.050 to 50 µg/L (0.002 to 2.0 mg/kg) y = 1.0281x - 0.0061839 r = 0.9995	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Pollen		0.01	0.01 0.1	89 – 99 (95) 81 – 82 (82)	5.8 (3) 0.7 (3)		
Spray solution		0.01	0.01 0.10	93 – 104 (97) 86 – 100 (93)	6.0 (3) 7.5 (3)		

B.5.1.2.7. ***Methods used in support of physical and chemical properties studies.***

BCS-CN88460 (isoflucypram)

Report:	KCA 2.5/01; Ziemer, F., Peschke, C., 2014; M-488486-01-1
Title:	BCS-CN88460, pure substance: Solubility in distilled water (column elution method)
Report No.:	PA14/030
Document No.:	M-488486-01-1
Guideline(s):	European Commission Council Regulation (EC) No 440/2008, Annex, Part A, method A.6. OECD-Guideline 105 US EPA Product Properties Test Guideline OCSPP 830.7840
Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCA 2.6/01; Eyrich, U., Ziemer, F., 2014; M-491820-01-1
Title:	BCS-CN88460, pure substance: Solubility in organic solvents
Report No.:	PA14/060
Document No.:	M-491820-01-1
Guideline(s):	European Commission Council Regulation (EC) No 440/2008, Annex, Part A, method A.6., OECD Guideline 105 and US EPA Product Properties Guideline OCSPP 830.7840
Guideline deviation(s):	None
GLP/GEP:	**yes**

Report:	KCA 2.12/01; Eyrich, U., Ziemer, F., 2014, M-488659-01-1
Title:	BCS-CN88460, pure substance: Determination of the surface tension
Report No.:	PA14/059
Document No.:	M-488659-01-1
Guideline(s):	OECD Guideline 115 European Commission Council Regulation (EC) No 440/2008, Annex, Part A, method A.5. US EPA OCSPP Not Applicable
Guideline deviation(s):	None
GLP/GEP:	**Yes**

The analytical method described in this section was used:

- to determine the concentration of isoflucypram in the column elution samples from the solubility in water study, PA14/030 (reported in Volume 3CA Section B.2.5/01) as well as the concentration of the isoflucypram coating remaining on the support material (after appropriate dilution into the range).

- to determine the concentration of isoflucypram in the organic solvents heptane and methanol (after appropriate dilution into the linear range) as part of the solubility in organic solvent measurement in study PA14/060 reported in Volume 3CA Section B.2.6/01.

- to determine the concentration in the aqueous solution used to measure surface tension in study PA14/059 (Volume 3CA Section B.2.12/01). These samples were injected directly and were quantified against a reference standard of similar nominal concentration (~1.5 mg/L) which was well within the established linear range of the method.

The method was validated within study PA14/030 and a summary of the method and its validation is presented below .

Principle of the method

Samples were analysed without any additional sample work-up or dilution by reverse phase HPLC-UV (218nm) using an Eclipse Plus C18 50mm x 4.6mm x 1.8µm analytical column at 40ºC and isocratic elution with acetonitrile/water (70:30v/v) mobile phase. Quantification was by external standards.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample.

Linearity
Linearity was demonstrated by the analysis (in duplicate) of six standards of increasing concentration. The range of standard concentrations used was 0.20 mg/L to 4 mg/L, which is directly equivalent to the levels in the samples as there was no sample work up or dilution. Due to the low solubility of isoflucypram in water, standards solution were prepared by dissolving in acetonitrile and then diluting 1:99 v/v with water

Accuracy
Five separate solutions were prepared at ~2 mg/L and analysed samples analysed by the method described. The RMS assumes that these samples were prepared in the same way as the standard solutions in order to achieve the desired concentration, but this is not specifically stated in the report. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 110 %.

Precision
Precision in the form of %RSD was calculated for the accuracy determinations described above. Acceptable precision is demonstrated by <20%RSD.

LOQ
The LOQ is defined by the lowest concentration at which acceptable recovery and precision data have been generated. In this case, it is 2 mg/L, which is above the solubility of 1.8 mg/L determined in the study, but in view of the large, well defined peak at that concentration and that fact that the calibration standards range from 0.02 to 4 mg/L, the RMS considers that the method is suitable for the analysis.

Extraction efficiency
Not applicable as no extraction is performed.

Assessment
In accordance with Reg (EU) 283/2013 and SANCO/3029/99 rev.4 (11/07/00), accuracy and precision data should be generated at 2 levels; the LOQ and either the likely residue levels or 10 times the LOQ. In this case, data have been generated at the expected residue level only however, given that the samples are injected directly without any sample work-up or dilution, the available precision and recovery data are very good, there is a large well defined peak at 2 mg/L and the linearity range extends from 10 to 200% of this expected concentration, the RMS considers that the method is acceptable and no further data are required.

Analyte	Recovery fortification level (mg/L)	% Recovery range (mean)	Repeatability % RSD (n)	Linearity	Specificity
BCS-CN88460	2.0	91 – 100 (95)	4.2 @ 2 mg/L (n=5)	0.20 to 4.0 mg/L (direct analysis) y = 0.200224x + 0.03047 r = 0.9956	Retention time match to reference standard. No significant interfering peaks observed in the blank formulation.

BCS-CY26497 (BCS-CN88460-carboxylic acid)

Report:	KCA 2.7/02; Ziemer, F., Peschke, C., 2015; M-519996-01-1
Title:	BCS-CY26497 (BCS-CN88460-carboxylic acid): Partition coefficients 1-octanol / water at pH 5, pH 7 and pH 9 (shake flask method)
Report No.:	PA14/151
Document No.:	M-519996-01-1
Guideline(s):	Not stated
Guideline deviation(s):	Not stated
GLP/GEP:	**yes**

The analytical method described in this section was used to determine the concentration of BCS-CY26497 in the octanol and water phases from the partition coefficient study, PA14/151 as reported in Volume 3CA Section B.2.7/01.

The method was validated within study PA14/151 and a summary of the method and its validation is presented below.

Principle of the method
Aliquots of the octanol and aqueous phases were diluted with acetoniltrile/water 1:1 v/v and analysed by reverse phase HPLC-DAD (215nm) using an Eclipse Plus C18 50mm x 4.6mm x 1.8µm analytical column at 40°C and isocratic elution with acetonitrile/0.01M phosphoric acid (50:50/v) mobile phase. Quantification was by external standards.

Note: the rates of dilution varied for each sample. The aqueous phase from the pH 5 dtermination was analysed without dilution.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample.

Linearity
Linearity was demonstrated by the analysis (in duplicate) of seven standards of increasing concentration. The range of standard concentrations used was 0.20 mg/L to 4 mg/L. The corresponding concentration in the octanol and aqueous phases varied depending on the dilution factor. Final concentrations were within the demonstrated linear range.

Accuracy
Five separate solutions were prepared at ~20 mg/L and analysed samples analysed by the method described. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 110 %.

Precision
Precision in the form of %RSD was calculated for the accuracy determinations described above. Acceptable precision is demonstrated by <20%RSD.

LOQ
The LOQ is defined by the lowest concentration at which acceptable recovery and precision data have been generated. In this case, it is 20 mg/L.

Extraction efficiency
Not applicable as no extraction is performed.

Assessment
In accordance with Reg (EU) 283/2013 and SANCO/3029/99 rev.4 (11/07/00), accuracy and precision data should be generated at 2 levels; the LOQ and either the likely residue levels or 10 times the LOQ. In this case, data have been generated at one level only however, given that the samples are injected after dilution only (no sample work-up), the available precision and recovery data are very good, there is a very large well defined peak at 20 mg/L

and the linearity range extends from 2.5 to 200% of this expected concentration, the RMS considers that the method is acceptable and no further data are required.

Analyte	Recovery fortification level (mg/L)	% Recovery range (mean)	Repeatability % RSD (n)	Linearity	Specificity
BCS-CY26497	20	99 – 101 (100)	0.6 @ ~20 mg/L (n=5)	0.50 to 40 mg/L y = 0.239021x + 0.00534 r = 0.99999	Retention time match to reference standard. No significant interfering peaks observed in the blank formulation.

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

B.5.2.1. Analytical method for the determination of residues in/on food and feed of plant origin.

Report:	KCA 4.2/04; Uceda, L.; 2017; M-588974-01-1
Title:	Analytical method 01520 for the determination of residues of BCS-CN88460 in/on plant by HPLC-MS/MS
Report No.:	17-02
Document No.:	M-588974-01-1
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and 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 - Guidance document on pesticide residue analytical methods, SANCO/825/00 rev.8.1 16/11/2010 - European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 - OECD Guidance Document on Pesticide Residue Analytical Methods, Series on Testing and Assessment Document 72 and Series on Pesticides: Document 39, August 2007 (OECD Guideline, ENV/JM/MONO (2007) 17, Aug 13, 2007) [4] - US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method of August 1996 [5]
Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCA 4.2/05; Schmiedt, S.; 2016; M-603219-01-1
Title:	Independent laboratory validation of analytical method 01520 for the determination of residues of BCS-CN88460 in/on plant by HPLC-MS/MS
Report No.:	P 4386 G
Document No.:	M-603219-01-1
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and 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; European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00; Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010; US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method; OECD Guidance document on pesticide residue analytical methods, ENV/JM/MONO (2007) 17, 2007-08-13
Guideline deviation(s):	--
GLP/GEP:	**yes**

Report:	KCA 4.2/01; Traub, M.; 2018; M-611065-02-1
Title:	Amendment no.1 to final report - Testing of the extraction efficiencies according QuEChERS using radioactive incurred residues of BCS-CN88460 in different wheat, soybean and oilseed rape RACs
Report No.:	S16-05413
Document No.:	M-611065-02-1
Guideline(s):	US EPA OCSPP Residue Chemistry Test Guideline OPPTS 860.1340: Residue

	Analytical Method OECD Series on Testing and Assessment No. 72 and Series on Pesticides No. 39 Guidance Document on Pesticide Residue Analytical Methods Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 110712009 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
Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCA 4.2/02; Lamshoeft, M.; Weuthen, M.; 2017; M-598222-01-1
Title:	Extraction efficiency testing of the QuEChERS analytical method for the determination of residues of BCS-CN88460 in the primary RAC tomato using incurred radioactive residues
Report No.:	EnSa-17-0483
Document No.:	M-598222-01-1
Guideline(s):	US EPA OCSPP Residue Chemistry Test Guideline OPPTS 860.1340: Residue Analytical Method Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009
Guideline deviation(s):	none
GLP/GEP:	**yes**

Definition of the residue

The proposed definition of residue in plants, plant products, foodstuff (of plant origin) and feeding stuff (of plant origin) is parent Isoflucypram (BCS-CN88460) only.

Suitability of available multi-residue methods

The extraction efficiency of the QuEChERS analytical method for the determination of BCS-CN88460 in/on primary crops of tomato, wheat (hay, straw and grain), soybean (forage, hay, straw and seed) and oilseed rape (intermediate harvest and seed) was assessed in Traub, M., 2018 (M-611065-02-1) and Lamshoef, M., Weuthen, M., 2017 (M-598222-01-1). In these studies, samples containing incurred residues of BCS-CN88460 were extracted using the QuEChERs analytical method (extraction solvent was acetonitrile for tomatoes, and acetonitrile/water 50:50 v/v for the other crops) and compared to the results obtained in the primary crop metabolism studies (there was a dichoromethane wash of the tomatoes then all crops were conventionally extracted with acetonitrile water 8:2 v/v followed, where necessary, by microwave extraction with acetonitrile/water/formic acid 50:50:1 v/v/v). A summary of the results is presented below and shows that the QuEChERs method is not suitable for all matrices, with particularly low extraction efficiencies being reported for straw, hay and forage sample.

Summary of extraction efficiencies of QuEChERs method for various crops as reported in Traub, M., 2018 (M611065-02-1).

Matrix	Distribution of radioactivity	Summary of Extraction				Efficiency of Extraction (%)
		QUECHERS method		Metabolism study		
		%TRR	mg/kg	%TRR	mg/kg	
Wheat hay	TRR	100	3.999	100	4.032	
	Extracted	56.7	2.267	95.8	3.864	
	- BCS-CN88460	24.7	0.986	50.0	2.016	49
	Unextracted	43.3	1.732	4.2	0.168	
Wheat straw	TRR	100	16.925	100	15.536	
	Extracted	50.2	8.504	98.7	15.330	
	- BCS-CN88460	35.1	5.955	64.0	9.933	55
	Unextracted	49.8	8.421	1.3	0.206	

Wheat grain	TRR	100	0.267	100	0.385	
	Extracted	84.0	0.224	93.6	0.360	
	- BCS-CN88460	78.6	0.210	92.0	0.354	85
	Unextracted	16.0	0.043	6.4	0.025	
Soybean forage	TRR	100	4.186	100	4.371	
	Extracted	52.6	2.201	97.2	4.248	
	- BCS-CN88460	8.4	0.354	18.7	0.819	45
	Unextracted	47.4	1.985	8.2	0.123	
Soybean hay	TRR	100	3.026	100	4.679	
	Extracted	43.8	1.326	94.3	4.413	
	- BCS-CN88460	4.5	0.138	10.4	0.487	43
	Unextracted	56.2	1.700	5.7	0.266	
Soybean straw	TRR	100	18.775	100	17.715	
	Extracted	67.8	12.724	96.6	17.110	
	- BCS-CN88460	47.2	8.859	64.5	11.424	73
	Unextracted	32.2	6.052	3.4	0.605	
Soybean seed	TRR	100	0.028	100	0.035	
	Extracted	67.1	0.019	87.7	0.031	
	- BCS-CN88460	67.1	0.019	76.6	0.027	88
	Unextracted	32.9	0.009	12.3	0.004	
Oilseed rape (intermediate harvest)	TRR	100	4.596	100	4.751	
	Extracted	76.3	3.507	99.5	4.730	
	- BCS-CN88460	60.8	2.795	81.9	3.890	74
	Unextracted	23.7	1.089	0.5	0.022	
Oilseed rape seed	TRR	100	0.093	100	0.099	
	Extracted	54.9	0.051	93.3	0.093	
	- BCS-CN88460	54.9	0.051	71.0	0.070	77
	Unextracted	45.1	0.042	6.7	0.006	

Summary of extraction efficiencies of QuEChERs method for tomatoes as reported in Lamshoef, M., Weuthen, M., 2017 (M598222-01-1).

Matrix	Distribution of radioactivity	Summary of Extraction				Efficiency of Extraction (%)
		QUECHERS method		Metabolism study		
		%TRR	mg/kg	%TRR	mg/kg	
Tomato	TRR	100	0.220	100	0.170	
	Extracted	84.4	0.186	99.7	0.170	
	- BCS-CN88460	82.3	0.181	96.7	0.165	85
	Unextracted	15.6	0.034	0.2	0.000	

The QuEChERs method does not extract sufficient available residue from all samples therefore an alternative method is proposed for monitoring. The proposed monitoring method for food and feed of plant origin, Method 01520, and its associated validation data are presented below and in tables B.5.2.1-1 to B.5.2.1-4.

Principle of the method

Samples were extracted twice by homogenisation (ultra turrax) with acetonitrile/water (8:2 v/v). The resulting extract was centrifuged, and the supernatants combined and made up to a fixed volume with extraction solvent to give Extract A, which was then filtered and further diluted with acetonitrile/water (17:73 v/v) to give the final extract. Final extracts were analysed by reverse phase HLPC-MS/MS using an Ascentis express C18, 50 mm x 2.1 mm x 2.7µm analytical column at 60 ºC and gradient elution using water/formic acid (1000/0.12 v/v) + 10 mM ammonium formate and water/methanol/formic acid (100/900/0.12 v/v/v) + 10 mM ammonium formate mobile phases. Quantification was by external standards. Two ion transitions were monitored; *m/z* 400.2→139.0 for quantification and *m/z* 400.2→177.0 for confirmation.

Note: for dry crops (wheat grain, rape seed, bean seed (dry) and coffee bean (green), the first aliquot of extraction solvent was added as separate components; the water first, then after 20 minutes, the acetonitrile. The purpose of this was to soak the dry crops, thus assisting in the extraction efficiency.

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) samples.

Linearity
Linearity was demonstrated by the analysis of ten standards of increasing concentration. The range of standard concentrations used was 0.004 to 2.0 µg/L which is equivalent to 0.002 to 1.0 mg/kg, and adequately encompasses the recovery concentrations of 0.01 (LOQ) and 0.10 mg/kg (10xLOQ). Standards in solvent as well as matrix matched standards were analysed with each set of samples for each ion transition.

Deviations from these ranges occurred during the orange and coffee validation, where the lowest calibration point was rejected from the solvent and matrix standard calibration graphs for orange (both transitions) and the matrix calibration graph for coffee (confirmatory transition only) resulting in linearity ranges of 0.005 to 2 µg/L for these matrix/transitions, which is equivalent to 0.0025 to 1.0 mg/kg.

Correlation coefficients were >0.99 for all graphs.

Accuracy
Five control samples were fortified with reference standards at each of two levels and the samples analysed by the method described. Levels in the fortified samples were determined using calibration graphs of solvent standards as well as matrix matched standards, and recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 60 to 120 % at residue levels at or below 0.01 mg/kg and 70 to120 % at residue levels between 0.01 and 0.10 mg/kg.

Matrix effects were mainly insignificant with the exception of orange and coffee bean samples which indicated an enhancement of between 9 and 18% in recovery values measured against solvent standards compared to those measured against matrix standards (across both quantification and confirmatory ion transitions). It is therefore recommended to use matrix matched standards to quantify residues of Isoflucypram (BCS-CN88460).

Precision
Precision in the form of %RSD was calculated for the accuracy determinations described above. Acceptable precision is demonstrated by <30%RSD at residue levels at or below 0.01 mg/kg and <20% RSD at residue levels between 0.01 and 0.10 mg/kg.

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 0.01 mg/kg.

Confirmation
Residues were confirmed simultaneously to the quantification method by monitoring a second ion transition. Validation data as described above were submitted for the confirmatory method and the results are summarised in Table B.5.2.1-2

Extraction efficiency
The extraction procedure used in this analytical method is the same as that in residue analytical method 01475, which has already been evaluated for its extraction efficiency (refer to section B.5.1.2.5.1.). Extraction efficiency was confirmed in high water, high oil and dry crops but not in high acid crops.

Stability
The stability of BCS-CN88460 in standard solutions was demonstrated during the validation of method 01475 where it was shown that BCS-CN88460 was stable for 107 days in standard solutions of 1 mg/L prepared in acetonitrile and stored in the dark at around 4°C ± 3°C

The stability of BCS-CN88460 in sample extracts was also assessed during the validation of method 01475. BCS-CN88460 is stable in Extract A stored at 4°C ± 3°C for at least 106 hours in tomato (fruit), 149 hours in orange (fruit), 82 hours in wheat (grain), 54 hours in rape (seed) and 77 hours in bean (dry seed).

No stability data were available for coffee bean extracts in method 01475; also the preparation of the final extracts for all matrices was slightly different across the two methods, so additional data on the stability of BCS-CN88460 in Extract A for coffee bean (green) and in final extracts for all matrices were generated within this

study. The data show that BCS-CN88460 is stable in coffee bean (green) extract A for at least 97 hours when stored at 4°C ± 3°C in the dark. In final extracts (and thus in matrix matched standards), BCS-CN88460 is stable for at least 318 hours in tomato (fruit), 194 hours in orange (fruit), 318 hours in rape (seed), 172 hours in bean (dry seed) and 173 hours in coffee (green bean) when stored at 10°C ± 3°C in the dark. For wheat (grain), instability after 343 hours was reported, however, this was due to an increase in concentration of BCS-CN88460 in extracts reanalysed after the storage period. The RMS considers that this may be due to a concentration effect rather than the instability reported.

Independent Laboratory Validation

Method 01520 was validated in a second facility, independent to where the original validation was performed. The method was used as written with only minor modifications reported (alternative glassware and slightly higher centrifugation speed). These are not expected to have an impact on the results.

In addition to these reported differences, the RMS notes that the temperature of the HPLC column oven was 40ºC in the independent validation and was 60ºC in the primary validation. This difference in temperature is a likely explanation for the slightly longer retention time of 2.4 mins for BCS-CN88460 in the ILV compared to 2.1 minutes in the primary validation. This difference does not affect the validity of the ILV.

Matrix matched standards were used for all matrices, as recommended in the primary method. Two ion transitions were monitored; *m/z* 400.2→139.1 for quantification and *m/z* 400.2→177.1 for confirmation, which are fractionally different to the primary method but this difference does not affect the validity of the ILV.

A summary of the ILV data are presented in tables B.5.2.1-3 and B5.2.1-4.

Assessment

The method is acceptably validated in accordance with SANCO/825/00 rev. 8.1 and is suitable for use as a monitoring method for the determination of isoflucypram in/on plants from high water, high oil and dry crops, with a LOQ of 0.01 mg/kg, which is suitable for monitoring. For high acid crops, further data are required to demonstrate the extraction efficiency of the method with respect to isoflucypram, but all other validation parameters have been met.

Table B.5.2.1-1 Summary of validation data for analytical method 01520 - **quantification** method for BCS-CN88460 residues in/on food and feed of plant origin

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Tomato	BCS-CN88460 (matrix standards) *m/z* 400.2→139.0	0.01	0.01 0.1	100 – 107 (103) 100 – 105 (102)	2.8 (5) 1.9 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 1580000x + 72.6 r = 1.0000	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (solvent standards) *m/z* 400.2→139.0	0.01	0.01 0.1	(98) (97)	2.7 (5) 2.0 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 1670000x – 274 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Orange	BCS-CN88460 (matrix standards) *m/z* 400.2→139.0	0.01	0.01 0.1	83 – 97 (91) 91 – 102 (95)	6.2 (5) 4.6 (5)	0.005 to 2 µg/L (0.0025 to 1.0 mg/kg) y = 1430000x + 1910 r = 1.0000	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (solvent standards) *m/z* 400.2→139.0	0.01	0.01 0.1	(94) (99)	6.1 (5) 4.3 (5)	0.005 to 2 µg/L (0.0025 to 1.0 mg/kg) y = 1370000x + 2240 r = 1.0000	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Wheat grain	BCS-CN88460 (matrix standards) *m/z* 400.2→139.0	0.01	0.01 0.1	98 – 117 (108) 97 – 101 (99)	8.5 (5) 1.7 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 1780000x + 5350 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (solvent standards) *m/z* 400.2→139.0	0.01	0.01 0.1	(124) (110)	8.1 (5) 1.9 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 1600000x + 3910 r = 1.0000	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Coffee bean (green)	BCS-CN88460 (matrix standards) *m/z* 400.2→139.0	0.01	0.01 0.1	89 – 94 (91) 91- 101 (98)	2.1 (5) 4.2 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 1720000x + 8740 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (solvent standards) *m/z* 400.2→139.0	0.01	0.01 0.1	(106) (107)	2.5 (5) 3.9 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 1580000x + 6580 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Rape seed	BCS-CN88460 (matrix standards) *m/z* 400.2→139.0	0.01	0.01 0.1	84 – 92 (88) 89 – 93 (91)	4.3 (5) 1.8 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 1690000x + 9150 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (solvent standards) *m/z* 400.2→139.0	0.01	0.01 0.1	(94) (97)	4.4 (5) 2.0 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 1590000x + 8950 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Bean seed (dry)	BCS-CN88460 (matrix standards) *m/z* 400.2→139.0	0.01	0.01 0.1	91 – 98 (94) 94 – 101 (97)	3.1 (5) 3.1 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 2040000x + 1560 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (solvent standards) *m/z* 400.2→139.0	0.01	0.01 0.1	(97) (102)	3.0 (5) 3.2 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 1940000x + 2080 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Note: for the recovery determinations based on solvent standards, only the mean value was reported.

Table B.5.2.1-2 Summary of validation data for analytical method 01520 - **confirmatory** method for BCS-CN88460 residues in/on food and feed of plant origin

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Tomato	BCS-CN88460 (matrix standards) *m/z* 400.2→177.0	0.01	0.01 0.1	96 – 105 (101) 99 – 104 (101)	3.2 (5) 1.9 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 871000x + 58.8 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (solvent standards) *m/z* 400.2→177.0	0.01	0.01 0.1	(96) (96)	3.0 (5) 1.8 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 921000x – 95.6 r = 1.0000	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Orange	BCS-CN88460 (matrix standards) *m/z* 400.2→177.0	0.01	0.01 0.1	91-104 (96) 91-102 (95)	5.2 (5) 4.4 (5)	0.005 to 2 µg/L (0.0025 to 1.0 mg/kg) y = 783000x + 510 r = 1.0000	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (solvent standards) *m/z* 400.2→177.0	0.01	0.01 0.1	(94) (98)	5.6 (5) 4.2 (5)	0.005 to 2 µg/L (0.0025 to 1.0 mg/kg) y = 754000x + 1420 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Wheat grain	BCS-CN88460 (matrix standards) *m/z* 400.2→177.0	0.01	0.01 0.1	98 -120 (108) 97-103 (99)	7.7 (5) 2.3 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 961000x + 2380 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (solvent standards) *m/z* 400.2→177.0	0.01	0.01 0.1	(124) (109)	7.1 (5) 2.2 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 878000x + 1300 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Coffee bean (green)	BCS-CN88460 (matrix standards) *m/z* 400.2→177.0	0.01	0.01 0.1	85-93 (89) 89 – 100 (96)	3.2 (5) 4.7 (5)	0.005 to 2 µg/L (0.0025 to 1.0 mg/kg) y = 956000x + 4910 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (solvent standards) *m/z* 400.2→177.0	0.01	0.01 0.1	(107) (107)	2.7 (5) 4.8 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 864000x + 3430 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Oilseed rape (seed)	BCS-CN88460 (matrix standards) *m/z* 400.2→177.0	0.01	0.01 0.1	86 – 90 (88) 88 – 93 (90)	1.7 (5) 2.0 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 931000x + 4310 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (solvent standards) *m/z* 400.2→177.0	0.01	0.01 0.1	(92) (96)	1.6 (5) 1.9 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 868000x + 4700 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Bean seed (dry)	BCS-CN88460 (matrix standards) *m/z* 400.2→177.0	0.01	0.01 0.1	93 – 103 (97) 92 – 99 (96)	4.0 (5) 3.0 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 1130000x + 664 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (solvent standards) *m/z* 400.2→177.0	0.01	0.01 0.1	(101) (101)	4.6 (5) 2.7 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 1060000x + 1040 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Table B.5.2.1-3 Summary of ILV data for analytical method 01520 - **quantification** method for BCS-CN88460 residues in/on food and feed of plant origin

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Tomato	BCS-CN88460 *m/z* 400.2→139.1	0.01	0.01 0.1	97 – 104 (101) 97 – 100 (99)	2.6 (5) 1.4 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 813000x + 365 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Orange		0.01	0.01 0.1	80 – 109 (96) 69 – 106 (91)	13 (5) 16 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 855000x + 317 r = 0.9994	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Wheat grain		0.01	0.01 0.1	91 – 100 (94) 96 – 100 (98)	4.0 (5) 1.4 (5)	0.005 to 2 µg/L (0.0025 to 1.0 mg/kg) y = 638000x + 2040 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Coffee bean (green)		0.01	0.01 0.1	64 – 98 (81) 84 – 93 (87)	18 (5) 4.2 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 643000x - 1100 r = 0.9993	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Oilseed rape (seed)		0.01	0.01 0.1	79 – 91 (84) 92 – 101 (96)	6.6 (5) 3.3 (5)	0.005 to 2 µg/L (0.0025 to 1.0 mg/kg) y = 552000x + 3140 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Bean seed (dry)		0.01	0.01 0.1	89 – 126 (104) 85 – 107 (98)	15 (5) 9.3 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 540000x - 450 r = 0.9994	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Table B.5.2.1-4 Summary of ILV data for analytical method 01520 - **confirmatory** method for BCS-CN88460 residues in/on food and feed of plant origin

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Tomato	BCS-CN88460 *m/z* 400.2→177.1	0.01	0.01 0.1	97 – 107 (102) 96 – 100 (98)	3.6 (5) 1.7 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 451000x + 193 r = 0.9996	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Orange		0.01	0.01 0.1	85 – 114 (99) 69 – 106 (91)	12 (5) 16 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 481000x + 1200 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Wheat grain		0.01	0.01 0.1	84 – 95 (90) 97 – 102 (98)	4.7 (5) 2.2 (5)	0.005 to 2 µg/L (0.0025 to 1.0 mg/kg) y = 350000x + 1090 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Coffee bean (green)		0.01	0.01 0.1	68 – 98 (82) 83 – 91 (87)	16 (5) 3.7 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 360000x - 556 r = 0.9993	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Oilseed rape (seed)		0.01	0.01 0.1	79 – 99 (85) 90 – 96 (94)	9.9 (5) 2.6 (5)	0.005 to 2 µg/L (0.0025 to 1.0 mg/kg) y = 306000x + 1490 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Bean seed (dry)		0.01	0.01 0.1	85 – 118 (99) 90 – 103 (98)	12 (5) 6.3 (5)	0.004 to 2 µg/L (0.002 to 1.0 mg/kg) y = 300000x + 134 r = 0.9995	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

B.5.2.2. Analytical method for the determination of residues in/on food and feed of animal origin

Report:	KCA 4.2/06; Hennes, M.; Glaubitz, J.; 2017; M-608768-01-1
Title:	Analytical method 01300/M034 for the determination of residues BCS-CN88460 and its metabolites, BCS-CY26497, BCS-CY24813 and BCS-CX99799 in/on animal tissues, milk and eggs and biota by HPLC-MS/MS following QuEChERS - Enforcement method animal
Report No.:	P683176031
Document No.:	M-608768-01-1
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and 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 Guidance document on residue analytical methods; SANCO/825/00 rev. 8.1, European Commission, Directorate General Health and Consumer Protection; 2010-11-16. US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 OECD Guidance document on pesticide residue analytical methods, ENV/JM/MONO (2007) 17, 2007-08-13
Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCA 4.2/07; Miller, A.; 2018; M-612340-01-1
Title:	Independent laboratory validation of analytical method 01300/M034 for the determination of residues BCS-CN88460 and its metabolites, BCS CY26497, BCS-CY24813 and BCS-CX99799 in/on animal tissues, milk and eggs and biota by HPLC-MS/MS following QuEChERS - Enforcement method animal
Report No.:	M-612340-01-1
Document No.:	M-612340-01-1
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and 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 Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, July 11, 2000 OECD Guideline, ENV/JM/MONO (2007) 17, August 13, 2007
Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCA 4.2/08; Bongartz, R.; Luks, A. K.; 2017; M-608765-01-1
Title:	Testing of the extraction efficiencies according to QuEChERS using radioactive incurred residues of BCS-CN88460 in animal origin from livestock metabolism studies (lactating goat and laying hen)
Report No.:	EnSa-17-0647

Document No.:	M-608765-01-1
Guideline(s):	US EPA OCSPP Residue Chemistry Test Guideline OPPTS 860.1340: Residue Analytical Method (Aug. 1996) Commission Regulation (EU) No 283/2013 (Mar. 2013) in accordance with Regulation (EC) No 1107/2009 (Oct. 2009)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Definition of the residue
The proposed definition of residue in foodstuff (of animal origin) is parent Isoflucypram (BCS-CN 88460) only.

Residue analytical method 01300/M034 was developed as an enforcement method for the determination of the residues of BCS-CN88460 (parent compound) and its metabolites BCS-CY26497, BCS-CY24813 and BCS-CX99799 in/on animal tissues, milk, eggs and biota. The validated matrices are: cattle muscle, cattle fat, cattle liver, cattle kidney, cattle milk, hen muscle and hen eggs. Data are available for all of the analytes listed but only those relevant to the proposed residue definition are presented below and in tables B.5.2.2-1 to B.5.2.2-4.

Principle of the method
Samples were extracted using the extraction procedure described in the QuEChERS multi-residue method and then analysed by Reverse phase HPLC-MS/MS using a YMC-TriartC18 ExRS, 100 mm x 2.1mm x 3.0µm analytical column at 60 ºC and gradient elution using water + 120 µL/L formic acid and acetonitrile + 120 µL/L formic acid mobile phases. Quantification was by matrix matched external standards or internal standards of deuterated analytes. Two ion transitions were monitored; *m/z* 400.1→167.0 for quantification and *m/z* 400.1→139.0 for confirmation.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) samples.

Linearity
Linearity was demonstrated by the duplicate analysis of 7 standards of increasing concentration* . The range of standard concentrations used was 0.025 to 5.0 µg/L which is equivalent to 0.0025 to 0.50 mg/kg, and adequately encompasses the recovery concentrations 0.01 mg/kg (0.005 mg/kg for milk) and 0.10 mg/kg (0.05 mg/kg for milk).

Several example calibration graphs were presented in the study report but they were not clearly identified and some had points rejected. The applicant was asked to clarify which calibration line related to which matrix/transition/method of quantification (internal or matrix matched standard). Their response (Heinemann, D., Kaussmann, M., Diot, R., 2018, M-644281-01-1) has been incorporated into Table B.5.2.2-1.

*Where data points have been removed, the calibration lines still consisted of at least 8 data points and covered the concentration range of interest.

The impact of matrix effects was addressed by the use of deuterated internal standards or matrix matched external standards, however the study reports issues with stability due to matrix effects – this is discussed in the stability section below.

Accuracy
At least five control samples were fortified with reference standards at each of two levels and the samples analysed by the method described. Levels in the fortified samples were determined using calibration graphs of solvent standards as well as matrix matched standards, and recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 60 to 120 % at residue levels at or below 0.01 mg/kg and 70 to120 % at residue levels between 0.01 and 0.10 mg/kg.

Precision

Precision in the form of %RSD was calculated for the accuracy determinations described above. Acceptable precision is demonstrated by <30%RSD at residue levels at or below 0.01 mg/kg and <20% RSD at residue levels between 0.01 and 0.10 mg/kg.

LOQ

The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 0.005 mg/kg for milk and 0.01 mg/kg for all other matrices.

Confirmation

Residues were confirmed simultaneously to the quantification method by monitoring a second ion transition. Validation data as described above were submitted for the confirmatory method and the results are summarised in Table B.5.2.2-2.

Extraction efficiency

The extraction efficiency study and the proposed monitoring method for residues of BCS-CN88460 in animal products both claim to use the QuEChERS extraction procedure, but examination of the actual procedures highlights several differences. On request, the applicant provided a comparative analysis of the extraction procedures used in both studies (Heinemann, D., Kaussmann, M., Diot, R., 2018, M-644281-01-1). This indicated that the proportions of matrix to extraction volume was different and that the extraction efficiency study omitted the addition of the QuEChERS salts, whereas it is included in the monitoring method.

Matrix	Method 01300/M034	Extraction efficiency study (M-608765-01-1)
Eggs	5 g sample material + 6.5 mL water Add 10 mL acetonitrile and shake vigorously for 1 min Overhead shaking for 20 min QuEChERS salts added shaking 1 min	50 g sample material + 12.5 mL water Add 50 mL ACN Overhead shaking for 20 min
Hen leg muscle		50 g sample material + 15 mL water + 50 mL ACN Overhead shaking for 20 min
Hen thorax muscle		50 g sample material + 14.5 mL water + 50 mL ACN Overhead shaking for 20 min
Goat muscle		48.55 g sample material + 14.5 mL water + 48.5 mL ACN Overhead shaking for 20 min
Hen fat	5 g sample material + 0 mL water + 10 mL ACN shaking 20 min QuEChERS salts added shaking 1 min	21.261 g sample material + 0 mL water + 21.3 mL ACN Overhead shaking for 20 min
Goat fat		50.07 g sample material + 0 mL water + 50 mL ACN Overhead shaking for 20 min
Hen liver	5 g sample material + 6.5 mL water + 10 mL ACN shaking 20 min QuEChERS salts added shaking 1 min	20 g sample material + 6 mL water + 20 mL ACN Overhead shaking for 20 min
Goat liver		50.15 g sample material + 15 mL water + 50 mL ACN Overhead shaking for 20 min
Goat milk	5 g sample material + 5.5 mL water + 10 mL ACN shaking 20 min QuEChERS salts added shaking 1 min	50 g sample material + 6.5 mL water + 50 mL ACN Overhead shaking for 20 min
Goat kidney	5 g sample material + 6.5 mL water + 10 mL ACN shaking 20 min QuEChERS salts added shaking 1 min	6.16 g sample material + 1.36 mL water + 6.16 mL ACN Overhead shaking for 20 min

The purpose of the QuEChERS salt is to remove some water and facilitate phase separation of the acetonitrile and water phases, allowing the concentration of analyte in the organic phase (assumed to be 10 ml) to be determined. Whilst this step could reasonably be considered to be a partition step, and thus outside the scope of the extraction efficiency assessment, it is referred to in the QuEChERS method as the second step of extraction and its absence may affect quantification (since the exact volume of the water/acetonitrile is not known). Conversely the use of QuEChERS salts could assist extraction as it produces an exothermic reaction, the heat from which could assist dissolution of the analytes.

The proportions of matrix to acetonitrile in the monitoring method and extaction efficiency method are not the same; 5 g matrix : 20 ml solvent in the monitoring method compared to 50 g matrix ; 100 ml solvent in extraction efficiency study (6.16 g matrix : 12.36 ml solvent for kidney). See below:

Matrix	Natural water content per 100g sample	Calculation of solvent ratio including natural water content of sample used in method 01300/M034	Calculation of solvent ratio including natural water content of sample used in extraction efficiency study (M-608765-01-1)
Eggs	74.4 g (=74.4%)	3.7 g natural water content in 5 g sample + 6.5 mL water = **10.2 g** total water content (=10.2 mL) + 10 mL acetonitrile **final ratio of acetonitrile / water = 1 / 1 (v/v)**	37.2 g natural water content in 50 g sample + 12.5 mL water = **49.7 g** total water content (=49.7 mL) + 50 mL acetonitrile **final ratio of acetonitrile / water = 1 / 1 (v/v)**
Hen leg muscle	70.0 g (=70.0%)	3.5 g natural water content in 5 g sample + 6.5 mL water = **10.0 g** total water content (=10.0 mL) + 10 mL acetonitrile **final ratio of acetonitrile / water = 1 / 1 (v/v)**	35.0 g natural water content in 50 g sample + 15 mL water = **50.0 g** total water content (=50.0 mL) + 50 mL acetonitrile **final ratio of acetonitrile / water = 1 / 1 (v/v)**
Hen thorax muscle			35.0 g natural water content in 50 g sample + 14.5 mL water = **49.5 g** total water content + 50 mL acetonitrile **final ratio of acetonitrile / water = 1 / 1 (v/v)**
Goat muscle			34.0 g natural water content in 48.55 g sample + 14.5 mL water = **48.5 g** total water content (=48.5 mL) + 48.5 mL acetonitrile **final ratio of acetonitrile / water = 1 / 1 (v/v)**
Hen fat	0 g (=0%)	0 g natural water content in 5 g sample + 0 mL water = **0 g** total water content + 10 mL acetonitrile **final ratio of sample / acetonitrile = 1/2 (w/v)**	0 g natural water content in 21.261 g sample + 0 mL water = **0 g** total water content + 21.3 mL acetonitrile **final ratio of sample / acetonitrile = 1/1 (w/v)**
Goat fat			0 g natural water content in 50.07 g sample + 0 mL water = **0 g** total water content + 50 mL acetonitrile **final ratio of sample / acetonitrile = 1/1 (w/v)**
Hen liver	70.4 g (=70.4%)	3.5 g natural water content in 5 g sample + 6.5 mL water = **10.0 g** total water content (=10.0 mL) + 10 mL acetonitrile **final ratio of acetonitrile / water = 1 / 1 (v/v)**	14.1 g natural water content in 20 g sample + 6 mL water = **20.1 g** total water content (=20.1 mL) + 20 mL acetonitrile **final ratio of acetonitrile / water = 1 / 1 (v/v)**
Goat liver			35.3 g natural water content in 50.15 g sample + 15 mL water = **50.3 g** total water content (=50.3 mL) + 50 mL acetonitrile **final ratio of acetonitrile / water = 1 / 1 (v/v)**
Goat milk	87.2 g (=87.2%)	4.4 g natural water content in 5 g sample + 5.5 mL water = **9.9 g** total water content (=9.9 mL) + 10 mL acetonitrile **final ratio of acetonitrile / water = 1 / 1 (v/v)**	43.6 g natural water content in 50 g sample + 6.5 mL water = **50.1 g** total water content (=50.1 mL) + 50 mL acetonitrile **final ratio of acetonitrile / water = 1 / 1 (v/v)**
Goat kidney	78.5 g (=78.5%)	3.9 g natural water content in 5 g sample + 6.5 mL water = **10.4 g** total water content (=10.4 mL) + 10 mL acetonitrile **final ratio of acetonitrile / water = 1 / 1 (v/v)**	4.8 g natural water content in 6.16 g sample + 1.36 mL water = **6.16 g** total water content (=6.16 mL) + 6.16 mL acetonitrile **final ratio of acetonitrile / water = 1 / 1 (v/v)**

The difference may have an impact on the extraction efficiency, although it would be expected that the higher volume of solvent relative to matrix that is used in the monitoring method would improve efficiency rather than decrease it.

The extraction efficiency study concludes that extraction efficiencies based on the sum of the relevant residuesin hen tissues rangesd from 73 to 85 % and for goat ranged from 70 to 126%. However, individual extraction efficiencies for parent compound were very varied, ranging from 69 to 206%. See below:

For the hens:

Relevant residue	**Sample**				
Report name: BCS-CN88460-	**Eggs** [%]	**Leg muscle** [%]	**Thorax muscle** [%]	**Fat** [%]	**Liver** [%]
desmethyl-carboxylic acid	---	62.1	83.7	---	78.6
desmethyl-propanol	82.2	87.2	81.5	67.7	85.9
carboxylic acid	125.1	80.5	67.5*	54.8*	85.1
propanol	86.4	69.1*	34.4*	73.5	77.2
parent compound	58.5*	165.6*	---	74.8	---
Sum of relevant residues	**85.4**	**82.4**	**73.4**	**76.5**	**82.0**

* Amount of metabolite close to limit of detection (LOD).

For the goat:

Relevant residue	**Sample**				
Report name: BCS-CN88460-	**Milk** [%]	**Muscle** [%]	**Fat** [%]	**Liver** [%]	**Kidney** [%]
desmethyl-carboxylic acid	---	---	---	95.0	55.7*
desmethyl-propanol	---	71.4	---	181.5**	---
carboxylic acid	---	50.6*	62.0*	70.0	84.7
propanol	---	98.0	88.8	148.2**	63.4
2-propanol	72.1	93.1	76.0	162.0**	79.1
parent compound	119.8	87.8	67.8	205.6**	85.5
Sum of relevant residues	**101.7**	**85.0**	**70.0**	**126.0**	**73.8**

* Amount of metabolite close to limit of detection (LOD).
** The extraction efficiencies of some single compounds of liver were considerably higher than 100% (highest value 206% for parent compound). However, based on the extraction efficiency for the sum of the relevant residues (126%) and the result for the extraction efficiency of liver from the hens (82%), it could be concluded that the QuEChERS method is suitable to analyse the relevant residues.

Based on this data it must be concluded that the extraction efficieny data are not reliable.

Stability

The stability of BCS-CN88460 in the final extracts was checked for the different matrices, monitoring both transitions and using quantification against both internal standards and matrix-matched standards and a freshly prepared mixed standard solution.

The data showed that BCS-CN88460 was stable (<20% deviation from initial value) in final extracts of eggs, fat, milk and kidney samples for at least 6 days when stored at 4°C ± 3°C under dark conditions, when quantified using the quantification ion transitions and by both internal and matrix matched calibration. However, issues with stability were identified in muscle samples (cattle and hen) when quantification was based on matrix matched standards, and in cattle liver when quantification was based on both internal and matrix matched

standards. The study report therefore recommends analysing extracts within 24 hours under the conditions described.

There does not appear to have been any stability data generated using the confirmatory transition despite the report stating that data were available for both transitions. The report is very confusing in this regard, indicating in some places that it is the qualitative (confirmatory) ion transition which is problematic but this is not supported by the reported data. The applicant has been asked for clarification.

Independent Laboratory Validation

Method 01300/M034 was validated in a second facility, independent to where the original validation was performed. ILV was performed on a reduced matrix set which included liver, muscle, milk and fat (all sourced from cows). This is acceptable since the same method is used for all animals. Eggs were not included in the ILV data set. The ILV gave good results for cow liver, fat and milk but the cow muscle recovery values were low, ranging from 63% to 74% (average of 68%). A reinjection of the extracts resulted in even lower recoveries, 52% to 63%. After speaking with the method developer, it was pointed out that the muscle extracts should be analysed within 24 hours (as explained in the original method). A second validation data set for cow muscle was therefore initiated, with a larger dilution factor and analysis on the same day as extraction. Recoveries for the second cow muscle validation trial were acceptable.

Mean recoveries were generally lower than those observed during the primary validation, but with the exception of the muscle recoveries at 0.10 mg/kg using the confirmatory method, all mean recoveries were in the range 70-120 % and demonstrated a high degree of precision. The mean recovery value for cow muscle at 0.10 mg/kg using the confirmatory method was 69% which is just outside the acceptable limits for mean recovery as specified in SANCO/825/00 rev.8.1, however, as SANCO/825/00 rev.8.1 only specifies that an ILV is required for the primary method, this is not an issue.

The calibration graph for the ILV ranged from 0.025 to 5 µg/L, which is consistent with the original validation, but the corresponding concentrations were reported as 0.00125 to 0.25 mg/kg for fat and liver, and 0.0025 to 0.50 mg/kg for milk and muscle, indicating that different dilution factors were used in the ILV compared to the original method. The RMS considers that this is unlikely to have a significant impact on the results and no further data are required.

A deviation from the original method was noted, in which the stock standard solutions were stored in the freezer instead of the fridge. The RMS considers that this is unlikely to have a significant impact on the results.

Matrix matched standards were used for all matrices, as recommended in the primary method. Two ion transitions were monitored; *m/z* 400.2→139.0 for quantification and *m/z* 400.2→177.0 for confirmation.

A summary of the ILV data is presented in Tables B.5.2.2-3 and B.5.2.2-4.

Assessment

The extraction efficiency data is not reliable and therefore this method cannot be considered acceptable as a monitoring method for the determination of BCS-CN88460 in animal products.

However, under Section B.5.1.2.5.3, Method 01511 was acceptably validated for BCS-CN88460 in animal products with an LOQ of 0.01 mg/kg for all matrices, except milk for which the LOQ is 0.005 mg/kg. There is also a validation data for a confirmatory HPLC-MS/MS transition, with the same LOQs. Method 01511 can therefore be used to fulfuil the requirement for a monitoring method for animal products.

Note: the method employs the use of a deuterated standard of isoflucypram as internal standard. In line with SANCO/825/00 rev 8.1, section 2.12, if this standard is not commercially available, then it should be made generally available by the applicant and contact details provided.

Table B.5.2.2-1 Summary of validation data for analytical method 01300/M034 - **Quantification** method for BCS-CN88460 residues in/on food and feed of animal origin

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity*	Specificity
Eggs (hen)	BCS-CN88460 (matrix standards) m/z 400.1→167.0	0.01	0.01 0.1	100 – 118 (110) 85 – 103 (93)	6.1 (10) 6.3 (12)	0.1 to 5 µg/L (0.01 to.0.5 mg/kg) y = 307835x - 5931 r = 0.99743	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (internal standards) m/z 400.1→167.0	0.01	0.01 0.1	75 – 100 (94) 95 – 102 (99)	10.2 (6) 3.0 (6)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 1.51770x + 0.00657 r = 0.99878	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Milk (cow)	BCS-CN88460 (matrix standards) *m/z* 400.1→167.0	0.005	0.005 0.05	95 – 115 (104) 88 – 96 (94)	6.4 (12) 3.0 (12)	0.05 to 5 µg/L (0.005 to.0.5 mg/kg) y = 885965x - 8251 r = 0.99603	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (internal standards) *m/z* 400.1→167.0	0.005	0.005 0.05	101 – 105 (103) 99 – 107 (104)	1.8 (6) 2.8 (6)	0.05 to 5 µg/L (0.005 to.0.5 mg/kg) y = 1.41835x + 0.00694 r = 0.99924	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Muscle (cow)	BCS-CN88460 (matrix standards) *m/z* 400.1→167.0	0.01	0.01 0.1	77 – 95 (86) 77 – 89 (84)	5.4 (12) 4.6 (10)	0.05 to 5 µg/L (0.005 to.0.5 mg/kg) y = 477116x - 3511 r = 0.99473	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (internal standards) *m/z* 400.1→167.0	0.01	0.01 0.1	105 – 113 (109) 106 – 113 (109)	2.9 (6) 2.1 (6)	0.05 to 5 µg/L (0.005 to.0.5 mg/kg) y = 1.34606x + 0.00104 r = 0.99904	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Muscle (hen)	BCS-CN88460 (matrix standards) *m/z* 400.1→167.0	0.01	0.01 0.1	84 – 102 (93) 70 – 114 (86)	5.3 (12) 17.2 (14)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 270656x + 2727 r = 0.99607	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity*	Specificity
	BCS-CN88460 (internal standards) *m/z* 400.1→167.0	0.01	0.01 0.1	115 – 119 (117) 114 – 118 (117)	1.5 (6) 1.3 (6)	0.05 to 5 µg/L (0.005 to.0.5 mg/kg) y = 1.30011x + 0.00494 r = 0.99994	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Fat (cow)	BCS-CN88460 (matrix standards) m/z 400.1→167.0	0.01	0.01 0.1	89 – 101 (95) 83 – 99 (92)	3.4 (12) 4.9 (12)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 693854x + 7425 r = 0.99819	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (internal standards) m/z 400.1→167.0	0.01	0.01 0.1	107 – 116 (114) 104 – 116 (111)	3.0 (6) 4.0 (6)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 1.55666x + 0.00657 r = 0.99938	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Liver (cow)	BCS-CN88460 (matrix standards) *m/z* 400.1→167.0	0.01	0.01 0.1	88 – 103 (95) 71 – 89 (77)	6.6 (12) 7.7 (12)	0.05 to 5 µg/L (0.005 to.0.5 mg/kg) y = 152396x - 2049 r = 0.99942	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (internal standards) *m/z* 400.1→167.0	0.01	0.01 0.1	104 – 119 (113) 115 – 120 (117)	4.8 (6) 1.4 (6)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 1.33958x + 0.00587 r = 0.99927	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Kidney (cow	BCS-CN88460 (matrix standards) *m/z* 400.1→167.0	0.01	0.01 0.1	60 – 100 (83) 65 – 89 (81)	11.8 (14) 13.2 (14)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 285174x + 1987 r = 0.99902	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (internal standards) *m/z* 400.1→167.0	0.01	0.01 0.1	82 – 87 (85) 87 – 92 (89)	2.2 (6) 2.2 (6)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 1.46867x + 0.00632 r = 0.99879	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

* information presented in a separate document (Heinemann, D., Kaussmann, M., Diot, R., 2018, M-644281-01-1)

Table B.5.2.2-2 Summary of validation data for analytical method 01300/M034 - **Confirmatory** method for BCS-CN88460 residues in/on food and feed of animal origin

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Eggs (hen)	BCS-CN88460 (matrix standards) m/z 400.1→139.0	0.01	0.01 0.1	102 – 119 (112) 85 – 104 (93)	5.0 (10) 7.1 (12)	0.1 to 5 µg/L (0.01 to.0.5 mg/kg) y = 376023x - 7777 r = 0.99739	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (internal standards) m/z 400.1→139.0	0.01	0.01 0.1	75 – 102 (95) 97 – 104 (100)	10.6 (6) 2.4 (6)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 1.84107x + 0.00895 r = 0.99904	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Milk (cow)	BCS-CN88460 (matrix standards) *m/z* 400.1→139.0	0.005	0.005 0.05	93 – 110 (103) 86 – 98 (93)	5.4 (12) 4.1 (12)	0.05 to 5 µg/L (0.005 to.0.5 mg/kg) y = 1082500x - 9154 r = 0.99579	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (internal standards) *m/z* 400.1→139.0	0.005	0.005 0.05	97 – 106 (100) 95 – 105 (101)	3.1 (6) 3.5 (6)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 1.76094x + 0.00895 r = 0.99913	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Muscle (cow)	BCS-CN88460 (matrix standards) *m/z* 400.1→139.0	0.01	0.01 0.1	81 – 93 (88) 81- 87 (84)	4.4 (12) 2.3 (10)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 581629x + 4210 r = 0.99552	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (internal standards) *m/z* 400.1→139.0	0.01	0.01 0.1	106 – 113 (109) 106 – 114 (110)	2.3 (6) 3.0 (6)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 1.63819x + 0.00514 r = 0.99973	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Muscle (hen)	BCS-CN88460 (matrix standards) *m/z* 400.1→139.0	0.01	0.01 0.1	89 – 104 (94) 66 – 112 (86)	5.6 (12) 17.5 (14)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 333853x + 2327 r = 0.99591	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
	BCS-CN88460 (internal standards) *m/z* 400.1→139.0	0.01	0.01 0.1	114 – 118 (115) 116 – 118 (117)	1.4 (6) 0.8 (6)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 1.60331x + 0.00697 r = 0.99986	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Fat (cow)	BCS-CN88460 (matrix standards) m/z 400.1→139.0	0.01	0.01 0.1	93 – 100 (96) 80 – 96 (92)	2.9 (12) 5.1 (12)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 847078x + 8737 r = 0.99839	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (internal standards) m/z 400.1→139.0	0.01	0.01 0.1	106 – 119 (113) 101 – 117 (110)	4.1 (6) 5.0 (6)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 1.47466x - 0.00207 r = 0.99982	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Liver (cow)	BCS-CN88460 (matrix standards) *m/z* 400.1→139.0	0.01	0.01 0.1	87 – 109 (94) 70 – 89 (78)	7.9 (12) 8.0 (12)	0.05 to 5 µg/L (0.005 to.0.5 mg/kg) y = 187680x - 1822 r = 0.99921	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (internal standards) *m/z* 400.1→139.0	0.01	0.01 0.1	108 – 123 (115) 111 – 119 (116)	4.6 (6) 2.6 (6)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 1.61541x + 0.00821 r = 0.99912	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Kidney (cow	BCS-CN88460 (matrix standards) *m/z* 400.1→139.0	0.01	0.01 0.1	62 – 101 (85) 64 – 95 (81)	13.1 (14) 13.5 (14)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 348203x + 2582 r = 0.99936	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (internal standards) *m/z* 400.1→139.0	0.01	0.01 0.1	82 – 89 (86) 86 – 90 (88)	3.4 (6) 1.7 (6)	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 1.82677x + 0.00447 r = 0.99893	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Table B.5.2.2-3 Summary of **ILV** data for analytical method 01300/M034 - **Quantification** method for BCS-CN88460 residues in/on food and feed of animal origin

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Liver (cow)	BCS-CN88460 (matrix standards) m/z 400.1→167.0	0.01	0.01 0.1	69 – 74 (71) 70 – 74 (71)	2.9 2.3	0.025 to 5 µg/L (0.00125 to 0.25 mg/kg) y = 61044x + 120 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Muscle (cow)		0.01	0.01 0.1	69 – 77 (74) 67 – 73 (70)	4.4 3.3	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 53153x + 146 r = 0.9996	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Fat (cow)		0.01	0.01 0.1	95 – 97 (96) 93 – 100 (96)	1.0 3.1	0.025 to 5 µg/L (0.00125 to 0.25 mg/kg) y = 61478x + 157 r = 0.9994	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Milk (cow)		0.005	0.005 0.05	72 – 81 (77) 69 – 70 (70)	4.4 0.8	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 58200x – 38.1 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Table B.5.2.2-4 Summary of **ILV** data for analytical method 01300/M034 - **Confirmatory** method for BCS-CN88460 residues in/on food and feed of animal origin

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Liver (cow)	BCS-CN88460 (matrix standards) m/z 400.1→139.0	0.01	0.01 0.1	70 – 73 (72) 69 – 72 (71)	1.6 1.6	0.025 to 5 µg/L (0.00125 to 0.25 mg/kg) y = 78217x – 0.484 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Muscle (cow)		0.01	0.01 0.1	70 – 74 (72) 66 – 72 (69)	2.3 3.2	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 68425x + 108 r = 0.9994	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Fat (cow)		0.01	0.01 0.1	93 – 98 (96) 92 – 98 (95)	2.3 2.3	0.025 to 5 µg/L (0.00125 to 0.25 mg/kg) y = 78716x + 85.2 r = 0.9996	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Milk (cow)		0.005	0.005 0.05	67 – 79 (73) 70 – 71 (70)	5.8 0.6	0.025 to 5 µg/L (0.0025 to.0.5 mg/kg) y = 74316x - 188 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

B.5.2.3. Analytical method for the determination of residues in/on soil

Report:	KCA 4.2/09; Koch, V.; 2016; M-553434-01-1
Title:	Analytical method 01479 for the determination of BCS-CN88460 and the metabolite BCS-CN88460-carboxylic acid in soil by HPLC-MS/MS
Report No.:	P681 14 1804
Document No.:	M-553434-01-1
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and 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 European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method
Guideline deviation(s):	none
GLP/GEP:	**yes**

Definition of the residue
The applicant has proposed the definition of residue in soil as parent Isoflucypram (BCS-CN88460) only.

The RMS consider the residue definition in soil to be ……..

Residue analytical method 01479 was developed as a monitoring method for the determination of the residues of BCS-CN88460 (parent compound) and its carboxylic acid metabolite BCS-CY26497 in soil. A summary of the method and its validation is presented below and in Tables B.5.2.3-1 and B.5.2.3-2. The method was validated in two soils: Höfchen (silt loam) and Laacher Hof (sandy loam).

Principle of the method
Soil samples were extracted with acetonitrile/water/acetic acid (40/10/0.3 v/v/v) in a microwave extractor. An aliquot of the resulting extract was centrifuged, if needed, to remove any fine particles and then analysed by reverse phase HLPC-MS/MS using a YMC Ultra HT Hydrosphere C18, 30mm x 2mm x 2µm analytical column at 60 °C and gradient elution using a water/formic acid (1000/1 v/v) and acetonitrile/formic acid (1000/1 v/v) mobile phases. Quantification was by external matrix matched standards and two ion transitions were monitored for each analyte:

BCS CN88460
Quantification: 400.1→139.0
Confirmation: 400.1→167.1

BCS CY26497
Quantification: 430.1→177.0
Confirmation: 430.1→412.1

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) samples.

Linearity
Linearity was demonstrated by the analysis of eight standards of increasing concentration. The range of standard concentrations used was 0.15 to 50 µg/L, which is equivalent to 0.0003 to 0.10 mg/kg, and adequately encompasses the recovery concentrations of 0.001 mg/kg (LOQ) and 0.010 mg/kg (10xLOQ). The correlation coefficients were >0.99.

The impact of matrix effects was addressed by the use of matrix matched standards in both Höfchen and Laarcher Hof soils.

Accuracy
Five control samples of each soil type were fortified with reference standards at each of two levels and the samples analysed by the method described. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 110 %.

Precision
Precision in the form of %RSD was calculated for the accuracy determinations described above. Acceptable precision is demonstrated by <20%RSD.

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 0.001 mg/kg.

Confirmation
Residues were confirmed simultaneously to the primary detection by monitoring a second ion transition. Validation data as described above were submitted for the confirmatory method and the results are summarised in Table B.5.2.3-2

Extraction efficiency
Not assessed for this method. The extraction procedure used in the soil degradation study reported in Section B.8.1.1.1.1. included triplicate extractions by shaking with acetonitrile/water (1:1 v/v), followed by two separate microwave extractions with different solvent/temperature combinations. This are summarise below:

Solvent	Volume	Minimum duration	Temperature	Extracts
ACN/H_2O 1/1 (*v/v*)	80 mL	30 min, shaking	ambient	3
ACN/H_2O 1/1 (*v/v*)	80 mL	10 min, stirring	microwave, 70°C	1
MeOH/H_2O 1/1 (*v/v*)	80 mL	10 min, stirring	microwave, 50°C	1

The majority of the radioactivity was extracted by shaking with acetonitrile/water (1:1 v/v) with at least 85% of the applied radioactivity being recovered in these extracts from all soils tested across all time points. There is no information relating to the proportion in each extract, but it can be assumed that the majority was extracted into the first extract, with smaller and smaller still amounts being extracted by the two successive extractions.

The extraction procedure used in method 01479 involves a single microwave extraction with acetonitrile/water/acetic acid (40/10/0.3 v/v/v) therefore, based on the available information, it is not possible to assess the extraction efficiency of this method at this time. The applicant has been asked to comment. However, whilst it is scientifically valid to require this data (on the basis that residues can be incorporated into soil) , it is accepted that under Regulation (EU) No. 283/2013 there is no explicit requirement to do so and therefore the absence of this data is not regarded as a data gap.

Stability
BCS-CN88460 and BCS-CY26497 were stable in primary and secondary standard solutions for at least 89 days when stored in a fridge.

BCS-CN88460 and BCS-CY26497 were stable in soil extracts for at least 14 days when stored in a fridge in sample tubes.

Assessment
The method is acceptably validated in accordance with SANCO/825/00 rev.8.1 and is suitable for the determination of isoflucypram and its carboxylic acid metabolite (BCS-CY26497) in soil samples with a LOQ of 0.001 mg/kg which is acceptable with respect to the lowest NOEC for non-target organisms; *Folsomia candida* with a NOEC of 2.59 mg a.s./kg soil.

Table B.5.2.3-1 Summary of validation data for analytical method 01479 – **Quantification** method for BCS-CN88460 and BCS-CY26497 residues in soil.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Soil (Höfchen) (Silt loam)	BCS-CN88460 400.1→139.0	0.001	0.001 0.010	101 – 110 (106) 92 – 111 (103)	3.3 (5) 8.9 (5)	0.15 to 50 µg/L (0.0003 to 0.10 mg/kg) y = 368352x + 11990 r = 0.99957	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CY26497 430.1→177.0	0.001	0.001 0.010	102 – 114 (107) 96 – 116 (109)	4.0 (5) 8.2 (5)	0.15 to 50 µg/L (0.0003 to 0.10 mg/kg) y = 235774x + 18831 r = 0.99787	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Soil (Laacher Hof) (Sandy loam)	BCS-CN88460 400.1→139.0	0.001	0.001 0.010	95 – 100 (96) 96 – 98 (97)	2.3 (5) 0.9 (5)	0.15 to 50 µg/L (0.0003 to 0.10 mg/kg) y = 359343x + 2816 r = 0.99994	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CY26497 430.1→177.0	0.001	0.001 0.010	102 – 110 (107) 103 – 109 (105)	3.0 (5) 2.2 (5)	0.15 to 50 µg/L (0.0003 to 0.10 mg/kg) y = 243565x + 552 r = 0.99928	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Table B.5.2.3-2 Summary of validation data for analytical method 01479 – **Confirmatory** method for BCS-CN88460 and BCS-CY26497 residues in soil.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Soil (Höfchen) (Silt loam)	BCS-CN88460 400.1→167.1	0.001	0.001 0.010	102 – 111 (106) 92 – 112 (104)	3.1 (5) 8.8 (5)	0.15 to 50 µg/L (0.0003 to 0.10 mg/kg) y = 305595x + 10215 r = 0.99950	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CY26497 430.1→412.1	0.001	0.001 0.010	92 – 113 (101) 98 – 115 (109)	8.0 (5) 6.8 (5)	0.15 to 50 µg/L (0.0003 to 0.10 mg/kg) y = 125290x + 13474 r = 0.99810	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Soil (Laacher Hof) (Sandy loam)	BCS-CN88460 400.1→167.1	0.001	0.001 0.010	95 – 99 (96) 96 – 99 (97)	1.7 (5) 1.3 (5)	0.15 to 50 µg/L (0.0003 to 0.10 mg/kg) y = 299119x + 1592 r = 0.99997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
	BCS-CY26497 430.1→412.1	0.001	0.001 0.010	90 – 111 (103) 102 – 111 (106)	7.7 (5) 3.4 (5)	0.15 to 50 µg/L (0.0003 to 0.10 mg/kg) y = 129517x - 973 r = 0.99931	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

B.5.2.4. Analytical method for the determination of residues in water

Report:	KCA 4.2/10; Krebber, R.; Leppelt, L.; 2017; M-589592-01-1
Title:	Modification M004 of analytical method 01387 for the determination of BCSCN88460 in drinking and surface water by HPLC-MS/MS
Report No.:	01387/M004
Document No.:	M-589592-01-1
Guideline(s):	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 EC Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1 of November 16, 2010 European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, July 11, 2000
Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCA 4.2/11; Schmiedt, S.; 2017; M-607474-01-1
Title:	Independent laboratory validation of analytical method 01387/M004 for the determination of BCS-CN88460 in surface water by HPLC-MS/MS
Report No.:	P 4615 G
Document No.:	M-607474-01-1
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and 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 European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 EC Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1, 16/11/10
Guideline deviation(s):	none
GLP/GEP:	**yes**

Definition of the residue
The applicant has proposed the definition of residue in drinking and surface water as parent Isoflucypram (BCS-CN88460) only.

The residue definition will be confirmed during the EFSA peer review process.

Residue analytical method 01387/M004 was developed as a post registration method for the determination of the residues of BCS-CN88460 (parent compound) in drinking and surface water. A summary of the method and its validation, including independent laboratory validation (ILV) is presented below and in Table B.5.2.4-1 and B.5.2.4-2. Validation data were generated for surface water only but as the LOQ of the method meets the requirements for drinking water, the method is also valid for drinking water.

Principle of the method
Samples of water are combined with acetonitrile in the ratio 4:1 and analysed directly by reverse phase HPLC-MS/MS) using an Ascentis Express C18, 50mm x 2.1mm x 2.7µm analytical column at 80°C and gradient elution using water/formic acid (1000/0.12 v/v) + 10 mM ammonium formate, methanol/formic acid (1000/0.12 v/v) + 10 mM ammonium formate and water/methanol/formic acid (500/500/0.12 v/v/v) mobile phases. Quantification was by external standards (in solvent) and two ion transitions were monitored; *m/z* 400→139 for quantification and *m/z* 400→167 for confirmation.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample.

Linearity
Linearity was demonstrated by the analysis (in quadruplicate) of seven standards of increasing concentration. The range of standard concentrations used was 0.015 µg/L to 10.0 µg/L, which is equivalent to 0.0188 to 12.5 µg/L in the samples. Correlation coefficients were as >0.99.

The impact of matrix effects was addressed by comparison of the peak areas of the isoflucypram peaks in a standard solution (prepared in deionised water) to that of a sample solution containing matrix. There was no difference in peak areas, therefore it was acceptable to use standard solutions prepared in deionised water for the calibration.

Accuracy
Not relevant as the samples were analysed directly.

Precision
As no recovery determinations were performed, the precision (repeatability) of the method was determined from the replicate analysis of control samples fortified with reference standards at each of two levels; 0.05 µg/L and 0.50 µg/L, which is equivalent to 0.0625 and 0.625 µg/L in the test samples. Acceptable precision is demonstrated by <20%RSD.

LOQ
The LOQ is defined by the lowest concentration at which acceptable recovery and precision data have been generated. However as recovery determinations are not relevant to this direct analysis method, the LOQ is taken as the lowest level at which acceptable precision data are available: 0.0625 µg/L. This is acceptable with respect to drinking water, for which the LOQ must be at least 0.1 µg/L. It is also acceptable for surface water as it is significantly lower than the lowest of the effect concentrations relevant to surface water, which is the NOEC in the long term fish study of 9.48 µg/L.

Confirmation
Residues were confirmed simultaneously to the primary detection by monitoring a second ion transition. Validation data as described above were submitted for the confirmatory method and the results are summarised in Table B.5.2.4-1

Extraction efficiency
Not applicable as the samples are analysed directly, without any extraction.

Stability
BCS-CN88460 was stable in surface water/acetonitrile (80/20, v/v) when stored in a freezer at ≤ -18 °C for a period of 7 days.

Independent Laboratory Validation (ILV)
Method 01387/M004 was validated in a second facility, independent to where the original validation was performed. No deviations from the method were reported however the RMS noted that the temperature of the column oven was lower in the ILV than the original validation and that the ILV used matrix matched standards whereas the original validation did not.

The temperature of the HPLC column oven was 60°C in the independent validation and was 80°C in the original validation. This difference in temperature is a likely explanation for the slightly longer retention time of 3.09 mins for BCS-CN88460 in the ILV compared to 2.7 minutes in the primary validation. This difference does not affect the validity of the ILV.

Matrix matched standards were used in the ILV whereas the original validation quantified samples against solvent (deionised water) standards. However, in the original validation, the absence of matrix effects was demonstrated therefore the use of matrix standarsd in the ILV does not affect the validity of the results.

A summary of the ILV data is presented in table B.5.2.4-2

Assessment

The method is acceptably validated (including ILV) in accordance with SANCO/825/00 rev.8.1 in surface water at 0.0625 µg/L. This LOQ is acceptable to determine levels of isoflucypram in water at the lowest of the effect concentrations relevant to surface water, which is the NOEC in the long term fish study of 9.48 µg/L. The LOQ is also acceptable with respect to drinking water, for which the LOQ must be at least 0.1 µg/L.

Table B.5.2.4-1 Summary of validation data for analytical method 01387/M004 for the determination of BCS-CN88460 residues in water.

Matrix	Analyte	LOQ (µg/L)	Sample concentration (µg/L)	Repeatability % RSD (n)	Linearity	Specificity
Surface water	BCS-CN88460 400.1→139.0	0.0625	0.0625 0.625	2.2 (10) 1.1 (10)	0.015 to 10.0 µg/L (0.0188 to 12.5 µg/L) y = 556000x + 1710 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 400.1→167.1	0.0625	0.0625 0.625	3.4 (10) 1.3 (10)	0.015 to 10.0 µg/L (0.0188 to 12.5 µg/L) y = 374000x + 1640 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

Table B.5.2.4-2 Summary of ILV for analytical method 01387/M004 for the determination of BCS-CN88460 residues in water.

Matrix	Analyte	LOQ (µg/kg)	Sample concentration (µg/L)	Repeatability % RSD (n)	Linearity	Specificity
Surface water	BCS-CN88460 400.1→139.0	0.0625	0.0625 0.625	4.9 (10) 4.3 (10)	0.015 to 10.0 µg/L (0.0188 to 12.5 µg/L) y = 1230000x + 16300 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 400.1→167.1	0.0625	0.0625 0.625	4.2 (10) 4.6 (10)	0.015 to 10.0 µg/L (0.0188 to 12.5 µg/L) y = 729000x + 8270 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

B.5.2.5. Analytical method for the determination of residues in air.

Report:	KCA 4.2/12; Bendig, P.; 2016; M-572420-01-1
Title:	Analytical method 01506 for the determination of BCS-CN88460 in air
Report No.:	01506
Document No.:	M-572420-01-1
Guideline(s):	European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 The OECD Principles of Good Laboratory Practice (OECD 1998) German Chemical Law (Chemikaliengesetz-ChemG), dated 02-Jul-2008, current version of annex 1 to § 19a, dated 28-Aug-2013. The national requirements are based on the OECD Principles of Good Laboratory Practice, which are accepted by regulatory authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHLW, MAFF and METI) on the basis of intergovernmental agreements.
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Definition of the residue
The applicant has proposed the definition of residue in air as parent Isoflucypram (BCS-CN88460) only.

The residue definition will be confirmed during the EFSA peer review process.

Residue analytical method 01506 was developed as a post-registration monitoring method for the determination of the residues of BCS-CN88460 in air. A summary of the method and its validation is presented below and in Table B.5.2.5-1.

Principle of the method
It is expected that air samples will be collected using XAD sorbent tubes by drawing air through the tubes at a rate of about 1.0 mL/min for 6 hours (total air volume of 0.36 m^3). The sorbent material is then extracted in triplicate by ultrasonication with acetonitrile. An aliquot of the acetonitrile extract is diluted ten-fold with acetonitrile/water (8:2 v/v) containing 0.1 % formic acid and analysed by phase HLPC-MS/MS using a Waters X Terra MS C18, 50mm x 4.6mm x 3.5µm analytical column at 40 °C and gradient elution using water + 0.5% formic acid and acetonitrile + 0.05% formic acid mobile phases. Quantification was by external standards and two ion transitions were monitored: *m/z* 400.1→167.1 for quantification and *m/z* 400.1→139.0 for confirmation.

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) samples.

Linearity
Linearity was demonstrated by the analysis of five standards of increasing concentration. The range of standard concentrations used was 1.5 to 30 ng/mL, which is equivalent to 0.42 to 8.3 µg/m^3, and adequately encompasses the LOQ recovery concentration of 4.2 µg/m^3. The higher recovery samples were further diluted prior to analysis to ensure that they were within the linear range. The correlation coefficients were >0.99.

Matrix effects were evaluated by comparing the peak areas of two matrix matched standards solutions with the peak areas of corresponding standard solutions in solvent. No significant matrix effects were observed.

Accuracy
Blank XAD sorbent tubes were fortified in triplicate with reference standard solution at 15 µg/sorbent tube (42 µg/m^3 air based on a total air volume of 0.36 m^3) and analysed directly by the method described. Recovery values

were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 110 %.

Accuracy at the LOQ of 4.2 µg/m^3 was not assessed directly but can be inferred from the results of the LOQ retention capacity experiment (see below).

Three replicates were analysed at 15 µg/m^3 rather than the requisite 5, but, again, accuracy can be inferred from the retention capacity experiment described below.

Retention Capacity:
Double layers of sorbent material, separated by plugs of glass wool, were inserted into the sampling tubes, and the front layers fortified with reference standard solution at rates of 1.5 and 15 µg/sorbent tube. Warm, humid air (35°C and 85% relative humidity) was then passed through the tubes at a rate of 1 L/min for 6 hours (0.36 m^3 total air volume), after which the layers were analysed separately by the method described. Retention/accuracy was calculated as a percentage of measured concentration in the first sorbent layer relative to fortified concentration. Breakthrough was calculated as a percentage of measured concentration in the second sorbent layer relative to fortified concentration. Breakthrough was only assessed at the higher fortification level and was only observed in 2 of the 5 samples (both ion transitions). The level of breakthrough was low; 2% in each of the two positive samples. This low level of breakthrough is acceptable.

Precision
Precision in the form of %RSD was calculated for the accuracy and retention capacity determinations described above. Acceptable precision is demonstrated by <20%RSD.

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 4.2 µg/m^3. This complies with the relevant monitoring concentration of 12 µg/m^3 calculated from the AOEL of 0.04 mg/kg bw/day according to the following equation taken from SANCO/825/00 rev.8.1 (the in the absence of an inhalative AOEL, the systemic AOEL has been used):

$$c = AOEL_{Inhalative} \cdot \frac{\text{safety factor} \cdot \text{body weight}}{\text{air intake}}$$

$$\text{with safety factor} : 0.1; \text{body weight} : 60\,[\text{kg}]; \text{air intake} : 20\,[\text{m}^3/\text{day}]$$

$$c = AOEL_{Inhalative} \cdot 300 \left[\frac{\mu g}{m^3}\right]$$

Confirmation
Residues were confirmed simultaneously to the primary detection by monitoring a second ion transition. Validation data as described above were submitted for the confirmatory method and the results are summarised in Table B.5.2.5-1.

Extraction efficiency
Assessed by the analysis of spiked accuracy samples since incurred residues are not possible for the sorbent material.

Stability
BCS-CN88460 was stable in stock and calibration standard solutions for at least 9 days when stored in a freezer.

BCS-CN88460 was stable in sorbent material and final extracts for at least 7 days when stored in a freezer.

Assessment
The method is acceptably validated in accordance with SANCO/825/00 rev.8.1 and is suitable for the determination of isoflucypram in air with a LOQ of 4.2 µg/m^3 which complies with the relevant monitoring concentration of 12 µg/m^3, as calculated from the AOEL.

Table B.5.2.5-1 Summary of validation data for analytical method 01506 for the determination of BCS-CN88460 in air

Matrix	Analyte	LOQ (µg/m^3)	Recovery fortification level (µg/m^3)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
XAD sorbent material (recovery)	BCS-CN88460 400→167	4.2	42	102 – 105 (104)	1.7 (3)	1.5 to 30 ng/mL (0.42 to 8.3 µg/m^3) y = 96600x + 3430 r = 0.9987	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS- CN88460 400→139	4.2	42	103 – 106 (105)	1.1 (3)	1.5 to 30 ng/mL (0.42 to 8.3 µg/m^3) y = 73700x + 3640 r = 0.9989	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
XAD sorbent material (retention)	BCS-CN88460 400→167	4.2	4.2 42	99 – 109 (105) 98 – 109 (105)	3.7 (5) 3.7 (5)	1.5 to 30 ng/mL (0.42 to 8.3 µg/m^3) y = 96600x + 3430 r = 0.9987	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS- CN88460 400→139	4.2	4.2 42	104 – 109 (107) 103 – 108 (106)	1.7 (5) 1.7 (5)	1.5 to 30 ng/mL (0.42 to 8.3 µg/m^3) y = 73700x + 3640 r = 0.9989	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

B.5.2.6. Analytical method for the determination of residues in body fluids and tissues

Report:	KCA 4.2/13; Desmaris, F.; 2017; M-601583-01-1
Title:	Analytical method 01534 for the determination of residues of BCS-CN88460 and its metabolite BCS-CX99799 in blood plasma by HPLC-MS/MS
Report No.:	17-03
Document No.:	M-601583-01-1
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and 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 Guidance for generating and reporting methods of analysis in support of preregistration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414, SANCO/3029/99 rev.4, July 2000 Guidance document on pesticide residue analytical methods, SANCO/825/00 rev.8.1 16/11/2010 OECD Guidance Document on Pesticide Residue Analytical Methods, Series on Testing and Assessment Document 72 and Series on Pesticides: Document 39, August 2007 (OECD Guideline, ENV/JM/MONO (2007) 17, Aug 13, 2007)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Definition of the residue
The applicant has proposed the residue definition for monitoring body fluids as parent Isoflucypram (BCS-CN88460) and its M11 metabolite, BCS-CX99799. For tissues, a residue definition of parent only is proposed. The RMS agrees with these proposals and confirms that plasma and liver are suitable matrices for monitoring the respective residues.

The residue definition will be confirmed during the EFSA peer review process.

Body Tissues (Liver)
For liver, the applicant makes reference to analytical method 01300/M034, which was developed as a post-registration monitoring method for the determination of the residues of BCS-CN88460 in animal products (including liver). This was assessed in Section B.5.2.2. of this volume 3CA but was found not be be acceptable due to unreliable data on extraction efficiency. The RMS proposes that Method 01511, which was used as a pre-registration method for the determination of BCS-CN88460 in animal products and is fully validated (including confirmatory data) for the determination of BCS-CN88460 in liver (ref Section B.5.1.2.5.3.) is proposed as the monitoring method instead, therefore no further data are required.

Note: the method employs the use of a deuterated standard of isoflucypram as internal standard. In line with SANCO/825/00 rev 8.1, section 2.12, if this standard is not commercially available, then it should be made generally available by the applicant and contact details provided.

Body Fluids (Plasma)
Residue analytical method 01534 was developed as a post-registration monitoring method for the determination of the residues of BCS-CN88460 and BCS-CX99799 in plasma. A summary of the method and its validation is presented below and in Table B.5.2.6-1.

Principle of the method
Samples of plasma were extracted by shaking on a vibratory mixer with acetonitrile/water (4:1 v/v). The resulting extract was centrifuged to give Extract A which was then diluted with acetonitrile/water (1:4 v/v) to give the final extract. Analysis was performed by reverse phase HLPC-MS/MS using an Ascentis express C18, 50mm x 2.1mm x 2.7µm analytical column at 60 °C and gradient elution using water + 0.12 % formic acid + 10 mM ammonium formate and methanol/water (10/90 v/v) +10mM ammonium formate + 0.12% formic acid mobile phases. Quantification was against external standards and two ion transitions were monitored for each analyte:

BCS CN88460
Quantification: 400.2→139.0

Confirmation: 400.2→177.0

BCS CX99799
Quantification: 416.0→236.0
Confirmation: 416.0→208.0

Specificity
Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a control (untreated) sample.

Linearity
Linearity was demonstrated by the analysis of seven standards of increasing concentration. The range of standard concentrations used was 0.025 to 2.5 µg/L, which is equivalent to 0.0125 to 1.25 mg/L in plasma samples and adequately encompasses the LOQ and 10 x LOQ recovery concentrations of 0.05 mg/L and 0.5 mg/L. Where necessary, samples were diluted into the linear range.

Matrix effects were assessed by comparison of recoveries calculated against standard solutions in solvent and also matrix matched standards. No significant matrix effects were observed, therefore it is recommended to use solvent standards to quantify residues of BCS-CN88460 and BCS-CX99799.

Accuracy
Five control samples were fortified with reference standard at each of two levels and the samples analysed by the method described. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 110 %.

Precision
Precision in the form of %RSD was calculated for the accuracy determinations described above. Acceptable precision is demonstrated by <20%RSD.

LOQ
The LOQ, as defined by the lowest concentration at which acceptable recovery and precision data have been generated, is 0.05 mg/L.

Extraction efficiency
Whilst residues could be incorportaed into plasma, there is no explicit requirement under Regulation (EU) No. 283/2013 to submit extraction efficiency data for body fluids, therefore the absence of this data is not regarded as a data gap.

Stability
The stability of BCS-CN88460 and BCS-CX99799 in standard solution has been demonstrated in previous studies. Reference is made to Section B.5.1.2.3.4.

BCS-CN88460 and BCS-CX99799 were found to be stable in plasma samples and final extracts for at least 103 hours stored at -18°C (plasma) or 10°C (final extact)

Assessment
The method is acceptably validated in accordance with SANCO/825/00 rev.8.1 and is suitable for the determination of isoflucypram and BCS-CX99799 in plasma with a LOQ of 0.05 mg/L.

Table B.5.2.6-1 Summary of validation data for analytical method 01534 for the determination of BCS-CN88460 and BCS-CX99799 in plasma

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	% Recovery range (mean)	Repeatability % RSD (n) *(based on recovery determinations)*	Linearity	Specificity
Rodent plasma	BCS-CN88460 (400.2→139.0)	0.05	0.05 0.50	103 – 111 (107) 99 – 105 (102)	2.7 (5) 2.3 (5)	0.025 to 2.5 µg/L (0.0125 to 1.25 mg/L) y = 1810000x - 14500 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CN88460 (400.2→177.0)	0.05	0.05 0.50	104 – 111 (108) 100 – 106 (103)	3.3 (5) 2.3 (5)	0.025 to 2.5 µg/L (0.0125 to 1.25 mg/L) y = 1340000x - 14700 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
Rodent plasma	BCS-CX99799 (416.0→236.0)	0.05	0.05 0.50	107 – 112 (110) 102 – 109 (106)	1.9 (5) 2.7 (5)	0.025 to 2.5 µg/L (0.0125 to 1.25 mg/L) y = 97400x - 246 r = 0.9999	Retention time match to reference standard. No significant interfering peaks observed in the control sample.
	BCS-CX99799 (416.0→208.0)	0.05	0.05 0.50	100 – 111 (104) 102 – 109 (107)	4.4 (5) 2.9 (5)	0.025 to 2.5 µg/L (0.0125 to 1.25 mg/L) y = 50500x - 261 r = 0.9997	Retention time match to reference standard. No significant interfering peaks observed in the control sample.

B.5.3. REFERENCES 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	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 4.1.1 / 01	Peiffer, C.; Uroic, K.	2015	BCS-CN88460 - Determination of technical grade active substance - HPLC - External standard Bayer Report No.: AM023714MP3 Edition Number: M-537692-01-1 Date: 2015-10-16 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.1.1 / 02	Uroic, K.; Peiffer, C.	2016	Validation of AM023714MP3 - BCS-CN88460 - Determination of technical grade active substance - HPLC-external standard Bayer Report No.: VB1-AM023714MP3 Edition Number: M-560314-01-1 Date: 2016-07-27 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.1.2 / 01	Koch, V.	2014	Analytical method 01432 for the determination of BCS-CN88460 and the metabolite BCS-CN88460-carboxylic acid in soil and sediment by HPLC-MS/MS Bayer Report No.: 01432 Edition Number: M-499794-01-1 Date: 2014-10-10 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.1.2 / 02	Menettrier, P.; Vincent, M.	2013	BCS-CN88460 - Determination by high performance liquid chromatography analysis in canine diet Bayer S.A.S., Bayer CropScience, Sophia Antipolis, France Bayer Report No.: SA 12136	No	Yes	New data for a new active substance	Bayer	N/A

			Edition Number: M-448255-01-1 Date: 2013-03-04 GLP/GEP: Yes, unpublished					
KCA 4.1.2 / 03	Amir Tahmasseb, L.	2013	BCS-CN88460 - Stability in canine diet Bayer S.A.S., Bayer CropScience, Sophia Antipolis, France Bayer Report No.: SA 12222 Edition Number: M-449932-01-1 Date: 2013-03-13 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.1.2 / 04	Gomez, C.; Vincent, M.	2012	BCS-CN88460 determination by high performance liquid chromatography analysis in 0.5 percent aqueous methylcellulose 400 Bayer S.A.S., Bayer CropScience, Sophia Antipolis, France Bayer Report No.: SA 12045 Edition Number: M-432731-01-1 Date: 2012-03-01 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.1.2 / 05	Amir Tahmasseb, L.	2012	BCS-CN88460 - Stability in aqueous 0,5 percent methylcellulose 400 Bayer S.A.S., Bayer CropScience, Sophia Antipolis, France Bayer Report No.: SA 12053 Edition Number: M-439523-01-1 Date: 2012-09-06 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.1.2 / 06	Vincent, M.; Amir Tahmasseb, L.	2012	BCS-CN88460 - Determination by high performance liquid chromatography analysis in ground rodent diet Bayer S.A.S., Bayer CropScience, Sophia Antipolis, France Bayer Report No.: SA 12003 Edition Number: M-435636-01-1	No	Yes	New data for a new active substance	Bayer	N/A

			Date: 2012-07-27 GLP/GEP: Yes, unpublished					
KCA 4.1.2 / 07	Vincent, M.	2012	BCS-CN88460 - Stability in ground rodent diet Bayer S.A.S., Bayer CropScience, Sophia Antipolis, France Bayer Report No.: SA 12032 Edition Number: M-439130-01-1 Date: 2012-08-17 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.1.2 / 08	Uceda, L.	2016	Analytical method 01475 for the determination of residues of BCS-CN88460 and its metabolite BCS-CR60082 in/on plant by HPLC-MS/MS Bayer S.A.S., Bayer CropScience, Lyon, France Bayer Report No.: 01475 Edition Number: M-558986-01-1 Method Report No.: MR-16/234 Date: 2016-07-11 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.1.2 / 09	Schulte, G.	2017	Amendment no. 1 to final report - Determination of the residues of BCS-CN88460 in/on barley after spray application of BCS-CN88460 EC 050 in Portugal, southern France and Spain Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: 15-2066 Report includes Trial Nos.: 15-2066-01 15-2066-02 15-2066-03 15-2066-04 Edition Number: M-584388-02-1 Date: 2017-03-24 **... amended: 2017-11-21** GLP/GEP: Yes, unpublished **... also filed:**	No	Yes	New data for a new active substance	Bayer	N/A

			KCA 6.3.1 / 05					
KCA 4.1.2 / 10	Schulte, G.	2017	Amendment no. 1 to final report - Determination of the residues of BCS-CN88460 in/on wheat and durum after spray application of BCS-CN88460 EC 050 in Portugal, southern France and Spain Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: 15-2069 Report includes Trial Nos.: 15-2069-01 15-2069-02 15-2069-03 15-2069-04 Edition Number: M-584384-02-1 Date: 2017-03-24 **... amended: 2017-11-21** GLP/GEP: Yes, unpublished **... also filed:** **KCA 6.3.2 / 05**	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.1.2 / 11	Freitag, T.; Effertz, C.	2017	Determination of the residues of BCS-CN88460 in/on soil and the field rotational crops barley, carrot, turnip and lettuce after spray application of BCS-CN88460 EC 050 to bare soil in Germany, the Netherlands, southern France and Italy Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: 15-2502 Report includes Trial Nos.: 15-2502-01 15-2502-02 15-2502-03 15-2502-04 Edition Number: M-605725-01-1 Date: 2017-10-26 GLP/GEP: Yes, unpublished **... also filed:** **KCA 6.6.2 / 01**	No	Yes	New data for a new active substance	Bayer	N/A

KCA 4.1.2 / 12	Freitag, T.; Hoffmeister, R.	2017	Determination of the residues of BCS-CN88460 in/on barley and the processed fractions (malt sprouts; brewer's malt; brewer's grain; hops draff; brewer's yeast; beer; pearl barley rub off and pearl barley) after spray application of BCS-CN88460 EC 050 in the field in the Netherlands and Spain Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: 15-3407 Report includes Trial Nos.: 15-3407-01 15-3407-02 Edition Number: M-579494-01-1 Date: 2017-02-02 GLP/GEP: Yes, unpublished **... also filed:** **KCA 6.5.3 / 01**	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.1.2 / 13	Traub, M.	2018	Amendment no.1 to final report - Extraction efficiency testing of the residue analytical method 01475 for the determination of residues of BCS-CN88460 in different wheat and soybean and oilseed rape RACs using incurred radioactive residues Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer Report No.: S15-06246 Edition Number: M-609382-02-1 Date: 2017-12-01 **... amended: 2018-01-19** GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.1.2 / 14	Lamshoeft, M.; Doebbe, A.	2017	Extraction efficiency testing of the residue analytical method 01475 (data gathering method) for the determination of residues of BCS-CN88460 in the primary RAC tomato using incurred radioactive residues Bayer AG, Crop Science Division, Monheim, Germany Bayer	No	Yes	New data for a new active substance	Bayer	N/A

			Report No.: EnSa-16-0204 Edition Number: M-598220-01-1 Date: 2017-08-14 GLP/GEP: Yes, unpublished					
KCA 4.1.2 / 15	Lamshoeft, M.; Doebbe, A.	2017	Extraction efficiency testing of the residue analytical method 01475 (data gathering method) for the determination of residues of BCS-CN88460 and its metabolite BCS-CR60082 in succeeding RACs (turnip, Swiss chard, wheat) using incurred radioactive residues Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EnSa-16-0179 Edition Number: M-595703-01-1 Date: 2017-07-21 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.1.2 / 16	Miller, A.	2017	An analytical method for the determination of residues of BCS-CN88460 in/on plant matrices using LC/MS/MS Bayer CropScience LP, RTP, NC, USA Bayer Report No.: LN-002-P16-01 Edition Number: M-606616-01-1 Date: 2017-06-24 GLP/GEP: No, unpublished	No	No		Bayer	N/A
KCA 4.1.2 / 17	Miller, A.; Arthur, E. L.	2017	Validation of analytical method LN-002-P16-01, an analytical method for the determination of BCS-CN88460 in/on plant matrices using LC-MS/MS Bayer CropScience LP, Stilwell, KS, USA Bayer Report No.: RALN0017 Edition Number: M-606610-01-1 Date: 2017-10-24 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.1.2 / 18	Harbin, A. M.	2017	BCS-CN88460: Magnitude of residues in/on wheat processed fractions following treatment with BCS-CN88460 EC50 Bayer CropScience, Inc., Saskatoon, SK, Canada	No	Yes	New data for a new active substance	Bayer	N/A

			Bayer Report No.: RALNN137 Report includes Trial Nos.: C1101-15PA C1102-15PA Edition Number: M-600505-02-1 Date: 2017-09-05 **... amended: 2017-12-04** GLP/GEP: Yes, unpublished **... also filed:** **KCA 6.5.3 / 02**					
KCA 4.1.2 / 19	Glaubitz, J.; Kuppels, U.; Eickstaedt, D.	2017	Residue analytical method 01511 for the determination of residues of BCS-CN88460 and its metabolites BCS-DC20298, BCS-CY26497, BCS-CY24813, BCS-DC22055 and BCS-CX99799 in/on animal tissues, milk and eggs by HPLC-MS/MS Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: P603166029 Edition Number: M-599206-01-1 Date: 2017-08-21 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.1.2 / 20	Diot, R.; Heinemann, D.	2017	Request for waiver of the requirement for radiovalidation of the analytical method for the determination of BCS-CN88460 residues in animal matrices Bayer S.A.S., Division Crop Science, Lyon, France; Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: M-605551-01-1 Date: 2017-11-06 GLP/GEP: n.a., unpublished	No	No		Bayer	N/A
KCA 4.1.2 / 21	Krebber, R.; Sandau, C.	2014	Method 01388 for the determination of BCS-CN88460 in test water by HPLC-MS/MS Bayer Report No.: MR-13/075 Edition Number: M-479643-01-1	No	Yes	New data for a new active substance	Bayer	N/A

			Date: 2014-03-10 GLP/GEP: No, unpublished					
KCA 4.1.2 / 22	Krebber, R.; Leppelt, L.	2014	Modification 001 of method 01388 for the determination of BCS-CN88460 in test water by HPLC-MS/MS Bayer Report No.: MR-14/160 Edition Number: M-502733-01-1 Date: 2014-11-21 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.1.2 / 23	Krebber, R.; Leppelt, L.	2016	Analytical method 01476 for the determination of BCS-CN88460-carboxylic-acid (BCS-CY26497) in test water from aquatic toxicity tests by HPLC-UV Bayer Report No.: P 604 157029 Edition Number: M-552469-01-1 Method Report No.: MR-15/182 Date: 2016-04-12 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.1.2 / 24	Mathieu, C.; Vincent, M.	2010	BCS-CN45153 - Determination by high performance liquid chromatography analysis in ground rodent diet Bayer S.A.S., Bayer CropScience, Sophia Antipolis, France Bayer Report No.: SA 10145 Edition Number: M-393802-01-1 Date: 2010-10-13 GLP/GEP: Yes, unpublished	No	Yes	Data requirement of Regulation EC 1107/2009	Bayer	N/A
KCA 4.2 / 01	Traub, M.	2018	Amendment no.1 to final report - Testing of the extraction efficiencies according QuEChERS using radioactive incurred residues of BCS-CN88460 in different wheat, soybean and oilseed rape RACs Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Bayer Report No.: S16-05413 Edition Number: M-611065-02-1	No	Yes	New data for a new active substance	Bayer	N/A

			Date: 2017-12-11 **... amended: 2018-01-19** GLP/GEP: Yes, unpublished					
KCA 4.2 / 02	Lamshoeft, M.; Weuthen, M.	2017	Extraction efficiency testing of the QuEChERS analytical method for the determination of residues of BCS-CN88460 in the primary RAC tomato using incurred radioactive residues Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EnSa-17-0483 Edition Number: M-598222-01-1 Date: 2017-08-14 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.2 / 03	Lamshoeft, M.; Weuthen, M.	2017	Extraction efficiency testing of the QuEChERS analytical method for the determination of residues of BCS-CN88460 and its metabolite BCS-CR60082 in turnip leaves, swiss chard and wheat grain using incurred radioactive residues Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EnSa-17-0551 Edition Number: M-600910-01-1 Date: 2017-09-11 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.2 / 04	Uceda, L.	2017	Analytical method 01520 for the determination of residues of BCS-CN88460 in/on plant by HPLC-MS/MS Bayer S.A.S., Division Crop Science, Lyon, France Bayer Report No.: 17-02 Edition Number: M-588974-01-1 Date: 2017-05-22 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.2 / 05	Schmiedt, S.	2016	Independent laboratory validation of analytical method 01520 for the determination of residues of BCS-CN88460 in/on plant by HPLC-MS/MS	No	Yes	New data for a new active substance	Bayer	N/A

			EAG Laboratories GmbH (formerly EAG Laboratories PTRL Europe GmbH), Ulm, Germany Bayer Report No.: P 4386 G Edition Number: M-603219-01-1 Date: 2017-09-19 GLP/GEP: Yes, unpublished					
KCA 4.2 / 06	Hennes, M.; Glaubitz, J.	2017	Analytical method 01300/M034 for the determination of residues BCS-CN88460 and its metabolites, BCS-CY26497, BCS-CY24813 and BCS-CX99799 in/on animal tissues, milk and eggs and biota by HPLC-MS/MS following QuEChERS - Enforcement method animal Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: P683176031 Edition Number: M-608768-01-1 Date: 2017-11-30 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.2 / 07	Miller, A.	2018	Independent laboratory validation of analytical method 01300/M034 for the determination of residues BCS-CN88460 and its metabolites, BCS CY26497, BCS-CY24813 and BCS-CX99799 in/on animal tissues, milk and eggs and biota by HPLC-MS/MS following QuEChERS - Enforcement method animal Bayer CropScience LP, RTP, NC, USA Bayer Report No.: M-612340-01-1 Date: 2018-01-15 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.2 / 08	Bongartz, R.; Luks, A. K.	2017	Testing of the extraction efficiencies according to QuEChERS using radioactive incurred residues of BCS-CN88460 in animal origin from livestock metabolism studies (lactating goat and laying hen) Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: EnSa-17-0647	No	Yes	New data for a new active substance	Bayer	N/A

			Edition Number: M-608765-01-1 Date: 2017-11-29 GLP/GEP: Yes, unpublished					
KCA 4.2 / 09	Koch, V.	2016	Analytical method 01479 for the determination of BCS-CN88460 and the metabolite BCS-CN88460-carboxylic acid in soil by HPLC-MS/MS Bayer Report No.: P681 14 1804 Edition Number: M-553434-01-1 Date: 2016-04-22 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.2 / 10	Krebber, R.; Leppelt, L.	2017	Modification M004 of analytical method 01387 for the determination of BCS-CN88460 in drinking and surface water by HPLC-MS/MS Bayer, Crop Science Division Bayer Report No.: 01387/M004 Edition Number: M-589592-01-1 Date: 2017-05-29 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.2 / 11	Schmiedt, S.	2017	Independent laboratory validation of analytical method 01387/M004 for the determination of BCS-CN88460 in surface water by HPLC-MS/MS EAG Laboratories GmbH, Ulm, Germany Bayer Report No.: P 4615 G Edition Number: M-607474-01-1 Date: 2017-11-21 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCA 4.2 / 12	Bendig, P.	2016	Analytical method 01506 for the determination of BCS-CN88460 in air PTRL Europe GmbH, Ulm, Germany Bayer Report No.: 01506 Edition Number: M-572420-01-1 Date: 2016-11-07 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A

KCA 4.2 / 13	Desmaris, F.	2017	Analytical method 01534 for the determination of residues of BCS-CN88460 and its metabolite BCS-CX99799 in blood plasma by HPLC-MS/MS Bayer S.A.S., Division Crop Science, Lyon, France Bayer Report No.: 17-03 Edition Number: M-601583-01-1 Date: 2017-09-01 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A