FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: SHA 7216 A

Product name: CIAZ

Chemical active substances:

Boscalid, 233 g/L

Difenoconazole, 66 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Sharda Cropchem España S.L.

Submission date: August 2021

Finalisation date: March 2022; December 2022

Version history

When	What
March 2022	ZRMS Poland Assessment
December 2022	Updated assessment after commenting period

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5 Analytical methods

5.1 Conclusion and summary of assessment

The proposed analytical methods are suitable for the determination of active substances – boscalid and difenoconazole and the relevant impurity – toluene in the formulation SHA 7216 A (CIAZ) and fulfill requirements of SANCO/3030/99 rev.4 and rev 5. Methods have been validated in terms of specificity, linearity, precision and accuracy.

Noticed data gaps are: none

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

Noticed data gaps are:
- ~~none~~

Boscalid (minor data gaps – post registration requirement)
Animal matrices:
- ILV method for eggs
- Primary and ILV method with LOQ of 0.01 mg/kg for muscle.

An independent laboratory validation (ILV) of the analytical method for boscalid in drinking water
An analytical method for boscalid in body fluids
Difenoconazole (minor data gaps – post registration requirement)
An independent laboratory validation (ILV) of the analytical method for difenoconazole in drinking water
An analytical method for difenoconazole in body fluids

Commodity/crop	Supported / Not supported
Winter wheat	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

An overview on the acceptable methods and possible data gaps for analysis of Boscalid and Difenoconazole in plant protection product SHA 7216 A (CIAZ) is provided as follows:

Reference:	KCP 5.1.1
Report	Boscalid 23.3% + Difenoconazole 6.6% SC – Method validation for determination the content of active substances, Pokrzywnicka S., 2017, Report No. BA-27/17
Guideline(s):	Yes, SANCO/3030/99 rev. 4 (11/07/00) and rev.5 (22/03/2019)

Deviations: No

GLP: Yes

Acceptability: Yes/~~No/Supplementary~~

Materials and methods

Test item

Boscalid 23.3% + Difenoconazole 6.6% SC
Batch number: SCL-20245
Test item code: 151/16/BA-27/17

Reference item
The following standards were used as reference material:
- Boscalid, 99.6%, IPO 924, batch Np. 1A/15
- Difenoconazole, 99.6%, IPO 830, batch No. 1A/16

Equipment
- Shimadzu liquid chromatograph equipped with UV-Vis
- Shimadzu liquid chromatograph equipped with UV-DAD
- Column: Luna C18(2), 250 x 4.6 mm, 5µm (Phenomenex)
- Typical laboratory equipment

Reagents
- Acetonitrile for HPLC, LiChrosolv
- Deionized water, ultra-pure, Millipore

Analytical method
The method is based on determination of Boscalid and difenoconazole using reversed phase high performance liquid chromatography (RP-HPLC) with UV-Vis detection at wavelength 206 nm and external standard.

Standard solution of difenoconazole and boscalid
The corresponding amount of difenoconazole standard or boscalid standard were weighed into a volumetric flask. Acetonitrile was added up to the corresponding volume. The dilutions needed were performed with acetonitrile.

Sample solution
The corresponding amount of the examined sample was weighted into the volumetric flask, 2 mL of water was added and the content was mixed. Acetonitrile was added up to the volume and the content was mixed. The sample solution was diluted 5 times with acetonitrile.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of Boscalid and Difenoconazole in plant protection product Boscalid 23.3% + Difenoconazole 6.6% SC

	Boscalid	**Difenoconazole**
Author(s), year	Pokrzywnicka S., 2017	
Principle of method	The method is based on determination of boscalid and difenoconazole using reversed phase high performance liquid chromatography (RP-HPLC) with UV-	

	Boscalid	**Difenoconazole**
	Vis detection at wavelength 206 nm and external standard.	
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Linear between 0.19 – 0.43 mg/mL (~ 67 - 155%) *n* = 5 R^2 = 0.9999 Calibration curve: y = 21282506x + 269652	Linear btween 0.05-0.13 mg/mL (~ 68 - 169%) *n* = 5 R^2 = 0.9998 Calibration curve: y = 22063839x - 36824
Precision – Repeatability Mean n = 6 (%RSD)	Boscalid mean content: 20.965 % w/w SD = 0.137 %RSD = 0.65% Acceptable Horwitz RSD: ≤ 1.67% H_r: 0.39	Difenoconazole mean content: 5.953%w/w SD = 0.061 %RSD = 1.02% Acceptable Horwitz RSD: ≤ 2.02% H_r: 0.5
Accuracy n = 6 (% Recovery)	Mean recovery = 100.54% Acceptable limit (SANCO rev. 4): 98-102% Acceptable limit (SANCO rev. 5): 97-103% SD = 0.67 %RDS = 0.67	Mean recovery = 100.76% Acceptable limit (SANCO rev. 4): 97-103% Acceptable limit (SANCO rev. 5): 90-110% SD = 0.53 %RSD = 0.53%
Interference/ Specificity	Chromatograms submitted. No interference, specific.	Chromatograms submitted. No interference, specific.
Comment	-	

Conclusion

The method for determination of the active substances Boscalid and Difenoconazole in Boscalid 23.3% + Difenoconazole 6.6% SC preparation was developed and validated according to EU requirements described in SANCO/3030/99 rev. 4 (11/07/00) and rev. 5 (22/03/2019) guidelines. Validation criteria are compliant with EU requirements given in SANCO/3030/99 rev.4 and rev 5.

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 impurity - Toluene in plant protection product CIAZ is provided as follows:

Reference:	KCP 5.1.2
Report	Boscalid 23.3 % + Difenoconazole 6.6 % SC – Analysis of relevant content of initial preparation after accelerated procedure (CIPAC MT 46.3). E. Nowakowska-Bogdan, 2020. Report No. 112/2020.
Guideline(s):	Yes SANCO/3030/99 rev.5 (22/03/2019)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Description of the method
The GC-MS analysis was performed according to the internal test procedure BA-AC/SPO-2 "Quantitative and qualitative determination of pesticides in plant protection formulations by GC/MS" and research method No. BA-AC/MB-11 Toluene."

Equipment
Gas chromatograph Agilent Technologies 7890A with MSD detector Agilent Technologies type 5977B and computer program "MassHunter'
Chromatographic column HP-5MS; 30 m x 0.25 mm i.d. 0.25 mm i.d. 0.25 µm film
Analytical balance Mettler AT200
Automatic pipette Brand Transferpette
Laboratory glassware

Reagents
Acetonitrile for HPLC

Sample solution preparation:
Amount of about 200 mg of initial formulation were diluted to 10 cm^3 with acetonitrile. Five independent test item solutions prepared from five individual weights of the test item. Were analysed 2 times using the same chromatographic conditions as during the calibration process.
Amount of about 200 mg of formulation after accelerated storage procedure were diluted to 10 cm^3 with acetonitrile. Five independent test item solutions prepared from five individual weights of the test item. Were analysed 2 times using the same chromatographic conditions as during the calibration process.

All sample solutions were analysed by GC/MS under stable chromatographic conditions.

Validation - Results and discussions

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

	Toluene
Author(s), year	E. Nowakowska-Bogdan, 2020
Principle of method	GC-MS
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	5.67 – 8.45 mg/L Calibration points = 5 Correlation coeff R=0.9993 Calibration curve: y = 19291x + 44451
Precision – Repeatability Mean n = 10 (%RSD)	%RSD: 3.46 % SD: 0.001 Acceptable Horwitz RSD: ≤ 6.67% %RSDr: 4.47% H_r: 0.77
Accuracy n = 7 (% Recovery)	Analyte conc. 80% - recovery 107.4 % Analyte conc. 100% - recovery 106.7 % Analyte conc. 120% - recovery 103.1 % Acceptable limit (SANCO rev. 5): 75-125% SD = 0.002 %RDS = 4.46
Interference/ Specificity	Interference <3 %

	Toluene
	Chromatograms submitted
LOQ	5.38 mg/L
RMS Comment	The proposed analytical method is suitable for the determination of the relevant impurity – toluene in the formulation CIAZ and fulfill requirements of SANCO/3030/99 and rev.5. Method has been validated in terms of specificity, linearity, precision and accuracy

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. 673 is available for Boscalid.
A CIPAC method No. 687 is available for Difenoconazole

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 Boscalid (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 not identical.

In the Draft Assessment Report, 2002, the residue definition was the following:
- for food of plant origin: Boscalid
- for food of animal origin: Boscalid, M510F01 (including its conjugates) calculated as Boscalid

However, the residue definition, stated in ~~Regulation (EU) No. 2016/156~~ Reg. (EU) 2021/590 is the following:

- for food of plant origin: Boscalid
- for food of animal origin: Sum of Boscalid and its hydroxy metabolites 2-chloro-*N*-(4'-chloro-5-hydroxybiphenyl-2-yl) nicotinamide (free and conjugated) expressed as Boscalid

Since, the Draft Assessment Report was finalised on 2002 and the last Commission regulation (EU) enter into force on 18 January 2016, the last established residue definition is therefore Difenoconazole for both plant and animal product. Analytical methods for residues in plant and animal matrices provided in this dossier are therefore complying with the last residue definition stated in ~~reg. (EU) 2016/156~~ Reg. (EU) 2021/590.

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	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	Boscalid	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	Sum of Boscalid and its hydroxy metabolites 2-chloro-*N*-(4'-chloro-5-hydroxybiphenyl-2-yl) nicotinamide (free and conjugated) expressed as Boscalid	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	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Boscalid	125 µg/L	Lowest NOEC from aquatic toxicity study on *O. mykiss*
Air	Boscalid	30 µg/m^3	AOEL sys: 0.1 mg/kg bw/d
Tissue (meat or liver)	-	Not required	Not classified as T / T+
Body fluids		Not required	Not classified as T / 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 Boscalid in plant matrices is given in the following tables.

zRMS: The methods of Funk & Mackenroth, 2000 for high water content, acidic and oily matrices and Weeren & Pelz, 1999 as well as Reichert, 2001 for high oil content in Table 5.3-2 showed higher LOQs than the required MRL of 0.01 mg/kg. Therefore, these methods are not suitable for monitoring of the MRLs.
However, as stated in EFSA Journal 2014;12(7):3799:
The multi-residue QuEChERS method in combination with HPLC-MS/MS, as described by CEN (2008), is also reported for analysis of parent boscalid with an LOQ of 0.01 mg/kg in dry commodities, high water content, high fat content and acidic commodities.
No additional enforcement method is required.

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: 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 (Funk and Mackenroth, 2000)
	ILV	0.01 mg/kg	GC-MS	DAR 2002 (Reichert, 2001)
	Confirmatory (if required)	-	-	-
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 (Funk and Mackenroth, 2000)
	ILV	0.01 mg/kg	GC-MS	DAR 2002 (Reichert, 2001)
	Confirmatory (if required)	-	-	-
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 (Funk and Mackenroth, 2000)
	ILV	0.02 mg/kg	GC-MS	DAR 2002 (Reichert, 2001)
	Confirmatory (if required)	-	-	-
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)~~
	Confirmatory (if required)	-	-	-
Difficult (if required, depends on intended use)	Primary	Not relevant	Not relevant	Not relevant
	ILV	Not relevant	Not relevant	Not relevant
	Confirmatory (if required)	Not relevant	Not relevant	Not relevant

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

Table 5.3-3: Statement on extraction efficiency

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

For the detailed evaluation of (additional) studies on extraction efficiency, it is referred to Appendix 2.

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 Boscalid in animal matrices is given in the following tables.

zRMS:
The LOQ of the method of Class, 2000 for eggs, and muscle are not sufficient for currently stated MRLs.
The method of Kampke Thiel, 2001 is not validated for eggs and meat.
Data gaps:
ILV method for eggs
Primary and ILV method with LOQ of 0.01 mg/kg for muscle.

Table 5.3-4: 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	DAR 2002 (Class, 2000)

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**
			HPLC-MS/MS	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)

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

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	No residues >LOQ are expected. Not presented in the DAR, 2002

For the detailed evaluation of (additional) studies on extraction efficiency please refer to Appendix 2.

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 Boscalid in soil is given in the following tables.

Table 5.3-6: 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, 1998)
Confirmatory	-	-	-

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

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 Boscalid in surface and drinking water is given in the following tables.

zRMS: An independent laboratory validation (ILV) of the analytical method for boscalid in drinking water is missing but required according to Regulation (EC) No 283/2013. This is a data gap.

Table 5.3-7: 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, 1998)
	ILV	-	-	-
	Confirmatory	-	-	-
Surface water	Primary	0.5 mg/kg	GC-MS	DAR 2002 (Grote, 2001)
	Confirmatory	-	-	-

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

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 Boscalid in air is given in the following tables.

Table 5.3-8: 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)
Confirmatory	-	-	-

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

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

Not required as the Boscalid is not classified as toxic or very toxic.

zRMS: An analytical method for boscalid in body fluids is missing but required according to Regulation (EC) No 283/2013. This is a data gap.

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 Difenoconazole (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.

In the Draft Assessment Report and EFSA Journal 2011; 9(1):1967, the residue definition was the following:

- for food of plant origin: Difenoconazole
- for food of animal origin: Difenoconazole alcohol (CGA 205375) expressed as difenoconazole

However, the residue definition, stated in Regulation (EU) No. 2019/552 is the following:

- for food of plant origin: Difenoconazole
- for food of animal origin: Difenoconazole

Since, EFSA Journal report was finalised on 2011 and the last Commission regulation (EU) enter into force on 24 April 2019, the last established residue definition is therefore Difenoconazole for both plant and animal product. Analytical methods for residues in plant and animal matrices provided in this dossier are therefore complying with the last residue definition stated in reg. (EU) 2019/552.

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Difenoconazole	0.05 mg/kg	Reg. (EU) 2019/552
Plant, high acid content		0.1 mg/kg	Reg. (EU) 2019/552
Plant, high protein/high starch content (dry commodities)		0.05 mg/kg	Reg. (EU) 2019/552
Plant, high oil content		0.05 mg/kg	Reg. (EU) 2019/552
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Reg. (EU) 2019/552
Muscle	Difenoconazole	0.05 mg/kg	Reg. (EU) 2019/552
Milk		0.005 mg/kg	Reg. (EU) 2019/552
Eggs		0.05 mg/kg	Reg. (EU) 2019/552
Fat		0.05 mg/kg	Reg. (EU) 2019/552
Liver, kidney		0.1 mg/kg	Reg. (EU) 2019/552
Soil (Ecotoxicology)	Difenoconazole	0.05 mg/kg	common limit
Drinking water (Human toxicology)	Difenoconazole	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Difenoconazole	5.6 µg/L	Lowest NOEC from aquatic toxicity study on *Daphnia magna*
Air	Difenoconazole	48 µg/m^3	AOEL sys: 0.16 mg/kg bw/d

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Tissue (meat or liver)	Not required.	Not required	Not classified as T / T+
Body fluids		Not required	Not classified as T / T+

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

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

Table 5.3-10: 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: Difenoconazole				
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	DFG method S19 (LC-MS/MS)	EFSA, 2011 (Steinhauer S., 2004)
	ILV	0.01 mg/kg	DFG method S19 (LC-MS/MS)	EFSA, 2011 (Schulz H., 2004)
	Confirmatory (if required)	-	-	-
High acid content	Primary	-	-	-
	ILV	-	-	-
	Confirmatory (if required)	-	-	-
High oil content	Primary	0.05 mg/kg	DFG method S19 (LC-MS/MS)	EFSA, 2011 (Steinhauer S., 2004)
	ILV	0.01 mg/kg	DFG method S19 (LC-MS/MS)	EFSA, 2011 (Schulz H., 2004)
	Confirmatory (if required)	-	-	-
High protein/high starch content (dry)	Primary	0.05 mg/kg	DFG method S19 (LC-MS/MS)	EFSA, 2011 (Steinhauer S., 2004)
	ILV	0.01 mg/kg	DFG method S19 (LC-MS/MS)	EFSA, 2011 (Schulz H., 2004)
	Confirmatory (if required)	-	-	-
Difficult (if required, depends on intended use)	Primary	-	-	-
	ILV	-	-	-
	Confirmatory (if required)	-	-	-

For any special comments or remarkable points concerning the analytical methods for the determination

of residues in plant matrices, please refer to Appendix 2.

Table 5.3-11: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	Not presented in the DAR, 2006

For the detailed evaluation of (additional) studies on extraction efficiency, it is referred to Appendix 2.

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 Difenoconazole in animal matrices is given in the following tables.

Table 5.3-12: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Difenoconazole alcohol (CGA 205375) expressed as Difenoconazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.005 mg/kg	LC-MS/MS	EU agreed (Crook S.J., 2004)
	ILV	0.005 mg/kg	LC-MS/MS	EU agreed (Benaseraf L., 2004)
	Confirmatory (if required)	-	-	-
Eggs	Primary	0.01 mg/kg	LC-MS/MS	EU agreed (Crook S.J., 2004)
	ILV	0.01 mg/kg	LC-MS/MS	EU agreed (Benaseraf L., 2004)
	Confirmatory (if required)	-	-	-
Muscle	Primary	0.01 mg/kg	LC-MS/MS	EU agreed (Crook S.J., 2004)
	ILV	0.01 mg/kg	LC-MS/MS	EU agreed (Benaseraf L., 2004)
	Confirmatory (if required)	-	-	-
Fat	Primary	0.01 mg/kg	LC-MS/MS	EU agreed (Crook S.J., 2004)
	ILV	0.01 mg/kg	LC-MS/MS	EU agreed (Benaseraf L., 2004)
	Confirmatory (if required)	-	-	-
Kidney, liver	Primary	0.01 mg/kg	LC-MS/MS	EU agreed (Crook S.J., 2004)
	ILV	0.01 mg/kg	LC-MS/MS	EU agreed (Benaseraf L., 2004)
	Confirmatory (if required)	-	-	-

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

Table 5.3-13: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Not presented in the DAR, 2006

For the detailed evaluation of (additional) studies on extraction efficiency please refer to Appendix 2.

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 Difenoconazole in soil is given in the following tables.

Table 5.3-14: Validated methods for soil (if appropriate)

Component of residue definition: Difenoconazole and difenoconazole alcohol (CGA 205375)			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	LC-MS/MS	EU agreed (Tummon O.J., 2004)
Confirmatory	-	-	-

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

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 Difenoconazole in surface and drinking water is given in the following tables.

zRMS: An independent laboratory validation (ILV) of the analytical method for difenoconazole in drinking water is missing but required according to Regulation (EC) No 283/2013. This is a **data gap**.

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

Component of residue definition: Difenoconazole				
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	GC-ECD	EU agreed (Tribolet R., 1999)
	ILV	-	-	-
	Confirmatory	0.05 µg/L	HPLC-UV	DAR, 2006
Surface water	Primary	0.1 µg/L	GC-ECD	EU agreed (Tribolet R., 1999)

Component of residue definition: Difenoconazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
	Confirmatory	0.05 µg/L	HPLC-UV	DAR, 2006

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

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 Difenoconazole in air is given in the following tables.

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

Component of residue definition: Difenoconazole			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.99 µg/m^3	LC-MS/MS	EU agreed (Tummon O.J., 2004)
Confirmatory	-	-	-

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

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

Not required as the Difenoconazole is not classified as toxic or very toxic.

zRMS: An analytical method for difenoconazole in body fluids is missing but required according to Regulation (EC) No 283/2013. This is a **data gap**.

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	Pokrzywnicka S.	2017	Boscalid 23.3% + Difenoconazole 6.6% SC – Method validation for determination the content of active substances Institute of Industrial Organic Chemistry report No. BA-27/17 GLP, Unpublished	N	Sharda Cropchem Limited
KCP 5.1.2	E. Nowakowska-Bogdan	2020	Boscalid 23.3 % + Difenoconazole 6.6 % SC – Analysis of relevant impurity content of initial preparation and preparation after accelerated procedure. Łukasiewicz Research Network, Report No. 112/2020 GLP, Unpublished	N	Sharda Cropchem Limited

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 Boscalid

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 2.1.3 Other Studies/ Information

No new or additional studies have been submitted

A 2.2 Analytical methods for Difenoconazole

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 2.2.3 Other Studies/ Information

No new or additional studies have been submitted