REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: GLOB182F

Product name(s): SURRENDER

Chemical active substance:

Fludioxonil, 100 g/L

Interzonal

Zonal Rapporteur Member State: PL

CORE ASSESSMENT

Applicant: Globachem NV

Submission date: January 2021

MS Finalisation date: October 2021 (initial Core Assessment)

March 2022 (final Core Assessment)

Version history

When	What
01/2021	Initial dRR - Globachem NV.
09/2021	Dossier updated on request of izRMS.
10/2021	Initial izRMS assessment. The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the izRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through and shaded for transparency~~.
03/2022	Final report (Core Assessment updated following the commenting period) Additional information/assessments included by the izRMS in the report in response to comments recieved from the cMS and the Applicant are highlighted in yellow. Information no longer relevant ~~is struck through and shaded~~.

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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 substance in the plant protection product.
Noticed data gaps are: none

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

- New method for the determination of residues of fludioxonil in animal matrices and ILV should be provided for the present product registration – post registration requirement.

- ILV for the method for the determination of residues of fludioxonil in high water content matrices should be provided at the renewal of fludioxonil.
- Complete demonstrations of the extraction efficiencies should be provided at the renewal of fludioxonil.
- An ILV of the method of determination of fludioxonil in drinking water should be provided at the renewal of fludioxonil.
- A method for determination of fludioxonil in body fluids and tissues should be provided at the renewal of fludioxonil.

Commodity/crop	Supported/ Not supported
Maize	Supported
Sweet corn	Supported
Sunflower	Supported

zRMS conclusions:

Fludioxonil
In EFSA Scientific Report (2007) 110, 1-85, Conclusion on the peer review of fludioxonil it is stated that *"Adequate analytical methods are available for the determination of fludioxonil residues in food of plant origin (grapes and wheat), soil, water, air. Recently submitted studies, regarding the validation of multi-residue method DFG S19 as the enforcement method for the determination of residues of fludioxonil in different plant matrices with LC-MS/MS detection and the independent laboratory validation of the DFG S19 method for the determination of residues of fludioxonil in plant matrices were summarised and accepted by the RMS in an addendum to the DAR (October 2006, B.5) and discussed in the PRAPeR 06 expert meeting.*
A confirmatory method for the determination of residues in soil by LC-MS/MS has also been evaluated by the RMS and discussed in the PRAPeR 06 expert meeting.
An analytical method for food of animal origin is not required due to the fact that no residue definition is proposed. Analytical methods for the determination of residues in body fluids and tissues are not required."

Residue definitions
Soil
Definitions for risk assessment: fludioxonil and soil photolysis metabolites CGA 265378, CGA 192155
Definitions for monitoring: fludioxonil
Water
Ground water
Definitions for exposure assessment: fludioxonil and soil photolysis metabolites CGA 265378, CGA 339833, CGA 192155
Definitions for monitoring: fludioxonil.
Based on the available information: CGA 339833 and CGA 192155 (to be confirmed by new modelling)
Surface water
Definitions for risk assessment: fludioxonil, CGA 192155, CGA 265378 and aqueous photolysis metabolite CGA 339833

Definitions for monitoring: fludioxonil
Air
Definitions for risk assessment: fludioxonil
Definitions for monitoring: fludioxonil
Food of plant origin
Definitions for risk assessment: Sum of fludioxonil and all metabolites containing the 2,2-difluorobenzo[1,3]dioxole-4-carboxylic moiety
Definitions for monitoring: Fludioxonil
Food of animal origin
Definitions for risk assessment: Not required (In case of use extension leading to significant livestock exposure, sum of fludioxonil and all metabolites containing the 2,2-difluoro-benzo[1,3]dioxole-4-carboxylic moiety)
Definitions for monitoring: Not required (In case of use extension leading to significant livestock exposure, sum of fludioxonil and all metabolites containing the 2,2-difluoro-benzo[1,3]dioxole-4-carboxylic moiety).

Monitoring/Enforcement methods

Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)	Method DFG-S19 (multi residue method), LC-MS/MS LOQ: 0.01 mg/kg (grapes and wheat grain)
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)	No analytical method is required, since no MRL are proposed.
Soil (analytical technique and LOQ)	HPLC-UV 0.02 mg/kg HPLC-MS-MS 0.01 mg/kg
Water (analytical technique and LOQ)	HPLC-UV 0.05 µg/L (drinking water) HPLC-UV 0.1 µg/L (drinking water)
Air (analytical technique and LOQ)	HPLC-UV 2 µg/m³
Body fluids and tissues (analytical technique and LOQ)	Not required [substance is not classified as toxic (T) or very toxic (T+)]

According to the EFSA Journal 2011;9(8):2335 the relevant residue for enforcement is proposed as parent fludioxonil. For risk assessment, the residue was defined as the sum of fludioxonil and its metabolites oxidized to metabolite 2,2-difluoro-benzo[1,3]dioxole-4 carboxylic acid, expressed as fludioxonil. Validated analytical methods for enforcement of the residue definition in foods of plant origin are available with a LOQ of 0.01 mg/kg in high water content, high oil content, acidic and dry commodities.
During the peer review under Directive 91/414/EEC, an analytical method using HPLC-UV, confirmed by the use of an alternative column in the HPLC system, and its ILV were evaluated and validated for the determination of the sum of fludioxonil and its metabolites that can be oxidised to metabolite CGA 19215513, expressed as fludioxonil, with a LOQ of 0.01 mg/kg in milk and meat and a LOQ of 0.05 mg/kg in liver, kidney, fat and eggs (FAO, 2004; Denmark, 2005). However, as the method is very complex, involving a laborious extraction method, the development of a more efficient method is still desirable.

In EFSA Journal 2019;17(8):5812 it is stated that "*In the framework of the MRL review, a possible simplification of the enforcement residue definition for certain animal products (muscle, fat and liver) was discussed. EFSA noted that a livestock feeding study would be required where fludioxonil and metabolites containing the 2,2-difluorobenzo[1,3]dioxole-4 carboxylic moiety are reported separately (EFSA, 2011). Since the new feeding study used the common moiety method (see Section 2.3), the residue definitions for enforcement and risk assessment set during the MRL review are still valid.*
Comparing the residue definition recommended by EFSA in the MRL review with the residue definition for enforcement established in Regulation (EC) No 396/2005, EFSA noted an inaccuracy, which should be corrected when the MRL regulation is updated, following the current assessment:
• Current residue definition established in Regulation (EC) No 396/2005 (applicable to animal products, except honey): sum of fludioxonil and its metabolites oxidized to metabolite 2,2-difluoro-benzo[1,3]dioxole-4 carboxylic acid
• Residue definition recommended by EFSA (2011): sum of fludioxonil and its metabolites oxidized to metabolite 2,2-difluoro-benzo[1,3]dioxole-4 carboxylic acid (CGA 192155), expressed as fludioxonil."

According to the current Regulation (EU) 2021/~~1098~~ 1807 the residue definition for animal products, except honey is established as sum of fludioxonil and its metabolites oxidized to metabolite 2,2-difluoro-benzo[1,3]dioxole-4 carboxylic acid, expressed as fludioxonil.

Note:
The active substance fludioxonil were evaluated on EU level according to the old data requirements. Now not Commission Regulation (EU) 545/2011 but the Commission Regulation (EU) No 284/2013 is applicable. Therefore,

general:
- an independent laboratory validation (ILV) for the method for the determination of residues of fludioxonil in drinking water is missing;
- an analytical method for the residues of fludioxonil in body fluids and tissues is required.
Additionally zRMS comments in individual points are presented in grey commenting boxes.

Conclusions:
1. The current values of MRL for eggs and fat for fludioxonil were decreased and equal 0.02 mg/kg for eggs and 0.01 mg/kg for fat (Reg. (EU) 2021/~~1098~~ 1807). The LOQ of the analytical method AG-616B equals 0.05 mg/kg in eggs and fat, so this method is not appropriate for post-authorization control and monitoring purposes.
Additionally, since HPLC-UV method for the determination of fludioxonil in animal matrices uses a two column switching system, which is considered as not "commonly available", it cannot be validated according to SANCO/825/00/rev.8.1.
Taking the above into account new method for the determination of residues of fludioxonil in animal matrices and ILV should be provided for the present product registration.
Applicants answer:
The applicant likes to mentioned that the dossier of Surrender was submitted well before the entrance into force of Reg. (EU) 2020/1633, amending the MRLs (25 May 2021). In consequence, the applicant should not be forced to comply with this regulation. Additionally, residues in commodities of animal origin after the use of Surrender are not expected (residue trials for the intended uses show a no residue situation and the dietary burden calculations are well below the trigger of 0.004 mg/kg bw/d) and, in any case, a new analytical method for monitoring compliant with the new MRLs is already available to the authorities so they would be capable to analyse Fludioxonil residues in this matrices.
However, the applicant acknowledges this issue and has already contacted several laboratories capable to match the available study. However not all necessary information on the extraction procedures is available (volumes and concentrations of reagents), which is the most important part of the methodology in order to mimic the metabolism data to prove extractability of the analytes, and therefore significant method development work would be required to make sure the method is fit for purpose before the validation can commence. It is also worth noting that, the new method validation guideline that came into force earlier this year (SANTE/2020/12830, Rev.1 Feb 2021) prohibits the use of dichloromethane in new studies and therefore a new extract purification step will also be required.
The applicant is therefore open to submit a new analytical method and ILV for commodities from animal origin, but request to be able to do it as a post-registration requirement.
zRMS-PL agrees with the Applicant's proposal: new method for the determination of residues of fludioxonil in animal matrices and ILV should be provided for the present product registration - post registration requirement.
It should be noted that the final report of new validation method is available and ILV for determination of residues in animal matrices is currently ongoing.

2. zRMS considers that the data requirement for
- ILV for the method for the determination of residues of fludioxonil in high water content matrices,
- complete demonstrations of the extraction efficiencies,
- an ILV of the method of determination of fludioxonil in drinking water,
- a method for determination of fludioxonil in body fluids and tissues

should be provided at the renewal of the active substance.

It should be noted that the report of the primary and confirmatory method for drinking and surface water (the same method is used for both matrices) is expected by the end of May/mid-June 2022. The ILV will start soon after.

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 analytical method has been developed for the determination of the active substance Fludioxonil in GLOB182F.

The following analytical method for the determination of the active substance in the plant protection product GLOB182F has not previously been reviewed according to the Uniform Principles and is provided in support of this assessment.

Comments of zRMS:	The method is considered to be sufficient for determination of the active substance fludioxonil in the plant protection product GLOB182F – the method has been validated in accordance with the SANCO 3030/99 rev 5 guidance.

Reference:	KCP 5.1.1-01
Report	Validation of the methods of determination of Fludioxonil in a FS formulation, in compliance with good laboratory practice. Pomeroy D., 2020, DNA5609.
Guideline(s):	Yes (SANCO/3030/99 rev.5)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The assay of Fludioxonil was performed using approximately 0.1g of formulated material. The mass of the formulation was accurately recorded, transferred to a 50ml volumetric flask and partially made to volume with Acetonitrile. The sample was sonicated for 5 minutes, alowed to cool to room temperature and made up to volume. These samples were then assayed by injecting each solution once into the HPLC-PDA under the following conditions:

HPLC-PDA Conditions:

Instrument:	Shimadzu LC-2030C 3D liquid Chromatograph HPLC-PDA
Mode:	Isocratic Reverse Phase
Column:	Inertsil ODS-3 (250mm X 4.6mm)
Packing:	ODS-3, 5 µm
Eluent:	55% Acetonitrile
	45% Deionised Water adjusted to pH3 with Phosphoric Acid
Wavelength:	254nm
Flow Rate:	1.0 ml/min
Injection Volume:	10µl
Column Temperature:	30°C
Data Collection:	LabSolutions
Retention Time:	Approximately 14.9-15.0 minutes

ULTIVO LC-QQQ Conditions – MS Spectral Analysis:

Instrument:	Agilent ULTIVO LC-QQQ Mass Spectrometer
Mode:	Isocratic Reverse Phase
Column:	Inertsil ODS-3 (250mm X 4.6mm)
Packing:	ODS-3, 5 µm
Eluent:	55% Acetonitrile: 45% Deionised Water adjusted to pH3 with Formic Acid
Flow Rate:	1.0 ml/min
Injection Volume:	10 µl
Column Temperature:	30°C
Retention Time:	Approximately 10.4 minutes
Data Acquisition:	MassHunter

Ionisation:	Negative	Sheath Gas Temperature:	250°C

Gas Temperature:	150°C	Sheath Gas flow:	8L/min
Gas flow:	6L/min	Capillary:	3500V
Nebulizer:	30psi	Nozzle Voltage:	2000V

MRM Precursor Ion (m/z)	MRM Product Ion (m/z)	Dwell Time (ms)	Fragmentor (V)	Collision Energy (V)
247	180	100	146	32
247	169	100	146	36
247	152	100	146	48
247	126	100	146	36

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances Fludioxonil in plant protection product GLOB182F

	Fludioxonil
Author(s), year	Pomeroy D., 2020
Principle of method	HPLC-PDA
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Determined from twenty injections of ten levels of standard ranging from a blank to 1.0 mg/mL. The method is linear with a correlation coefficient of 0.9999.
Precision – Repeatability Mean n = 6 (%RSD)	The method is repeatable with a sample precision ranging from 98.48 g/L to 101.8 g/L, a mean of 99.94 g/L, a standard deviation of 1.287 and a percentage relative standard deviation of 1.287%. Hr=0.679 Hr<≤1
Accuracy n = 6 (% Recovery)	The method is accurate with values of percentage recovery ranging from 100.0% to 101.2%, a mean of 100.8% and a standard deviation of 0.501. Hr=0.265 Hr<≤1
Interference/ Specificity	The method was found to be specific by comparing a Fludioxonil standard, a sample of GLOB182F, a solvent blank and the formulation blank. UV Spectral analysis The Fludioxonil reference standard gave a peak at 14.9 minutes with a spectral maxima at 215nm, a secondary maxima at 265nm, reducing to extinction by 300nm. The sample of GLOB182F formulation produced also gave a peak at 14.9 minutes with a spectral maxima at 215nm, a secondary maxima at 265nm, reducing to reducing to extinction by 300nm, in a similar manner to the Fludioxonil reference standard. There were no other peaks present in these chromatograms at the same elution time as Fludioxonil. Tjhis demonstrates that there were no analyte interferences. MS Spectral analysis The Fludioxonil reference standard gave a peak at 10.4 minutes showing the molecular ion of $[M-H]^-$ at 247.0m/z, with fragment ions present at 180.0m/z, 169.0m/z, 152.0m/z and 126.0m/z. The sample of GLOB182F formulation gave a peak at 10.4 minutes showing the molecular ion of $[M-H]^-$ at 247.0m/z, with fragment ions present at 180.0m/z, 169.0m/z, 152.0m/z and 126.0m/z in a similar manner to the Fludioxonil reference standard. This shows that the method is shown to be specific for Fludioxonil.
Comment	/

Conclusion

The analytical method is suitable for the specific and accurate determination of Fludioxonil in GLOB182F, with acceptable accuracy and precision. The validation complies with the criteria of SANCO/3030/99 rev. 5 guidelines.

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

There are no relevant impurities which are of toxicological, ecotoxicological and/or environmental concern.

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

Under current EU legislation methods on formulants are not required however if a formulant is defined as relevant for toxicity (environment, health) then a method needs to be provided. There are however no formulants in GLOB182F that are defined as relevant for toxicity.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There are no CIPAC methods available for the determination of Fludioxonil.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of Fludioxonil for the generation of pre-authorization data is given in the following table.

Table 5.2-2: Validated methods for the generation of pre-authorization data

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Component of residue definition: Fludioxonil				
Food/feed of plant origin (Residues)	Primary	0.02 mg/kg (strawberries, grapes, apples, wheat grain) 0.05 mg/kg (wine)	HPLC-UV	DK, 2006
		0.01 mg/kg (avocados, kiwi, citrus, wheat)	DFG-S19 multi residue method using HPLC-MS/MS	EFSA, 2007 EFSA, 2011
	ILV	0.01 mg/kg (avocados, kiwi, citrus, wheat)	DFG-S19 multi residue method using HPLC-MS/MS	EFSA, 2007 EFSA, 2011
	Confirmatory	Not required		
Component of residue definition: Sum of Fludioxonil and its metabolites, which can be oxidised to metabolite CGA192155 (2,2-difluro-benzo[1,3]dioxole-4-carboxylic acid)				
Food/feed animal origin (Residues)	Primary	0.01 mg/kg (milk, meat) 0.05 mg/kg (liver, kidney, fat, eggs)	HPLC-UV	DK, 2006 EFSA, 2011
	ILV	0.01 mg/kg (milk, meat) 0.05 mg/kg (liver, kidney, fat, eggs)	HPLC-UV	DK, 2006 EFSA, 2011
	Confirmatory	0.01 mg/kg (milk, meat) 0.05 mg/kg (liver, kidney, fat, eggs)	HPLC-UV	DK, 2006 EFSA, 2011

Component of residue definition: Fludioxonil				
Soil (Environmental fate, Efficacy, Ecotoxicology)	Primary	0.02 mg/kg	HPLC-UV	DK, 2006 EFSA, 2007
		0.01 mg/kg	HPLC-MS-MS	EFSA, 2007
	Confirmatory	0.02 mg/kg	HPLC-UV, GC-MS	DK, 2006
Water (Environmental fate, Efficacy, Ecotoxicology)	Primary	0.05 µg/L (drinking water) 0.1 µg/L (surface water)	HPLC-UV	DK, 2006 EFSA, 2007
	Confirmatory	0.05 µg/L (drinking water) 0.1 µg/L (surface water)	HPLC-UV	DK, 2006
Air (Exposure)	Primary	2 µg/m^3	HPLC-UV	DK, 2006 EFSA, 2007
	Confirmatory	2 µg/m^3	HPLC-UV	DK, 2006
Body fluids and tissues (Exposure)	Primary	Not required		DK, 2006 EFSA, 2007
	Confirmatory	Not required		

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 Fludioxonil (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	Fludioxonil	0.01 mg/kg	EFSA, 2007 Reg. (EU) 2021/~~1098~~1807
Plant, high acid content		0.01 mg/kg	EFSA, 2007 Reg. (EU) 2021/~~1098~~1807
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	EFSA, 2007 Reg. (EU) 2021/~~1098~~1807
Plant, high oil content		0.01 mg/kg	EFSA, 2007 Reg. (EU) 2021/~~1098~~1807
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	~~EU MRL (Reg. (EU) 2016/1902)~~ Reg. (EU) 2021/~~1098~~1807
Muscle	Sum of Fludioxonil and its metabolites, which can be oxidised to metabolite	0.01 mg/kg	DK, 2006 Reg. (EU) 2021/~~1098~~1807
Milk		~~0.01 mg/kg~~	DK, 2006

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
	CGA192155	0.04 mg/kg	Reg. (EU) 2021/~~1098~~ 1807
Eggs		~~0.05 mg/kg~~ 0.02 mg/kg	DK, 2006 Reg. (EU) 2021/~~1098~~ 1807
Fat		~~0.05 mg/kg~~ 0.01 mg/kg	DK, 2006 Reg. (EU) 2021/~~1098~~ 1807
Liver, kidney		~~0.05 mg/kg~~ 0.1 mg/kg	DK, 2006 Reg. (EU) 2021/~~1098~~ 1807
Soil (Ecotoxicology)	Fludioxonil	0.01 mg/kg	EFSA, 2007
Drinking water (Human toxicology)	Fludioxonil	0.05 µg/L	EFSA, 2007
Surface water (Ecotoxicology)	Fludioxonil	0.1 µg/L	EFSA, 2007
Air	Fludioxonil	2 µg/m^3	EFSA, 2007
Tissue (meat or liver)	Fludioxonil	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 Fludioxonil 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: Fludioxonil				
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	HPLC-UV	DK, 2006
	ILV	/	/	missing
	Confirmatory	/	/	
High acid content	Primary	0.02 mg/kg	HPLC-UV	DK, 2006
		0.01 mg/kg	DFG-S19 multi residue method using HPLC-MS/MS	EFSA, 2007 EFSA, 2011
	ILV	0.01 mg/kg	DFG-S19 multi residue method using HPLC-MS/MS	EFSA, 2007 EFSA, 2011
	Confirmatory	/	/	
High oil content	Primary	0.01 mg/kg	DFG-S19 multi residue method using HPLC-MS/MS	EFSA, 2007 EFSA, 2011
	ILV	0.01 mg/kg	DFG-S19 multi residue method using HPLC-MS/MS	EFSA, 2007 EFSA, 2011
	Confirmatory	/	/	
High protein/high starch content (dry)	Primary	0.02 mg/kg	HPLC-UV	DK, 2006
		0.01 mg/kg	DFG-S19 multi residue method using HPLC-MS/MS	EFSA, 2007 EFSA, 2011
	ILV	0.01 mg/kg	DFG-S19 multi residue method using HPLC-MS/MS	EFSA, 2007 EFSA, 2011

Component of residue definition: Fludioxonil				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	Confirmatory	/	/	
Difficult	Primary	No intended use		
	ILV			
	Confirmatory			

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	DK, 2006
Not required, because:	/

The extraction method used in the analytical method for food and feed of plant origin is the same as the one used in the storage stability studies. The extraction efficiency does not need to be proven again.

zRMS comments:
1. According to the Commission Regulation (EU) No 283/2013 an independent laboratory validation (ILV) for the HPLC-UV method for high water content matrix is required.
2. According to the EFSA Journal 2011;9(8):2335: "*The multi-residue QuEChERS method using HPLC-MS/MS described in the European Standard EN 15662:2008 validated with a LOQ of 0.01 mg/kg for the determination of residues in high water content and acidic commodities is also applicable. Hence, according to the peer review and the CEN, it is concluded that parent fludioxonil can be enforced in food of plant origin with a LOQ of 0.01 mg/kg in high water content, high oil content, acidic and dry commodities.*"
3. According to the EFSA Journal 2020;18(1):5994: *"Fully validated multiresidue DFG S19 and QuEChERS methods in combination with high performance liquid chromatography with tandem mass spectrometry (HPLC–MS/MS) are available for the analysis of fludioxonil; in high water-, high acid-, high oil content and in dry commodities the LOQ was 0.01 mg/kg (EFSA, 2007, 2011).*
For oilseeds, adequate analytical methods for monitoring of residues are available."
4. In our opinion complete demonstrations of the extraction efficiencies should be provided at the renewal of fludioxonil.

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 Fludioxonil 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: Sum of Fludioxonil and its metabolites, which can be oxidised to metabolite CGA192155 (2,2-difluro-benzo[1,3]dioxole-4-carboxylic acid)				
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	DK, 2006 EFSA, 2011
	ILV	0.01 mg/kg	HPLC-UV	DK, 2006 EFSA, 2011
	Confirmatory	0.01 mg/kg	HPLC-UV	DK, 2006 EFSA, 2011
Eggs	Primary	0.05 mg/kg	HPLC-UV	DK, 2006 EFSA, 2011

Component of residue definition: Sum of Fludioxonil and its metabolites, which can be oxidised to metabolite CGA192155 (2,2-difluro-benzo[1,3]dioxole-4-carboxylic acid)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
	ILV	0.05 mg/kg	HPLC-UV	DK, 2006 EFSA, 2011
	Confirmatory	0.05 mg/kg	HPLC-UV	DK, 2006 EFSA, 2011
Muscle	Primary	0.01 mg/kg	HPLC-UV	DK, 2006 EFSA, 2011
	ILV	0.01 mg/kg	HPLC-UV	DK, 2006 EFSA, 2011
	Confirmatory	0.01 mg/kg	HPLC-UV	DK, 2006 EFSA, 2011
Fat	Primary	0.05 mg/kg	HPLC-UV	DK, 2006 EFSA, 2011
	ILV	0.05 mg/kg	HPLC-UV	DK, 2006 EFSA, 2011
	Confirmatory	0.05 mg/kg	HPLC-UV	DK, 2006 EFSA, 2011
Kidney, liver	Primary	0.05 mg/kg	HPLC-UV	DK, 2006 EFSA, 2011
	ILV	0.05 mg/kg	HPLC-UV	DK, 2006 EFSA, 2011
	Confirmatory	0.05 mg/kg	HPLC-UV	DK, 2006 EFSA, 2011

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	DK, 2006
Not required, because:	/

The extraction method used in the analytical method for food and feed of animal origin is the same as the one used in the storage stability study. The extraction efficiency does not need to be proven again.

zRMS comments:

In the EFSA Journal 2011;9(8):2335 it is stated that *during the peer review under Directive 91/414/EEC, an analytical method using HPLC-UV, confirmed by the use of an alternative column in the HPLC system, and its ILV were evaluated and validated for the determination of the sum of fludioxonil and its metabolites that can be oxidised to metabolite CGA 19215513, expressed as fludioxonil, with a LOQ of 0.01 mg/kg in milk and meat and a LOQ of 0.05 mg/kg in liver, kidney, fat and eggs (FAO, 2004; Denmark, 2005). However, as the method is very complex, involving a laborious extraction method, the development of a more efficient method is still desirable.*

Component of residue definition: sum of fludioxonil and its metabolites that can be oxidised to metabolite CGA 192155, expressed as fludioxonil				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk, Eggs, Muscle,	Primary	0.01 mg/kg for milk and meat 0.05 mg/kg for	HPLC-UV	AG-616B, Vienneau, K. P., 1996, EU agreed, Denmark, 2005, EFSA Journal 2011; 9(8):2335

Fat, Kidney, liver		liver, kidney, fat and eggs		
	ILV	0.01 mg/kg for milk 0.05 mg/kg for liver and eggs	HPLC-UV	AG-616B ILV, Tang, J. and Baldi, B. G., 1996, EU agreed, Denmark, 2005, EFSA Journal 2011; 9(8):2335

1. The analytical method AG-616B (Vienneau K.P., 1996) using HPLC/UV has been evaluated in DAR of Fludioxonil (2005) for the determination of fludioxonil and metabolites as CGA192155 in foodstuff of animal origin with a LOQ of 0.01 mg/kg in muscle and milk and 0.05 mg/kg in eggs, fat, kidney and liver. Determination was performed by column switching reversed-phase HPLC with UV detection. Residues were expressed as fludioxonil equivalents. An alternative column in the HPLC system was used as confirmatory method.
The method was independently validated (Tang J., Baldi B., 1996) using HPLC/UV. The LOQ equals 0.01 mg/kg in milk and 0.05 mg/kg in eggs and liver.

The current values of MRL for eggs and fat for fludioxonil were decreased and equal 0.02 mg/kg for eggs and 0.01 mg/kg for fat (Reg. (EU) 2021/1098 1807). The LOQ of the analytical method AG-616B equals 0.05 mg/kg in eggs and fat, so this method is not appropriate for post-authorization control and monitoring purposes.
Additionally, in our opinion since HPLC-UV method for the determination of fludioxonil in animal matrices uses a two column switching system, which is considered as not "commonly available", it cannot be validated according to SANCO/825/00/rev.8.1.

Taking above into account a new method and ILV should be provided by Applicant.

2. In our opinion complete demonstrations of the extraction efficiencies should be provided at the renewal of fludioxonil.

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 Fludioxonil in soil is given in the following tables.

Table 5.3-6: Validated methods for soil

Component of residue definition: Fludioxonil			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.02 mg/kg	HPLC-UV	DK, 2006 EFSA, 2007
	0.01 mg/kg	HPLC-MS-MS	EFSA, 2007
Confirmatory	0.02 mg/kg	HPLC-UV, GC-MS	DK, 2006

zRMS comments:

The analytical method Tribolet R., 2001 and Mair P., 1994 using HPLC/UV has been evaluated in DAR of Fludioxonil (2005) for the determination of fludioxonil in soil with a LOQ of 0.02 mg/kg. No confirmatory method was provided. Determination was by normal-phase HPLC using a two column switching system.
In our opinion since HPLC-UV method for the determination of fludioxonil in soil uses a two column switching system, which is considered as not "commonly available", it cannot be validated according to SANCO/825/00/rev.8.1. However, this method can be considered acceptable because it is an agreed EU method in the DAR (2005) and because the renewal for fludioxonil has not yet been finalized.

Taking above into account a new method should be provided at the renewal of fludioxonil.

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 Fludioxonil 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: Fludioxonil				
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-UV	DK, 2006 EFSA, 2007
	ILV	/	/	missing
	Confirmatory	0.05 µg/L	HPLC-UV	DK, 2006 EFSA, 2007
Surface water	Primary	0.1 µg/L	HPLC-UV	DK, 2006 EFSA, 2007
	Confirmatory	0.1 µg/L	HPLC-UV	DK, 2006 EFSA, 2007

zRMS comments:

1. According to the Commission Regulation (EU) No 283/2013 an independent laboratory validation (ILV) for the HPLC-UV method for drinking water is required and should be provided at the renewal of fludioxonil.

2. The analytical method (Tribolet R., 1999) using HPLC/UV has been evaluated in DAR of Fludioxonil (2005) for the determination of fludioxonil in drinking and surface water with LOQ of 0.05 µg/L and 0.10 µg/L respectively.
Proposed HPLC-UV method for the determination of fludioxonil in water uses a two column switching system, which is not considered acceptable anymore.
However, this method can be considered acceptable because it is an agreed EU method in the DAR (2005) and because the renewal for fludioxonil has not yet been finalized.
Taking above into account a new method should be provided at the renewal of fludioxonil.

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 Fludioxonil in air is given in the following tables.

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

Component of residue definition: Fludioxonil			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	2 µg/m^3	HPLC-UV	DK, 2006 EFSA, 2007
Confirmatory	2 µg/m^3	HPLC-UV	DK, 2006

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

Not required as Fludioxonil is not classified as toxic or acutely toxic.

zRMS comments:

According to the "EFSA Scientific Report (2007) 110, 1-85, Conclusion on the peer review of fludioxonil" a method of analysis for body fluids and tissues is not required as the active substance is not proposed for classification as toxic (T) or very toxic (T+).
However in Commission Regulation (EU) No 283/2013 it is stated that "…*methods, with a full description, shall be submitted for the analysis in body fluids and tissues for active substance and relevant metabolites*".

In our opinion the method for determination of fludioxonil in body fluids and tissues is required and should be provided at the renewal of fludioxonil.

5.3.2.8 Other studies/ information

In several ecotoxicological studies summarized in section B9 of the dRR (toxicity to aquatic organisms and honeybees), analytical methods were used for the detection of the active substance Fludioxonil in the different test mediums. The analytical part of these studies is summarized in Appendix 2.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
KCP 5.1.1	Pomeroy, D.	2020	Validation of the methods of determination of Fludioxonil in a FS formulation, in compliance with good laboratory practice. DNA5609 David Norris Analytical Laboratories Ltd. GLP Unpublished	N	Globachem NV
KCP 5.2 **(submitted as KCP 2.10.1-2.10.2)**	De Vos P.	2021a	Fludioxonil 100 FS. Adhesion to and distribution on treated maize and sunflower seeds. Laboratory: CRA-W – Centre wallon de Recherches agronomiques Study number: 25152 GLP Unpublished	N	Globachem NV
KCP 5.2 **(submitted as KCP 2.11)**	De Vos P.	2021b	Fludioxonil 100 FS. Residues in dust of on treated maize and sunflower seeds. Laboratory: CRA-W – Centre wallon de Recherches agronomiques Study number: 25155 GLP Unpublished	N	Globachem NV
KCP 5.2 **(submitted as KCP 10.2.1)**	Renner, P.	2021a	Acute toxicity of GLOB182F to *Daphnia magna* in a 48-hour static test. 20 48 ADL 0012 BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 5.2 **(submitted as KCP 10.2.1)**	Renner, P.	2021b	Effects of GLOB182F on *Pseudokirchneriella subcapitata* in an algal growth inhibition test. 20 48 AAL 0015 BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 5.2 **(submitted as KCP 10.3.1.1)**	Amsel, K.	2020	Acute toxicity of Fludioxonil 100 FS to the bumblebee *Bombus terrestris* L. under laboratory conditions, 20 48 BBA 0026. BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP Unpublished	N	Globachem NV
KCP 5.2 **(submitted as KCP 10.3.1.2)**	Deβler, K.	2020	Chronic toxicity of GLOB182F to the honey bee *Apis mellifera* L. under laboratory conditions. 20 48 BAC 0051 BioChem agrar, Labor für biologische und chemische Analytik GmbH GLP	N	Globachem NV

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
			Unpublished		

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
-	-	-	-	-	-

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 Fludioxonil

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 A.2.A.9 Other Studies/ Information

Comments of zRMS:	The method is suitable for determination of the fludioxonil content on maize and sunflower seeds treated with Fludioxonil 100 FS according to the guidance SANCO/3029/99 rev. 4. The study is acceptable.

Reference:	KCP 5.2 (submitted as KCP KCP 2.10.1-2.10.2)
Report	Fludioxonil 100 FS. Adhesion to and distribution on treated maize and sunflower seeds, De Vos P., 2021, report No 25152.
Guideline(s):	SANCO/3029/99 rev. 5 (22/03/2019)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Fludioxonil is extracted from treated seeds with acetonitrile / water (80/20, v/v). The final extract is analysed by Ultra High-Performance Liquid Chromatography with UV-visible Diode Array Detection

(UHPLC-DAD) for determination of fludioxonil using the external standard calibration.

HPLC-MS/MS Conditions:

Instrument:	Waters Acquity UPLC H-Class Bio + PDA or Waters Acquity UPLC + PDA	
Column:	Phenomenex Kinetex XB C18, 100 mm x 4.6 mm i.d., 2.6 µm particle size (or equivalent)	
Eluent:	A: water	
	B: acetonitrile	
Gradient:	0.00 min	50% B
	0.05min	50% B
	2.00 min	95% B
	2.30 min	95% B
	2.50 min	50% B
	3.00 min	50% B
Flow Rate:	1 ml/min	
Injection Volume:	5 µl	
Detection:	266 nm for Fludioxonil	
Retention Time:	±1.9 minutes	

Results and discussions

	Fludioxonil - Maize seeds
Specificity	No interference likely to affect the chromatographic peak of fludioxonil (≤ LOQ)
Linearity	The response of fludioxonil is linear in the range 0.05 - 25 µg / mL. N = 9 r^2 = 1.0000 (0.05 - 25 µg/mL)
Accuracy	Exraction kinetic The fludioxonil concentration after 45, 75 and 90 minutes ultrasonication remains respectively at 98%, 100% and 103% of the concentration after 60 minutes ultrasonication. Recoveries (N = 5) Average loading (Level 5 g / 100 kg seeds): Mean: 99 % Relative Standard Deviation: 1.3 % (Level 0.5 g / 100 kg seeds): Mean: 96 % Relative Standard Deviation: 1.3 % Single seed analysis (Level 5 g / 100 kg seeds): Mean: 97 % Relative Standard Deviation: 1.3 %
Repeatibility	Mean: 4.03 g / 100 kg seeds Relative Standard Deviation: 1.6 % N = 6
LOQ	LOQ = 0.051 g / 100 kg seeds (seed loading) LOQ = 0.146 g / 100 kg seeds (single seed analysis)
	Fludioxonil - Sunflower seeds
Specificity	No interference likely to affect the chromatographic peak of fludioxonil (≤ LOQ)
Linearity	The response of fludioxonil is linear in the range 0.01 - 25 µg / mL. N = 11 R^2 = 1.0000 (0.01 - 25 µg/mL)
Accuracy	Exraction kinetic The fludioxonil concentration after 30, 60 and 75 minutes ultrasonication remains respectively at 96%, 101% and 100% of the concentration after 45 minutes ultrasonication.

	Recoveries (N = 5) Average loading (Level 15 g / 100 kg seeds): Mean: 101 % Relative Standard Deviation: 1.8 % (Level 1.5 g / 100 kg seeds): Mean: 94 % Relative Standard Deviation: 1.9 % Single seed analysis (Level 15 g / 100 kg seeds): Mean: 97 % Relative Standard Deviation: 0.4 %
Repeatibility	Mean: 13.82 g / 100 kg seeds Relative Standard Deviation: 1.0 % N = 6
LOQ	LOQ = 0.010 g / 100 kg seeds (seed loading) LOQ = 0.192g / 100 kg seeds (single seed analysis)

Conclusion: the method is acceptable.

Comments of zRMS:	The method is suitable for determination of residues of the fludioxonil in the dust of seeds treated with Fludioxonil 100 FS according to the guidance SANCO/3029/99 rev. 4. The study is acceptable.

Reference: KCP 5.2 (submitted as KCP KCP 2.11)

Report: Fludioxonil 100 FS. Residues in dust of on treated maize and sunflower seeds., De Vos P., 2021, report No 25155.

Guideline(s): SANCO/3029/99 rev. 5 (22/03/2019)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Fludioxonil is extracted from dust on filter paper with acetonitrile followed by water. The final extract is analysed by Ultra High-Performance Liquid Chromatography with UV-visible Diode Array Detection (UHPLC-DAD) for determination of fludioxonil using the external standard calibration.

HPLC-MS/MS Conditions:

Instrument:	Waters Acquity UPLC H-Class Bio + PDA	
Column:	Phenomenex Kinetex XB C18, 100 mm x 4.6 mm i.d., 2.6 µm particle size (or equivalent)	
Eluent:	A: water B: acetonitrile	
Gradient:	0.00 min	50% B
	0.05min	50% B
	2.00 min	95% B
	2.30 min	95% B
	2.50 min	50% B
	3.00 min	50% B
Flow Rate:	1 ml/min	
Injection Volume:	5 µl	
Detection:	266 nm for Fludioxonil	
Retention Time:	±1.95 minutes	

Results and discussions

<table>
<tr><th></th><th>Fludioxonil</th></tr>
<tr><td>Specificity</td><td>The analysis of blank (method without sample), unloaded filter paper sample (before and after the Heubach test) and filter paper samples loaded with dust of untreated seeds in comparison with the analysis of calibration solutions, spiked filter paper samples and filter paper samples loaded with dust of treated seeds showed the absence of compound interfering with the determination of fludioxonil.</td></tr>
<tr><td>Linearity</td><td>The response of fludioxonil is linear in the range 0.01 - 25 µg / mL.
N = 11
R^2 = > 0.99</td></tr>
<tr><td>Accuracy</td><td>Recovery values were calculated by fortifying unloaded filter paper samples with known amounts of fludioxonil. An unloaded filter paper sample was analysed prior to spiking and showed the absence of compound interfering with the determination of fludioxonil (≤ LOQ).
<table>
<tr><th>Specimen</th><th>Fortification level (µg / filter paper as fludioxonil)</th><th>Recovery (*) (%)</th></tr>
<tr><td rowspan="8">Filter paper</td><td>50.75</td><td>97.9</td></tr>
<tr><td>50.75</td><td>96.8</td></tr>
<tr><td>10.15</td><td>93.8</td></tr>
<tr><td>10.15</td><td>95.1</td></tr>
<tr><td>5.07</td><td>95.0</td></tr>
<tr><td>5.07</td><td>95.9</td></tr>
<tr><td>1.01</td><td>93.1</td></tr>
<tr><td>1.01</td><td>91.2</td></tr>
</table>
The individual recoveries for fludioxonil on filter paper range between 70 - 120 %.</td></tr>
</table>

Conclusion: the method is acceptable.

Comments of zRMS:	Concentrations of fludioxonil were determined in the test solutions of a *Daphnia magna* 48-hour static test. Limit of Quantification: 23.72 µg/L. Validation blank samples had peak areas of less than 30% of the method LOQ. The specificity of the method was assured by MS/MS-detection and absence of interfering peaks. The mean recovery values ranged from 105.0 to 106.0% for fludioxonil. The corresponding RSD values were below 20%. The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met. The study is acceptable.

Reference: KCP 5.2 (submitted as KCP 10.2.1)

Report: Acute toxicity of GLOB182F to *Daphnia magna* in a 48 hour static test, Renner P., 2021, report No 20 48 ADL 0012.

Guideline(s): SANCO/3029/99 rev. 4 (11/07/2000)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

A reversed phase HPLC method with MS/MS-detection for the determination of Fludioxonil in the aquatic test medium was validated according to the guidance document SANCO/3029/99 rev.4 and used for the analytical determination.

HPLC-MS/MS Conditions:

Instrument:	Agilent 1200 HPLC system with a 6460 triple quadrupole mass spectrometric detector
Column:	Ace Excel 3 C18, 3 µm, 100 x 2.1 mm
Eluent:	A: 0.1% formic acid and 5 mM ammonium formate in water B: 0.1% formic acid and 5 mM ammonium formate in methanol
Gradient:	0.00 min 45% B 4.00 min 75% B 6.00 min 100% B 7.00 min Stop 3 min Post time
Flow Rate:	0.350 ml/min
Injection Volume:	5 µl
Detection:	ESI positive, MRM: m/z 229 → 185; m/z 229 → 158
Retention Time:	5.2 minutes

Results and discussions

The method was validated with test medium spiked with test item at 23.72 and 405.5 µg/L Fludioxonil.

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

Matrix	Analyte	Fortification level (µg/L)	Mean recovery (%)	RSD (%)	Comments
Dilution water	Fludioxonil	23.72	106.0	0.41	Mean recovery between 70 and 110%. RSD < 20%
		405.5	105.0	0.94	

Table A 2: Characteristics for the analytical method used for validation of Fludioxonil residues in aquatic test medium

	Fludioxonil
Specificity	The specificty of the method was assured by MS/MS detection and the absence of interfering peaks. blank value < 30 % LOQ
Calibration (type, number of data points)	An external matrix-matched calibration with the analytical reference items including 7 calibration levels, measured in two repetitions each, was performed from 3.510 µg/L (29.62% of the lowest validation concentration) to 48.75 µg/L (120.3% of the highest validation concentration). N = 7
Calibration range	The detector signal for Fludioxonil (transition 229.0->158.0) was linear in the range from 3.51 to 48.75 µg/L. The corresponding calibration range regarding analytical dilution (DFanalytical = 2 for lower calibration limit and DFanalytical = 10 for upper calibration limit) was from 7.020 µg/L to 376.1 µg/L. r^2 = 0.9994.
Assessment of matrix effects is presented	Matrix effects were not assessed but a 7-point matrix-matched calibration was performed to account for possible matrix effects.
Limit of determination/quantification	The limit of quantification (LOQ) was defined as the lowest successfully validated fortification level. LOQ = 23.72 µg/L Fludioxonil

Conclusion

The method given above is suitable for the determination of Fludioxonil in the aquatic test medium as the following criteria are fulfilled:
- blank values do not exceed 30% of the lowest validated concentration.
- mean recoveries for each level are in the range 70 – 110%.
- the RSD is < 20%.

Comments of zRMS:	Concentrations of fludioxonil were determined in the test solutions on *Pseudokirchneriella subcapitata* in an algal growth inhibition test . Limit of Quantification: 6.159 µg/L. Validation blank samples had peak areas of less than 30% of the method LOQ. The specificity of the method was assured by MS/MS-detection and absence of interfering peaks. The mean recovery values ranged from 102.7 to 104.0% for fludioxonil. The corresponding RSD values were below 20%. The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met. The study is acceptable.

Reference:	KCP 5.2 (submitted as KCP 10.2.1)
Report	Effects of GLOB182F on *Pseudokirchneriella subcapitata* in an algal growth inhibition test, Renner P., 2021, report No 20 48 AAL 0015.
Guideline(s):	SANCO/3029/99 rev.4 (11/07/2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

A reversed phase HPLC method with MS/MS-detection for the determination of Fludioxonil in the aquatic test medium was validated according to the guidance document SANCO/3029/99 rev.4 and used for the analytical determination.

HPLC-MS/MS Conditions:

Instrument:	Agilent 1200 HPLC system with a 6460 triple quadrupole mass spectrometric detector
Column:	Ace Excel 3 C18, 3 µm, 100 x 2.1 mm
Eluent:	A: 0.1% formic acid and 5 mM ammonium formate in water B: 0.1% formic acid and 5 mM ammonium formate in methanol
Gradient:	0.00 min 45% B 4.00 min 75% B 6.00 min 100% B 7.00 min Stop 3 min Post time
Flow Rate:	0.350 ml/min
Injection Volume:	5 µl
Detection:	ESI positive, MRM: m/z 229 → 185; m/z 229 → 158
Retention Time:	5.2 minutes

Results and discussions

The method was validated with test medium spiked with test item at 6.159 and 993.3 µg/L Fludioxonil.

Table A 3: Recovery results from method validation of Fludioxonil using the analytical method

Matrix	Analyte	Fortification level (µg/L)	Mean recovery (%)	RSD (%)	Comments
Dilution water	Fludioxonil	6.159	102.7	1.18	Mean recovery between 70 and 110%. RSD < 20%
		993.3	104.0	0.97	

Table A 4: Characteristics for the analytical method used for validation of Fludioxonil residues in aquatic test medium

	Fludioxonil
Specificity	The specificty of the method was assured by MS/MS detection and the absence of interfering peaks. blank value < 30 % LOQ
Calibration (type, number of data points)	An external matrix-matched calibration with the analytical reference items including 7 calibration levels, measured in two repetitions each, was performed from 1.820 µg/L (29.55% of the lowest validation concentration) to 30.33 µg/L (122.1% of the highest validation concentration). N = 7
Calibration range	The detector signal for Fludioxonil (transition 229.0->158.0) was linear in the range from 1.820 to 30.33 µg/L. The corresponding calibration range regarding analytical dilution ($DF_{analytical}$ = 1 for lower calibration limit and $DF_{analytical}$ = 40 for upper calibration limit) was from 0.0018 mg/L to 1.213 mg/L. R^2 = 0.9996
Assessment of matrix effects is presented	Matrix effects were not assessed but a 7-point matrix-matched calibration was performed to account for possible matrix effects.
Limit of determination/quantification	The limit of quantification (LOQ) was defined as the lowest successfully validated fortification level. LOQ = 6.159 µg/L Fludioxonil

Conclusion

The method given above is suitable for the determination of Fludioxonil in the aquatic test medium as the following criteria are fulfilled:
- blank values do not exceed 30% of the lowest validated concentration.
- mean recoveries for each level are in the range 70 – 110%.
- the RSD is < 20%.

Comments of zRMS:	Concentrations of fludioxonil were determined in the test item solutions of acute toxicity tests on bumblebees. Limit of Quantification: 1521 µg/L fludioxonil for the contact toxicity test and 961 µg/L fludioxonil for the oral toxicity test. No fludioxonil was detected in the control samples. The recoveries of fludioxonil were between 84.4-111% for the contact toxicity test and 90.8 – 99.3% for the oral toxicity test. The mean recoveries for each level were in the range 70-110%. The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met. The study is acceptable.

Reference: KCP 5.2 (submitted as KCP 10.3.1.1)

Report: Acute toxicity of Fludioxonil 100 FS to the bumblebee *Bombus terrestris* L. under laboratory conditions, Amsel K., 2020, report No. 20 48 BBA 0026.

Guideline(s): SANCO/3029/99 rev.4 (11/07/2000)

Deviations: No

GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

The purpose of the analytical phase of the study was the verification of the concentration of fludioxonil in test item solutions of acute toxicity tests on bumblebees. The determination was conducted by an in-house developed method using reversed phase high performance liquid chromatography (RP-HPLC) with mass-spectrometric (MS-MS) detection.

The analytical method was validated according to SANCO/3029/99 rev. 4.

HPLC Conditions:

Instrument:	Agilent 1200 HPLC system with a 6460 triple quadrupole mass spectrometric detector
Column:	ACE Excel 3 C18, 2.1 x 100 mm, 3 µm
Eluent:	A: Water with 0.1% (v/v) formic acid, 5mM ammonium formate. B: Methanol with 0.1% (v/v) formic acid
Gradient:	0.00 min 45% B 4.00 min 75% B 6.00 min 100% B 7.00 min Stop
Injection volume:	5 µl
Flow Rate:	0.350 ml/min
Detection:	Retention time 5.2-5.3 min ESI positive, MRM Fludioxonil: m/z 229 → 158 (Quantifier) 229 → 185 (Qualifier)

Results and discussions

The method was validated with test item diluted with each test matrix. For the contact toxicity test the method was validated at 48.0% of the lowest nominal test concentration and at 130% of the highest nominal test concentration (contact toxicity test: 1521 to 66132 mg/L for fludioxonil). For oral toxicity test the method was validated at 47.9% of the lowest nominal test concentration and at 132% of the highest nominal concentration (oral toxicity test: 961 to 13353 mg/L for fludioxonil).

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

Matrix	Analyte	Fortification level (mg/L)	Mean recovery (%)	RSD (%)	Comments
Contact test: Water + 0.5% (v/v) Triton X	Fludioxonil	66132	107	0.412	Mean recovery between 70 and 110%. RSD < 20%
		1521	106	1.29	
Oral test: Sucrose solution (50%, w/v)		13353	97.1	2.28	
		961	97.0	3.61	

Table A 6: Characteristics for the analytical method used for validation of Fludioxonil residues in OECD medium

	Fludioxonil
Specificity	Validation blank samples had peak areas of less than 30% of the lowest validated concentration. No interfering peaks were detected.
Calibration (type, number of data points)	An external calibration with the analytical reference item was performed from 20% of the lowest validation measuring concentration to 133% of the highest validation measuring concentration for the contact toxicity test (14.89 to 331 µg/L of fludioxonil), and from 18% of the lowest validation measuring concentration to 153% of the highest validation measuring concentration for the oral toxicity test 15.35 to 409 µg/L of fludioxonil). N = 6
Calibration range	The calibration functions for fludioxonil were quadratic in the range of 14.9 to 332 µg/L for contact and for oral in the range of 15.3 to 409 µg/L. A correlation coefficient of > 0.99 was obtained
Assessment of matrix effects is presented	Matrix effects for the contact test were not taken into account since the samples of the biological study as well as the validation samples were diluted with a dilution factor of at least 20833. For the oral test a matix matched calibration were used.
Limit of determination/quantification	Limit of quantification representing the lowest validated level with sufficient recovery and precision. LOQ = 1521 µg/L fludioxonil for the contact toxicity test. LOQ = 961 µg/L fludioxonil for the oral toxicity test.

Conclusion

The method given above is suitable for the determination of Fludioxonil in sucrose medium as the following criteria are fulfilled:
- blank values do not exceed 30% of the lowest validated concentration.
- mean recoveries for each level are in the range 70 – 110%.
- the RSD is < 20% per level.

Comments of zRMS:	Concentrations of fludioxonil were determined in feeding solutions of a chronic toxicity test on honey bees (sample matrix: sucrose solution containing 50% (w/v) sucrose +0.1% (w/v) xanthan). Limit of Quantification: 160 mg/kg fludioxonil in sample matrix sucrose solution containing 50% (w/v) sucrose with 0.1% (w/v) xanthan) corresponding to 95.9 µg/L in diluted extracts. In the control specimens, the concentrations of the active ingredient were below 30% of LOQ. The recoveries of fludioxonil were between 92.8% and 102% with RSD<20%. The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met. The study is acceptable.

Reference:	KCP 5.2 (submitted as KCP 10.3.1.2)
Report	Chronic toxicity of GLOB182F to the honey bee *Apis mellifera* L. under laboratory conditions, Deβler K., 2020, report No 20 48 BAC 0051.
Guideline(s):	SANCO/3029/99 rev. 4 (11/07/2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

An in-house developed HPLC method using reversed phase HPLC (RP-HPLC) with mass-spectrometric (MS/MS) detection was used for the determination of Fludioxonil in the test medium and was validated according to SANCO/3029/99 rev. 4.

HPLC Conditions:

Instrument:	Agilent 1200 HPLC system with a 6460 triple quadrupole mass spectrometric detector
Column:	ACE Excel C18, 2.1mm x 150 mm, 5 µm
Eluent:	A: Water with 0.1% (v/v) formic acid, 5mM ammonium formate. B: Methanol with 0.1% (v/v) formic acid
Gradient:	0.00 min 45% B 4.00 min 75% B 6.00 min 100% B 7.00 min Stop
Injection volume:	5 µl
Flow Rate:	0.4 ml/min
Detection:	Retention time 5.6 min for Fludioxonil ESI positive, MRM Fludioxonil: m/z 229 → 158 (Quatifier, used for calculation). 229 → 185 (Qualifier, monitored, but not reported)

Results and discussions

The method was validated with test matrix spiked with test item at 49% of the lowest nominal test concentration (160 mg/kg of fludioxonil) and at 136% of the highest nominal test concentration (7013 mg/kg of fludioxonil).

Table A 9: Recovery results from method validation of Fludioxonil using the analytical method

Matrix	Analyte	Fortification level (µg/L)	Mean recovery (%)	RSD (%)	Comments
Sucrose solution (50%, w/v) + 0.1% (w/v) xanthan	Fludioxonil	95.9	100	1.8	Mean recovery between 70 and 110%. RSD < 20%
		281	89.1	10.9	

Table A 10: Characteristics for the analytical method used for validation of Fludioxonil residues in OECD medium

	Fludioxonil
Specificity	Validation blank samples had peak areas of less than 30% of the lowest validated concentration. No interfering peaks were detected.
Calibration (type, number of data points)	An external calibration with the analytical reference item was performed from 18% of the lowest validation measuring concentration to 121% of the highest validation measuring concentrations (16.9 to 338 µL of Fludioxonil). N = 6
Calibration range	Calibration range: 16.9 to 338 mg/L Fludioxonil $R^2 > 0.99$
Assessment of matrix effects is presented	Matrix effects were taken into account by addition of the same amount of blank extract to the analysis samples.
Limit of determination/quantification	Limit of quantification representing the lowest validated level with sufficient recovery and precision LOQ = 95.9 µg/L Fludioxonil

Conclusion

The method given above is suitable for the determination of Fludioxonil in sucrose medium as the following criteria are fulfilled:
- blank values do not exceed 30% of the lowest validated concentration.
- mean recoveries for each level are in the range 70 – 110%.
- the RSD is < 20%per level.