

FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: SHA 7273 A

Product name: CASINO ROYALE

Chemical active substances:

Boscalid, 267 g/kg

Pyraclostrobin, 67 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Sharda Cropchem España S.L.

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When	What
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5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substances (Boscalid and Pyraclostrobin) and relevant impurity (Dimethylsulfate (DMS)) in the plant protection product formulation SHA 7273 A (CASINO ROYALE).

Noticed data gaps are: no data gaps

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

Noticed data gaps are: none

Commodity/crop	Supported/ Not supported
High starch/protein content (sugar beet, carrot, beetroot, celery root, parsnip, parsley, radish, horseradish, turnip, swedes, rutabagas, chicory roots, salsifies)	Supported
High water content (Onion, tomatoes, cabbage, cherry, shallot, onion “seven years old”, aubergines/eggplants, jerusalem artichokes)	Supported
High acid content (Strawberries, Raspberry, Blackcurrant, Redcurrant, White currant)	Supported
Ornamentals	Not required

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

An overview on the acceptable methods and possible data gaps for analysis of Pyraclostrobin and Boscalid in plant protection product is provided as follows:

Comments of zRMS:	Sufficiently sensitive and selective analytical method is available for determination of the active substances Boscalid and Pyraclostrobin in the formulation SHA 7273 A (CASINO ROYALE). Method has been validated in terms of specificity, linearity, precision (repeatability) and accuracy and fulfil the requirements of EEC guideline SANCO/3030/99 rev. 4.
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Reference: KCP 5.1.1/01

Report Pyraclostrobin 6.7% + Boscalid 26.7% WG: Validation of the analytical method for the determination of the active ingredients content, Elena Riga-

	monti, 2017, report No. CH - 866/2017
Guideline(s):	Yes, SANCO/3030/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Principle of method:

The determination of the active ingredient is performed by HPLC using an internal standard and UV detector. The quantification of both Pyraclostrobin and Boscalid is achieved by comparing the ratio of each reference materials peak area versus Dibutyl phthalate internal standard peak area and the same ratio determined for a sample containing a known amount of internal standard.

Equipment:

- HPLC Agilent mod. 1200 equipped with DAD detector, autosampler and Agilent OpenLab software for data processing, Internal code No. 676
- HPLC column, Internal code No. LCN 292
- Analytical balance, Mettler ML204, Internal code No. 417
- Freezer Liebherr, mod. G 5216, Internal code No. 746
- Refrigerator Fiocchetti, mod. Labor Lux 1000, Internal code No. 418
- Plastic syringe without needle
- Syringe filter 25 mm PTFE 0.45 µm
- Ultrasonic Bath, VWR International Ultrasonic Cleaner, Internal code No. 479
- Laboratory Water Purification Systems, Sartorius Arium® 611, Internal code No. 355
- Volumetric glassware: pipettes, flasks, measuring cylinders (Class A)
- Usual laboratory glassware.

Reagents:

- Water, HPLC grade obtained by a Laboratory Water Purification Systems
- Acetonitrile, HPLC grade (VWR Chemicals)
- Phosphoric acid 85% (H₃PO₄), reagent grade (Sigma-Aldrich)
- Dibutyl phthalate, analytical grade used as internal standard (Sigma-Aldrich).

Reference material:

- Pyraclostrobin, analytical standard
- Boscalid, analytical standard.

Validation - Results and discussions

Specificity

A comparison of the chromatograms of the solvent wash (acetonitrile), Boscalid reference material, Boscalid test substance, Pyraclostrobin reference material, Pyraclostrobin test substance, Dibutyl phthalate internal standard, test item, Placebo and fortified solutions (spike medium), shows that, following the operating conditions recommended in the analytical method, the active ingredient and internal standard peaks are well separated and there is no evidence of interferences with the test item peaks.

Therefore, by using the conditions stated in the method, interferences can be avoided and the active ingredient can be reliably determined in Pyraclostrobin 6.7% + Boscalid 26.7% WG samples.

Linearity and System Precision

To check linearity and system precision, five working standard solutions were prepared as previously described in the experimental section and each solution was injected.

The peak areas obtained from five consecutive injections of the middle working standard solution (WSS 3) were then used to determine the mean value, the standard deviation (S.D.) and the relative standard deviation (RSD%) of the system precision.

The ranges tested from 15.58 to 36.36 µg/mL for Pyraclostrobin (± 40 % of the solution concentration used for the quantification analysis) and from 61.74 to 144.06 µg/mL for Boscalid (± 40 % of the solution concentration used for the quantification analysis) were determined to be linear (correlation coefficient > 0.99).

Results showed that the analytical method is linear over the range tested for the active ingredient (correlation coefficient > 0.99).

Repeatability (Precision)

The test was performed by six determinations of the test item (labelled from A to F).

The relative standard deviation was 1.29 % for Pyraclostrobin and 1.31 % for Boscalid. Since the relative standard deviation was lower than the Horwitz RSDr (2.00 at a Pyraclostrobin concentration of 7.0 % w/w and 1.64 at a Boscalid concentration of 26.5 % w/w), the repeatability test for the active ingredients was considered acceptable.

The values of 0.1 % w/w and 0.3 % w/w for the precision of the analytical method for Pyraclostrobin and for Boscalid, calculated as the standard deviation, can be considered acceptable for this test item with a declared nominal purity of 6.7 % w/w as Pyraclostrobin and 26.7 % w/w as Boscalid.

Data and results were used to determine the following precision:

Pyraclostrobin : 7.0 ± 0.1 % w/w

Boscalid : 26.5 ± 0.3 % w/w

Recovery (Accuracy)

For the accuracy, the SANCO/3030/99 rev. 4 guideline requires mean recovery values in the range 98 to 102 % for active ingredient content higher than 10 % w/w and in the range from 97 to 103 % for active ingredient content higher than 1 % w/w but lower than 10 % w/w.

Pyraclostrobin	Tests No.	Mean Recovery
Low level	(2 det.)	101.2%
Medium level	(2 det.)	100.9%
High level	(2 det.)	101.3%

Boscalid	Tests No.	Mean Recovery
Low level	(2 det.)	101.2%
Medium level	(2 det.)	101.1%
High level	(2 det.)	100.3%

The test was performed by spiking six aliquots of the Placebo (2927564-001) with the Pyraclostrobin test substance (2929561-001) and with the Boscalid test substance (2937128-001) at three levels in duplicate, corresponding to additions of 80 %, 100 % and 120 % of the nominal concentration of active ingredients. Each fortified sample was analyzed in duplicate.

Since all mean recovery values were in the correct range, these criteria were fulfilled and therefore accuracy of the analytical method can be considered acceptable.

Table 5.2-1: Methods suitable for the determination of Pyraclostrobin and Boscalid in plant protection product Pyraclostrobin 6.7% + Boscalid 26.7% WG

	Pyraclostrobin	Boscalid
Author(s), year	Elena, Rigamonti, 2017	
Principle of method	High performance liquid chromatography using an internal standard and UV detector	
Linearity	Range: 15.58 to 36.36 µg/mL (4.08	Range: 61.74 to 144.06 µg/mL (17.17 –

	Pyraclostrobin	Boscalid
(linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	- 9.72% w/w) ± 40% of the solution concentration used for the quantification analysis Correlation coefficient > 0.99 $y = 264561x - 239395$ n = 5	39.7% w/w) ± 40% of the solution concentration used for the quantification analysis Correlation coefficient > 0.99 $y = 68467x - 37887$ n = 5
Precision – Repeatability Mean n = 6 (%RSD)	Mean Pyraclostrobin content: 7.0 ± 0.1 % w/w % RSD: 1.29% Acceptable % RSD (Horwitz): 2.00	Mean Boscalid content: 26.5 ± 0.3 % w/w % RSD: 1.31% Acceptable % RSD (Horwitz): 1.64
Accuracy n = 6 (% Recovery)	Lower level % recovery: 101.2 Nominal level % recovery: 100.9 Upper level % recovery: 101.3 Mean % recovery: 101.1 Acceptable limit (SANCO): 97 - 103	Lower level % recovery: 101.2 Nominal level % recovery: 101.1 Upper level % recovery: 100.3 Mean % recovery: 100.9 Acceptable limit (SANCO): 98 – 102
Interference/ Specificity	No interference. Chromatograms submitted	
Comment	-	

Conclusion

From the results of the analytical method validation, it is concluded that the analytical method is specific, sensitive, precise, and accurate for the analysis of Pyraclostrobin 6.7% + Boscalid 26.7% WG. The results of validation criteria are within the specified limits of SANCO/3030/99 rev.4 dated 11/07/00.

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

An overview on the acceptable methods and possible data gaps for analysis of relevant impurities in plant protection product is provided as follows:

Comments of zRMS:	Sufficiently sensitive and selective analytical method is available for determination of the impurity Dimethylsulfate (DMS) in the formulation SHA 7273 A (CASINO ROYALE). Method has been validated in terms of specificity, linearity, precision (repeatability) and accuracy and fulfil the requirements of EEC guideline SANCO/3030/99 rev. 4.
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Reference: KCP 5.1.1/02

Report Pyraclostrobin 6.7% + Boscalid 26.7% WG: Validation of the Analytical Method for the Determination of Dimethylsulfate (DMS) as Relevant Impurity Content, Elena Rigamonti, 2017, report No. CH – 867/2017

Guideline(s): Yes, SANCO/3030/99 rev. 4 and SANCO/1420/2001-Final

Deviations: No

GLP: Yes

Acceptability: ~~Yes/No/Supplementary~~ Yes

Materials and methods

Principle of method:

The determination of the Dimethyl sulphate (DMS) relevant impurity is performed by Gas Chromatography using an external standard and MS/MS detector in the MRM mode.

Equipment:

- Gas chromatograph Agilent Mod 7890A, equipped with split/splitless injector, coupled with a 7000 Triple Quadrupole mass detector, autosampler and Agilent MSD ChemStation for data processing, Internal code No. 569
- GC column, Internal code No. GCN 179
- Analytical balance, Mettler ML204, Internal code No. 417
- Ultrasonic Bath, VWR International Ultrasonic Cleaner, Internal code No. 479
- Plastic syringe without needle
- Syringe filter 25 mm PTFE 0.45 µm
- Refrigerator Fiocchetti, mod. Labor Lux 1000, Internal code No. 418
- Volumetric glassware: pipettes, flasks, measuring cylinders (Class A)
- Usual laboratory glassware.

Reagents:

- Methyl-tert-butylether, MTBE (Merck)

Reference material:

- Dimethylsulfate (DMS), analytical standard.

Validation - Results and discussions

Specificity

The analysis was conducted using the GC/MS/MS technique and monitoring reaction mode (MRM) (two product ions at m/z 95 (quantifier) and 45 (qualifier) from the same precursor ion at m/z 125 for Dimethylsulfate (DMS).

A comparison of the chromatograms of the wash (MTBE), Dimethylsulfate (DMS) reference material solution (at about 50.00 ng/mL), test item solution (at about 1000000 µg/mL), fortified test item solution at low level (at 0.020 µg/g) and fortified test item solution at high level (at 0.070 µg/g) shows that, following the operating conditions recommended in the analytical method, the Dimethylsulfate (DMS) relevant impurity peak is well detected and there is no evidence of interferences with the test item peak.

Therefore, by using the conditions stated in the method, interferences can be avoided and the Dimethylsulfate (DMS) relevant impurity can be reliably determined in Pyraclostrobin 6.7% + Boscalid 26.7% WG formulation samples.

Linearity and System Precision

To check linearity and system precision, five working standard solutions were prepared as previously described in the experimental section and each solution was injected.

The peak areas obtained from five consecutive injections of the middle working standard solution (WSS 3) were then used to determine the mean value, the standard deviation (S.D.) and the relative standard deviation (% RSD) of the system precision.

The injected solutions from 10.00 ng/mL to 150.00 ng/mL for Dimethylsulfate (DMS), considering the test item preparation described in Appendix A, correspond to a nominal content in the test item ranging from 0.010 µg/g to 0.150 µg/g and therefore complies with the Pyraclostrobin SANCO/1420/2001 (maximum 0.07 µg/g for Dimethylsulfate (DMS) impurity in formulation sample).

No significant memory peak was detected in the washing injected after the highest working standard solution and the range tested for Dimethylsulfate (DMS) was found to be linear (correlation coefficient > 0.99).

The limit of quantification (L.O.Q.), as the lowest fortification level tested, was a final injected nominal solution for Dimethylsulfate (DMS) in the test item of 20.00 ng/mL, corresponding to 0.020 µg/g.

The limit of detection (L.O.D.), defined as half of the lowest calibration level, was 5.00 ng/mL, corresponding to nominal 0.005 µg/g for Dimethylsulfate (DMS) relevant impurity in the test item.

Impurity results calculated as < 0.005 µg/g (L.O.D.) are classified as not detected (n.d.).

Impurity results calculated as greater than the limit of detection but less than the limit of quantification, are designated as < 0.020 µg/g.

If Dimethylsulfate (DMS) content is calculated as greater than 0.150 µg/g, the test item solution must be suitably diluted using volumetric glassware.

Repeatability (Precision)

The repeatability test was performed by preparing six replicates of the test item (labelled from A to F), weighting, into six volumetric flasks, about 10 g of the test item.

The Dimethylsulfate (DMS) relevant impurity content was calculated using its own reference material.

Data and results were used to determine the following precision.

Impurity	Test No.	Mean value	RSD%	Horwitz %RSDr	Precision
Dimethylsul- fate (DMS) (*)	6 det.	n.d.	-	-	-

n.d.: Lower than the Limit of Detection (L.O.D.: 0.005 µg/g).

Since the Dimethylsulfate (DMS) impurity content was not detectable in repeatability test, the precision was determined via the accuracy test with the lowest fortification level and was fixed at ± 10 % of the lowest fortification level. The results are presented here below.

Impurity	Spike level	Total conc.(*)	Tests No.	Mean	RSD%	Horwitz % RSDr (**)	Precision
Dimethyl- sulfate (DMS)	0.020 µg/g	0.020 µg/g	(6 det.)	0.016 µg/g	3.64	19.32	± 0.002

(*) Total concentration in the test item after spiking (as the test item does not contain any impurity)

(**) Calculated in the total concentration found in the test item.

The acceptability of the % RSD, assessed using the modified Horwitz equation, is considered acceptable and therefore the precision of the analytical method is considered acceptable too.

Recovery (Accuracy)

For the Dimethylsulfate (DMS) relevant impurity, the test was performed by spiking a test item solution with the fortification standard solution (FSS) six times at two fortification levels corresponding to a nominal content for Dimethylsulfate (DMS) Impurity in the test item of 0.020 µg/g and of 0.070 µg/g,

For the accuracy, the SANCO/3030/99 rev. 4 guideline requires mean recovery values:

- in the range 75 to 125 % for impurity content lower than 0.1 % w/w (1000 µg/g).

Dimethylsulfate (DMS)	Tests No.	Recovery	Mean Recovery
Low level (0.020 µg/g)	(6 det.)	from 79.76 to 86.14%	82.7%
High level (0.070 µg/g)	(6 det.)	from 80.21 to 87.08%	81.8%

Since all recovery values for Dimethylsulfate (DMS) resulted to be in the correct ranges, these criteria were fulfilled and therefore accuracy of the analytical method can be considered acceptable.

The limit of quantification (L.O.Q.), as the lowest fortification level tested, was a final injected solution

for Dimethylsulfate (DMS) in the test item of about 10.00 ng/mL (corresponding to 0.020 µg/g).

Spike level	Total conc.(*)	Tests No.	Mean	RSD%	Horwitz %RSDr(**)
Dimethyl-sulfate (DMS)	0.020 µg/g	0.020 µg/g (6 det.)	0.016 µg/g	3.64	19.32

(*) Total concentration in the test item after spiking (as the test item does not contain any impurity)

(**) Calculated in the total concentration found in the test item.

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) Pyraclostrobin 6.7% + Boscalid 26.7% WG

	Dimethylsulfate maximum 0.07 µg/g in formulation sample
Author(s), year	Elena Rigamonti, 2017
Principle of method	Gas Chromatography using an external standard and MS/MS detector in the MRM mode
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	Concentration range: 10 ng/mL to 150 ng/mL Concentration range (nominal content): 0.010 µg/g to 0.150 µg/g Correlation coefficient > 0.99 $y = 7x + 24$ for m/z 125 → 95 (quantification) n = 5
Precision – Repeatability Mean n = 6 (%RSD)	Mean DMS content: lower than the limit of detection (LOD = 0.005 µg/g) Since the Dimethylsulfate impurity content was not detectable in repeatability test, the precision was determined via the accuracy test with the lowest fortification level and was fixed at ±10 % of the lowest fortification level. Mean DMS content: 0.016 ± 0.002 µg/g % RSD: 3.64 Acceptable % RSD (Horwitz): 19.32
Accuracy n = 6 (% Recovery)	Low level (0.020 µg/g) % recovery: from 79.76 to 86.14 Low level % mean recovery: 82.7 High level (0.070 µg/g) % recovery: from 80.21 to 87.08 High level % mean recovery: 81.8 Acceptable limit (SANCO): 75 – 125
Interference/ Specificity	No interference. Chromatograms submitted
LOQ	The limit of quantification (L.O.Q.), as the lowest fortification level tested, was a final injected solution for Dimethylsulfate (DMS) in the test item of about 20.00 ng/mL (corresponding to 0.020 µg/g).
Comment	-

Conclusion

The analytical method was shown to be specific for Dimethylsulfate (DMS) as relevant impurity content in Pyraclostrobin 6.7% + Boscalid 26.7% WG formulation samples.

The SANCO/1420/2001-Final, 8 September 2004 for relevant impurity in technical, requires a limit of 1 µg/g (0.0001 % w/w) for Dimethyl sulphate content in Pyraclostrobin technical. Considering a nominal content in the test item of about 6.7 % w/w, the limit becomes 0.07 µg/g (0.000007 % w/w).

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

5.1.1)

Not relevant.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

A CIPAC method No. 657 is available for Pyraclostrobin.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Please refer to post-registration methods.

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

Analytical methods for the determination of the active substance and relevant impurities in the plant protection product shall be submitted, unless the applicant shows that these methods already submitted in accordance with the requirements set out in point 5.2.1 can be applied.

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

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

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Pyraclostrobin	0.02 mg/kg	Reg. (EU) 2020/856
Plant, high acid content		0.02 mg/kg	Reg. (EU) 2020/856
Plant, high protein/high starch content (dry commodities)		0.02 mg/kg	Reg. (EU) 2020/856
Plant, high oil content		0.02 mg/kg	Reg. (EU) 2020/856
Plant, difficult matrices (hops, spices, tea)		0.1mg/kg	Reg. (EU) 2020/856
Muscle	Pyraclostrobin	0.05 mg/kg	Reg. (EU) 2020/856
Milk		0.01 mg/kg	Reg. (EU) 2020/856
Eggs		0.05 mg/kg	Reg. (EU) 2020/856
Fat		0.05 mg/kg	Reg. (EU) 2020/856

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Liver, kidney		0.05 mg/kg	Reg. (EU) 2020/856
Soil (Ecotoxicology)	Pyraclostrobin	0.05 mg/kg	Common limit
Drinking water (Human toxicology)	Pyraclostrobin	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Pyraclostrobin	2 µg/L	Lowest NOEC for aquatic toxicity study on <i>O. mykiss</i>
Air	Pyraclostrobin	4.5 µg/m ³	AOEL sys/AOEL inhal: 0.015 mg/kg bw/d
Tissue (meat or liver)	Pyraclostrobin	0.1 mg/kg	Classified as T
Body fluids		0.05 mg/kg	Classified as T

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

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

Table 5.3-2: Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

Component of residue definition: Pyraclostrobin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
High water content	Primary	0.02 mg/kg	LC-MS/MS HPLC-UV	DAR 2001 (Reinhard and Mackenroth, 1999a) DAR 2001 (Abdel-Baky and Riley, 2000)
	ILV	-	-	-
High acid content	Primary	0.02 mg/kg	LC-MS/MS HPLC-UV	DAR 2001 (Reinhard and Mackenroth, 1999a) DAR 2001 (Abdel-Baky and Riley, 2000)
	ILV	0.02 mg/kg	LC-MS/MS HPLC-UV	DAR 2001 (Perez and Perez, 2000) DAR 2001 (Jordan, 2000)
High oil content	Primary	0.02 mg/kg	LC-MS/MS HPLC-UV	DAR 2001 (Reinhard and Mackenroth, 1999a) DAR 2001 (Abdel-Baky and Riley, 2000)
	ILV	-	-	-
High protein/high starch content (dry)	Primary	0.02 mg/kg	LC-MS/MS HPLC-UV	DAR 2001 (Reinhard and Mackenroth, 1999a) DAR 2001 (Abdel-Baky and Riley, 2000)

Component of residue definition: Pyraclostrobin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	ILV	0.02 mg/kg	LC-MS/MS HPLC-UV	DAR 2001 (Perez and Perez, 2000) DAR 2001 (Jordan, 2000)
Difficult (if required, depends on intended use)	Primary	-	-	-
	ILV	-	-	-

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	Not provided in Germany, 2001

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

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

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

Component of residue definition: Pyraclostrobin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk	Primary	0.01 mg/kg	HPLC-UV GC-MS HPLC-MS/MS	DAR 2001 (Kampke-Thiel, 1999) DAR 2001 (Tilting, 1999) DAR 2001 (Tilting, 1999)
	ILV	0.01 mg/kg	HPLC-UV	DAR 2001 (Levsen and Kruppa, 1999)
	Confirmatory (if required)	0.01 mg/kg	RPLC-UV	DAR 2001 (Kampke-Thiel, 1999)
Eggs	Primary	0.05 mg/kg	HPLC-UV HPLC-MS/MS	DAR 2001 (Kampke-Thiel, 1999) DAR 2001 (Tilting, 1999)
	ILV	-	-	-
	Confirmatory (if required)	0.05 mg/kg	RPLC-UV	DAR 2001 (Kampke-Thiel, 1999)
Muscle	Primary	0.05 mg/kg	HPLC-UV HPLC-MS/MS	DAR 2001 (Kampke-Thiel, 1999) DAR 2001 (Tilting, 1999)
	ILV	0.05 mg/kg	HPLC-UV	DAR 2001 (Levsen and Kruppa, 1999)

Component of residue definition: Pyraclostrobin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
	Confirmatory (if required)	0.05 mg/kg	RPLC-UV	DAR 2001 (Kampke-Thiel, 1999)
Fat	Primary	0.05 mg/kg	HPLC-UV HPLC-MS/MS	DAR 2001 (Kampke-Thiel, 1999) DAR 2001 (Tilting, 1999)
	ILV	0.05 mg/kg	HPLC-UV	-
	Confirmatory (if required)	0.05 mg/kg	RPLC-UV	DAR 2001 (Kampke-Thiel, 1999)
Kidney, liver	Primary	0.05 mg/kg	HPLC-UV HPLC-MS/MS	DAR 2001 (Kampke-Thiel, 1999) DAR 2001 (Tilting, 1999)
	ILV	0.05 mg/kg	HPLC-UV	-
	Confirmatory (if required)	0.05 mg/kg	RPLC-UV	DAR 2001 (Kampke-Thiel, 1999)

Table 5.3-5: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	Not provided in Germany, 2001

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

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

Table 5.3-6: Validated methods for soil (if appropriate)

Component of residue definition: Pyraclostrobin			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.01 mg/kg	HPLC-UV	DAR 2001 (Ziegler, 1998b)
Confirmatory	0.01 mg/kg	LC-MS/MS	DAR 2001 (Zangmeister, 1999b)

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

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

Table 5.3-7: Validated methods for water (if appropriate)

Component of residue definition: Pyraclostrobin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	0.05 µg/L	HPLC-MS-MS	DAR 2001 (Zangmeister, 1999c)
Surface water	Primary	0.05 µg/L	HPLC-MS-MS	DAR 2001 (Zangmeister, 1999c)

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

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

Table 5.3-8: Validated methods for air (if appropriate)

Component of residue definition: Pyraclostrobin			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.3 µg/m ³	HPLC-UV	DAR 2001 (Zangmeister, 1999)

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

An overview on the acceptable methods and possible data gaps for analysis of Pyraclostrobin in body fluids and tissues is given in the following table.

Table 5.3-9: Methods for body fluids and tissues (if appropriate)

Component of residue definition: Pyraclostrobin			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.05 mg/kg (kidney/liver)	HPLC-UV HPLC-MS/MS	DAR 2001 (Kampke-Thiel, 1999) DAR 2001 (Tilting, 1999)

5.3.2.8 Other studies/ information

No new or additional studies have been submitted

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

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

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is not identical.

Indeed, the previous residue definition for Boscalid was different for all the commodities under the pesticide-code 1000000 (products of animal origin-terrestrial animals), where residue definition was sum of Boscalid and its hydroxy metabolite 2-chloro-N-(4'-chloro-5-hydroxybiphenyl-2-yl)nicotinamide (free and conjugated) expressed as Boscalid, and now the residue definition is different for pesticide-code number 1000000 except 1040000, 1011010, 1011020, 1011050, 1012010, 1012020, 1012050, 1013010, 1013020, 1013050, 1014010, 1014020, 1014050, 1015010, 1015020, 1015050, 1016010, 1016020, 1017010, 1017020, 1017050, 1020000 and 1030000.

Table 5.3-10: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Boscalid	0.01 mg/kg	Reg. (EU) 2016/156 Reg. (EU) 2021/590
Plant, high acid content		0.01 mg/kg	Reg. (EU) 2016/156 Reg. (EU) 2021/590
Plant, high protein/high starch content (dry commodities)		3.0 mg/kg	Reg. (EU) 2016/156 Reg. (EU) 2021/590
Plant, high oil content		0.01 mg/kg	Reg. (EU) 2016/156 Reg. (EU) 2021/590
Plant, difficult matrices (hops, spices, tea)		0.01 mg/kg	Reg. (EU) 2016/156 Reg. (EU) 2021/590
Muscle	Sum of Boscalid and its hydroxy metabolite 2-chloro-N-(4'-chloro-5-hydroxybiphenyl-2-yl)nicotinamide (free and conjugated) expressed as Boscalid (except for the pesticide-code numbers mentioned above)	0.01 mg/kg	Reg. (EU) 2016/156 Reg. (EU) 2021/590
Milk		0.02 mg/kg	Reg. (EU) 2016/156 Reg. (EU) 2021/590
Eggs		0.01 mg/kg	Reg. (EU) 2016/156 Reg. (EU) 2021/590
Fat		0.07 mg/kg	Reg. (EU) 2016/156 Reg. (EU) 2021/590
Liver, kidney		0.05 mg/kg	Reg. (EU) 2016/156 Reg. (EU) 2021/590
Soil (Ecotoxicology)	Boscalid	0.05 mg/kg	Common limit
Drinking water (Human toxicology)	Boscalid M510F47, M510F49	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Boscalid M510F47, M510F49	125 µg/L	Lowest NOEC from aquatic toxicity study on <i>O. mykiss</i>
Air	Boscalid	30 µg/m ³	AOEL sys/AOEL inhal: 0.1 mg/kg bw/d
Tissue (meat or liver)	-	Not required	Not classified as T / T+

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

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

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

Table 5.3-11: Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

Component of residue definition: Boscalid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
High water content	Primary	0.01 mg/kg 0.05 mg/kg	GC-MS HPLC-MS/MS	DAR 2002 (Weeren and Pelz, 1999) DAR 2002; FAO, 2006 (Funk and Mackenroth, 2000)
	ILV	0.01 mg/kg	GC-MS	DAR 2002 (Reichert, 2001)
High acid content	Primary	0.01 mg/kg 0.05 mg/kg	GC-MS HPLC-MS/MS	DAR 2002 (Weeren and Pelz, 1999) DAR 2002; FAO, 2006 (Funk and Mackenroth, 2000)
	ILV	0.01 mg/kg	GC-MS	DAR 2002 (Reichert, 2001)
High oil content	Primary	0.02 mg/kg 0.05 mg/kg	GC-MS HPLC-MS/MS	DAR 2002 (Weeren and Pelz, 1999) DAR 2002; FAO, 2006 (Funk and Mackenroth, 2000)
	ILV	0.02 mg/kg	GC-MS	DAR 2002 (Reichert, 2001)
High protein/high starch content (dry)	Primary	0.01 mg/kg	GC-MS	DAR 2002 (Weeren and Pelz, 1999)
	ILV	0.01 mg/kg	GC-MS	DAR 2002 (Reichert, 2001)
Difficult (if required, depends on intended use)	Primary	0.05 mg/kg	GC-MS *	DAR 2002 (Reichert, 2001)

* Low recovery

Table 5.3-12: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Germany, 2002
Not required, because:	-

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

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

Table 5.3-13: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Sum of Boscalid and its hydroxy metabolite 2-chloro-N-(4'-chloro-5-hydroxybiphenyl-2-yl)nicotinamide (free and conjugated) expressed as Boscalid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk	Primary	0.01 mg/kg	GC-ECD HPLC-MS/MS	DAR 2002 (Class, 2000) DAR 2002 (Grosshans, 2000)
	ILV	0.01 mg/kg	GC-ECD	DAR 2002 (Kampke-Thiel, 2001)
	Confirmatory (if required)	0.01 mg/kg	GC-MS	DAR 2002 (Class, 2000)
Eggs	Primary	0.025 mg/kg 0.01 mg/kg	GC-ECD HPLC-MS/MS	DAR 2002 (Class, 2000) DAR 2002 (Grosshans, 2000)
	ILV	0.01 mg/kg 0.025 mg/kg	GC-ECD	DAR 2002 (Kampke-Thiel, 2001)
	Confirmatory (if required)	0.025 mg/kg	GC-MS	DAR 2002 (Class, 2000)
Muscle	Primary	0.025 mg/kg	GC-ECD HPLC-MS/MS	DAR 2002 (Class, 2000) DAR 2002 (Grosshans, 2000)
	ILV	0.025 mg/kg	GC-ECD	DAR 2002 (Kampke-Thiel, 2001)
	Confirmatory (if required)	0.025 mg/kg	GC-MS	DAR 2002 (Class, 2000)
Fat	Primary	0.025 mg/kg	GC-ECD HPLC-MS/MS	DAR 2002 (Class, 2000) DAR 2002 (Grosshans, 2000)
	ILV	0.025 mg/kg	GC-ECD	DAR 2002 (Kampke-Thiel, 2001)
	Confirmatory (if required)	0.025 mg/kg	GC-MS	DAR 2002 (Class, 2000)
Kidney, liver	Primary	0.025 mg/kg	GC-ECD HPLC-MS/MS	DAR 2002 (Class, 2000) DAR 2002 (Grosshans, 2000)
	ILV	0.025 mg/kg	GC-ECD	DAR 2002 (Kampke-Thiel, 2001)
	Confirmatory (if required)	0.025 mg/kg	GC-MS	DAR 2002 (Class, 2000)

Table 5.3-14: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	No residues >LOQ are expected.

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

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

Table 5.3-15: Validated methods for soil (if appropriate)

Component of residue definition: Boscalid			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.01 mg/kg	GC-MS	DAR 2002 (Keller, 1998a)

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

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

Table 5.3-16: Validated methods for water (if appropriate)

Component of residue definition: Boscalid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	0.05 mg/kg	GC-MS	DAR 2002 (Keller, 1998b)
Surface water	Primary	0.5 mg/kg	GC-MS	DAR 2002 (Grote, 2001)

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

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

Table 5.3-17: Validated methods for air (if appropriate)

Component of residue definition: Boscalid			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	1.5 µg/m ³	GC-MS	DAR 2002 (Zangmeister, 2000)

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

Since Boscalid is not classified as toxic or highly toxic, no residue method for body fluids and tissues is required.

5.3.3.8 Other studies/ information

No new or additional studies have been submitted

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1/01	Rigamonti E.	2017	Pyraclostrobin 6.7% + Boscalid 26.7% WG: Validation of the Analytical Method for the Determination of the Active Ingredients Content Company Report No CH – 866/2017 ChemService GLP Unpublished	N	Sharda Cropchem Ltd.
KCP 5.1.1/02	Rigamonti E.	2017	Pyraclostrobin 6.7% + Boscalid 26.7% WG: Validation of the Analytical Method for the Determination of Dimethylsulfate (DMS) as Relevant Impurity Content Company Report No CH – 867/2017 ChemService GLP Unpublished	N	Sharda Cropchem Ltd.

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Pyraclostrobin

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

No new or additional studies have been submitted

A 2.1.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted

A 2.2 Analytical methods for Boscalid

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

No new or additional studies have been submitted

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

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.2.2.7 Other Studies/ Information

No new or additional studies have been submitted