REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product name(s): PYRIX 20 SL

Chemical active substance:

PYRIPROXYFEN, 20 g/l

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: AGROFARM SP. Z O.O.

Submission date: November 2025, updated June 2026

MS Finalisation date: July 2026 (initial Core Assessment)

September 2026 (final Core Assessment)

Version history

When	What
November 2025	Initial dRR – Agrofarm Sp. z o.o. Validation of analytical methods
June 2026	Applicant's update points: -5.3.2.3; 5.3.2.4; 5.3.2.5; 5.3.2.6; 5.3.2.7 Update APPENDIX 2 : - points: A 2.1.2.1.1.1a/A 2.1.2.1.1.1b -description validation of the analytical method - Table A1 - Table A2 - points : A2.1.2.2; A2.1.2.3; A2.1.2.4; A2.1.2.5; A2.1.2.6 - insert Table A3
July 2026	Initial zRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency. Following the evaluation and before sending the document for commenting, all coloured highlighting was removed, from the parts updated by the Applicant, for better legibility.
September 2026	Final report (Core Assessment updated following the commenting period). Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency.

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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 ~~and relevant impurities~~ in the plant protection product.

Noticed data gaps are:
The validation of a method for the determination of toluene in plant protection products should be presented. The method must be sufficiently sensitive to determine toluene in accordance with its possible content, calculated on the basis of the known content of the active substance in the PPP.

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

Noticed data gaps are:
~~- an analytical method and its independent laboratory validation (ILV) for the determination of residues of pyriproxyfen in animal matrices are required and should be provided by the Applicant as a post-registration requirement;~~
- an analytical method and its independent laboratory validation (ILV) for the determination of residues of pyriproxyfen in water are required and should be provided by the Applicant as a post-registration requirement;
- an analytical method for the determination of residues of pyriproxyfen in body fluids and tissues is required and should be provided at the renewal of the active substance and/or re-evaluation of plant production product.

Commodity/crop	Supported/Not supported
mushrooms	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 method and possible data gaps for analysis of pyriproxyfen in plant protection product PYRIX 20 SL is provided as follows:

-Analytical method for determining the content of pyriproxyfen in the product Pyrix 20 SL. Agrofarm A310322. (Procedure for determining pyriproxyfen in the product)
author(s) : Agrofarm Sp. z o.o.
Year: March 2022
GLP : YES

-Final report. Validation of analytical method for the determination of active substance (pyriproxyfen) content in the test item PYRIX 20SL
Test item :PYRIX 20 SL
Study code :0012/0003/FA
Author(s) : SORBOLAB Research Laboratory LLC ; May 2025
GLP: YES

Comments of zRMS:	The analytical method for the determination of the active substance pyriproxyfen in Pyrix 20 SL is sufficiently described and validated in accordance with the requirements of SANCO/3030/99 rev. 5.

Reference:	Determination of the pyriproxyfen concentration in product Pyrix 20 SL / Validation of analytical method
Report	-Analytical method for determining the content of pyriproxyfen in the product Pyrix 20 SL. A030625 author(s) : Agrofarm Sp. z o.o. -Final report.Validation of analytical method for the determination of active substance (pyriproxyfen) content in the test item PYRIX 20SL Test item :PYRIX 20 SL Study code :0012/0003/FA Author(s) : SORBOLAB Research Laboratory LLC ; May 2025
Guideline(s):	Yes: Experimental procedure SPB-FA/11 and Guideline SANCO/3030/99, rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
Analytical method for the determination of the active substance pyriproxyfen by HPLC method in the test item PYRIX 20SL (Procedure for determining pyriproxyfen in the product)

1.Reagents and solutions
- pyriproxyfen standard
- acetonitrile HPLC grade
- orthophosphoric acid, min 85%

- deionized water
- ultrapure water
- 0.1% (v/v) orthophosphoric acid solution (prepared by transferring acc. 1.18 mL of concentrated orthophosphoric acid into a flask and filling with ultrapure water up to 1000 mL)

2.Equipment
- liquid chromatography system with PDA detector
- analytical balance with an accuracy of at least 0.01 mg
- automatic pipettes
- deionizer
- system for obtaining ultrapure water
- ultrasonic bath
- stopwatch
- volumetric flask class A
- syringes and syringe hydrophobic filters 0.22 µm

3.Chromatographic conditions
Column Kinetex 5 µm C18 100Å 150 x 4.60 mm
Detection 206 nm
Injection volume 2 µL
Column thermostat temperature 30°C
Mobile phase acetonitrile : 0.1% H_3PO_4 (75% : 25%)
Flow of mobile phase 1.0 mL/min
Analysis Time 11 min

4.Current check of chromatograph operation
Prepare a standard solution of pyriproxyfen in acetonitrile with a concentration within the calibration curve range of 42.623 - 124.672 mg/L
Perform injection repeatability analysis by analyzing the standard solution three times.
Read the peak area of the analyzed standard peak and complete Appendix 1 (see Report appendixes).
Acceptance criterion:
RSD between peak areas of the triplicately injected sample ≤ 5%.

5.Calibration curve preparation
5.1. Weigh minimum of 10 mg of pyriproxyfen standard into a 10 mL volumetric flask with an accuracy of 0.01 mg. Fill the flask to the 10 mL with acetonitrile and mix thoroughly.
5.2. Take the standard solution prepared according to point 4.1 into 10 mL flasks in such a volume as to obtain standard solutions at three concentration levels within the calibration curve range of 42.623 - 124.672 mg/L (each concentration level in duplicate). Fill the flask with acetonitrile to their nominal volume.
5.3. Calculate the concentration of the active substance in the standard solution according to Appendix 2 (see Report appendixes).
5.4. Perform chromatographic analysis of the prepared standard solutions.
5.5. After performing the chromatographic analysis, use the instrument control software to plot a calibration curve showing the relationship between peak area and active substance concentration, and determine the linear correlation coefficient.
5.6. Acceptance criterion for the generated calibration curve:
- The established linearity range should allow for the determination of active substance concentrations from 80% to 120% of the nominal concentration of the substance being analyzed.
- $r \geq 0.99$.

6. Determination of active substance content in the test item
Weigh approximately 100 mg of the test item into a 25 mL volumetric flask with an accuracy of 0.01 mg. Fill the flask up to 25 mL with acetonitrile and sonicate for 15 minutes to allow complete dissolution of the test substance. Filter the prepared solution through a syringe filter into a chromatography vial.

Calculate the concentration of the test item solution using the following formula:

$$C_{TI} = \frac{m * 1000}{V}$$

where:
C_{TI} concentration of the test item solution in acetonitrile [mg/L]
m mass of the test item [mg]
V volumetric flask volume [mL]
1000 unit conversion factor [mL/L]

Calculate the concentration of the active substance pyriproxyfen in test item using the following formula:

$$\%C = \frac{C_{det} * 100}{C_{TI}}$$

where:
%C active substance concentration in the test item [% (w/w)]
C_{det} concentration of the active substance read from calibration curve [mg/L]
C_{TI} concentration of the test item solution [mg/L]
100 unit conversion factor [% (w/w)]

Report the final determined concentration as the arithmetic mean of all replicates with an accuracy of 0.01% (w/w)
Calculate the concentration of the active substance in the test item in units of g/L using the following formula:

$$C_{w/v} = \frac{C_{\%} \cdot d \cdot 1000}{100}$$

where:
C% concentration of the active substance in the test item [% (w/w)]
$C_{w/v}$ concentration of the active substance in the test item [g/L]
d density of the test item [g/mL]
100 unit conversion factor [%]
1000 unit conversion factor [mL/L]

Report the final determined concentration as the arithmetic mean of all replicates with an accuracy of 0.01 g/L.
Calculations of the active substance pyriproxyfen should be performed in a spreadsheet (see Report appendixes -Appendix 3).

7. Checking the calibration curve

Before each series of analyses, perform a calibration curve check:
Prepare a standard solution of pyriproxyfen with a concentration within the calibration curve range of 42.623 - 124.672 mg/L.
Perform chromatographic analysis and complete the spreadsheet (see Report appendixes -Appendix 4).
Acceptance criterion: difference between determined and theoretical concentration are within the range specified based on the RSDr value calculated from the modified Horwitz equation for the theoretical concentration.
Calculate the difference between determined and theoretical concentration using the following formula:

$$D = \left|\frac{C_N - C_{DT}}{C_N}\right| \cdot 10$$

where:
D difference between determined and theoretical concentration [%]
C_N theoretical active substance concentration [mg as/L]
C_{DT} determined active substance concentration [mg as/L]
100 units conversion factor [%]

Calculate the value from the modified Horwitz equation according to the formula:

$$RSD_r = 0.67 * 2^{\left(1-0.5*\log\left(\frac{c}{100}\right)\right)}$$

where:

RSDr	the value calculated from the modified Horwitz equation [%]
c	concentration of active substance in the test item [% (w/w)]
100	unit conversion factor [% (w/w)]

Calculate the Horrat factor using the formula:

$$Hr = \frac{RSD}{RSD_r}$$

where:

Hr	Horrat factor
RSD	relative standard deviation [%]
RSDr	value calculated from the modified Horwitz equation [%]

If the calibration curve is not stable, prepare standard solutions to generate a new calibration curve according to point 5.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances pyriproxyfen in plant protection product PYRIX 20 SL

	Pyriproxyfen
Study	Final report.Validation of analytical method for the determination of active substance (pyriproxyfen) content in the test item PYRIX 20SL Test item :PYRIX 20 SL / Study code :0012/0003/FA
Author(s), year	Author(s) : B.Cirga, M. Wlodarczyk /SORBOLAB Research Laboratory LLC ;
Principle of method	Validation of analytical method was performed according to experimental procedure SPB-FA/11 and Guideline SANCO/3030/99, rev. 5.

Parameter	**Required criterion**	**The result**		
Selectivity	In the analysis conditions, active substance retention time in standard solution and test item solution should be comparable. No interfering peaks with area larger than 3% of the value of active substance peak area in test item solution, in the place of active substance peak. UV spectrum comparison should allow for identification of the active substance in the test item solution.	In the analysis conditions, active substance retention time in standard solution and test item solution is comparable. No interfering peaks with area larger than 3% of the value of active substance peak area in test item solution, in the place of active substance peak. UV spectrum comparison allows for identification of the active substance in the test item solution.		
Linearity	r ≥0.99	r = 0.999 (42.623 mg/L – 124.672 mg/L)		
Accuracy [%]	90-110	level I*	98.14	99.18
		level II*	99.79	
		level III*	99.62	
Precision [%]	RSD ≤ RSD_r (2.42) Hr ≤ 1	RSD = 0.66 Hr = 0.27		

* Nominal concentration: level I: 63.934 mg/L, level II: 83.115 mg/L, level III: 104.426 mg/L.

	Pyriproxyfen
Comment	Results of the validation of the analytical method confirmed that this method is suitable for analysis the content of the active substance (pyriproxyfen) in the test item.

Conclusion
Validation of analytical method for determination of the active substance (pyriproxyfen) in the test item PYRIX 20SL was performed.
During the validation of the analytical method the following parameters: selectivity, linearity, accuracy and precision (repeatability) were determined. Validation of analytical method was performed according to experimental procedure SPB-FA/11 and Guideline SANCO/3030/99, rev. 5.
Results of the validation of the analytical method confirmed that this method is suitable for analysis the content of the active substance (pyriproxyfen) in the test item.

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

Not applicable - there are no significant impurities in the Pyrix 20 SL product

Comments of zRMS:	**Not agree**. According to Commission Implementing Regulation (EU) No 540/2011 for Pyriproxyfen: Max. impurity: Toluene 5 g/Kg. The validation of a method for the determination of toluene in plant protection products should be presented. The method must be sufficiently sensitive to determine toluene in accordance with its possible content, calculated on the basis of the known content of the active substance in the PPP.

The applicant's position	With reference to the requirements concerning the development of analytical methods for relevant impurities in plant protection products, we present the technical justification for the difficulty in developing a direct method for determining toluene in the product Pyrix 20 SL, together with a proposed-approach for controlling this impurity. Following initial review, we found that we are unable to use an analytical method for determining the Toluene content in the Product using liquid chromatography with an HPLC instrument, because toluene does not have a sufficiently specific UV spectrum and we must attempt to develop a gas chromatography method. Our product is formulated on a water basis, and a product sample containing water cannot be used for injection onto the capillary of a gas chromatograph. Toluene should be quantitatively extracted from the Product and the extract dried before preparing the working solution for injection into the gas chromatograph. The main difficulty is the composition of the product and the relatively low toluene content. 1L of Pyrix 20 SL contains 446 grams of water and 579.6 grams of various surfactants, 20.42 g of Pyriproxyfen 98%. This clear Product may contain a max. of 0.1020 g toluene/litre of product (at the permissible max. toluene content: 5g/kg in the Pyriproxyfen Technical used). We anticipate that the most difficult and most time-consuming task will be developing the quantitative extraction of Toluene from the Product. It can be assumed that during the extraction of toluene - using a solvent - a large part of the hydrophilic surfactants will dissolve in the aqueous phase, simultaneously dissolving part of the toluene, which would result in an incomplete and potentially variable recovery of toluene. Only the portion extracted with the solvent can be analysed under gas chromatography

	conditions. If 100% of the Toluene contained in the product is not extracted with the solvent, we will need to determine the partition coefficient of toluene between the solvent phase and the aqueous phase saturated with surfactants. Difficulties in establishing such a coefficient may compromise the accuracy of the analytical method. It is expected that the analytical method should confirm the amount of toluene introduced into the product together with the Pyriproxyfen technical. **(Guidelines** :*The method must be sufficiently sensitive to determine toluene in accordance with its possible content, calculated on the basis of the known content of the active substance in the PPP.)* Until the expected method for determining Toluene in the Product has been developed, we propose to use a calculation of the Toluene content in the product based on the Toluene content in the Pyriproxyfen technical - as determined by the manufacturer of Pyriproxyfen and documented by the Certificate of Analysis for this active substance. According to the declarations received from the manufacturers, none of the functional raw materials used in the formulation of the Product contains toluene, and therefore only the Pyriproxyfen technical concentrate can be the source of Toluene in our Product . We request acceptance of the calculation-based method for controlling the toluene content in the product Pyrix 20 SL, based on the documented toluene content in the Pyriproxyfen technical (according to the certificate of analysis of the active substance issued by manufacturer) and on the written declarations obtained from the surfactant manufacturers confirming the absence of toluene in their functional raw materials. We request that the principle of calculating the toluene content in the product remain in force until completion of the validation trial stage for the extraction of toluene from the product by **Łukasiewicz Research Network – Institute of Industrial Organic Chemistry in Warsaw** and any possible extension thereof shall be subject to separate assessment. Encl.: Declarations by the manufacturers of the functional raw materials of Pyrix 20 SL confirming the absence of toluene in their products: please refer part C (Appendix 2).
Comments of zRMS:	We agree with the applicant's proposal. The product may be registered conditionally, and the validation should be completed as soon as it becomes available.

Materials and methods
Not applicable - there are no significant impurities in the Pyrix 20 SL product.

Validation - Results and discussions
Not applicable - there are no significant impurities in the Pyrix 20 SL product.

Not applicable.

Conclusion
Not applicable - there are no significant impurities in the Pyrix 20 SL product.

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

Not required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There are no methods for pyriproxyfen SL formulation.

Available CIPAC method :
1)TC- technical material
Pyriproxyfen content (715/TC/M/3, CIPAC Handbook M, p.181, 2009)
2) EC
Pyriproxyfen content (715/EC/M/3, CIPAC Handbook M, p.183, 2009)
Relevant impurities-Water (MT 30.5, CIPAC Handbook J, p.120, 2000) [Maximum: 3 g/kg]
3) EW
Pyriproxyfen content (715/EW/M/3, CIPAC Handbook M, p.184, 2009)

5.2.2 Methods for the determination of residues (KCP 5.1.2)

An overview on the methods and possible data gaps for analysis of residues of active substance pyriproxyfen for authorization product PYRIX 20 SL are given in the following table.
For the detailed evaluation of new studies it is referred to Appendix 2.

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

Component of residue definition: pyriproxyfen active ingredient				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
~~Plants, mushrooms (Champinions)~~ ~~[*Agaricus biosporus*] (Residues)~~	~~Primary (also EU MRL)~~	~~LOQ 0,01mg/kg~~	~~Method for the determination of Pyriproxyfen was validated in accordance to the guidance document:~~ ~~1.Guidance on pesticide Residue Analytical Methods for Risdk Assessment and Post-approval Control and Monitoring Puposes.~~ ~~Final determination by HPLC-MS/MS~~ ~~2.“ Food of plant origin-Multimethod for determination of pesticide residue using GC- and LC-based analysis following acetronitrile extration/partitioning and clean-up by dispersive SPE- Modular QuEChERS0method”.~~	~~SANTE/2020/12830, Rev.2~~ ~~European Commission~~ ~~European Commite for Standarization (CEN): EN 15662:2018~~
	~~Confirmatory (if required)~~	~~LOQ mg/kg~~	~~**Not required**~~	~~author(s), year / missing / EU agreed~~
Plants, mushrooms (Champinions) [*Agaricus biosporus*] (Residues)	Primary Residues in mushrooms	0.01 mg/kg	HPLC- MS/MS	Joanna Kicińska, 2025, Study code: 25/FSL/07 New study, see KCP 5.2 in Appendix 2
	Confirmatory	Not required, primary method is specific		
Animal products, food of animal origin,... (Residues)	Primary	NOT APPLICABLE Mushrooms are not food for animals. No new methods submitted.		
	Confirmatory (if required)			
Soil, water, sediment,... (Environmental fate)	Primary	NOT APPLICABLE No new methods submitted. According the specific nature of mushroom cultivation: buildings that are isolated from the external environment. Such buildings are fully closed with non-transparent walls and a non-transparent roof, with a tightly sealed, smooth concrete floor most often covered with ceramic tiles. The growing rooms are equipped with the necessary air conditioning and irrigation systems with a microclimate control system. The solid, hole-free shelves as well as the computerised low-water systems prevent nutrients from being washed away, thus also preventing pyriproxyfen residues from flowing onto the lower shelves or onto the sealed floors. These differences make mushroom cultivation chambers functionally and structurally distinct from greenhouses. This all points more towards CLOSED BUILDING.		
	Confirmatory (if required)			
Soil, water,... (Efficacy)	Primary			
	Confirmatory (if required)			

Component of residue definition: pyriproxyfen active ingredient				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
		There is no possibility of pyriproxyfen being released into the environment and therefore there is no need to carry out tests for pyriproxyfen residues in soil, water, surface waters, ground waters. Please see the appendix attached to this document: *Expert Statement on the Classification of Indoor Mushroom Cultivation [Author: Marielle Fortin-McCuaig / EUROFINS Agroscience regulatory/ Completion date: 26 June 2025]*		
Feed, body fluids,... (Toxicology)	Primary Confirmatory (if required)	NOT APPLICABLE No new methods submitted. The limit of quantification (LOQ) was established at 0.01 mg/kg for analyte, interfering signal in control speciment were negligible, and thus the limit of detection (LOD) is 0,0025mg/kg for mushrooms. All results of pyriproxyfen in mushrooms samples from field triald was under limito of detection (< LOD ➔ < 0,0025mg/kg). Based on the results, we believe that there is no need to conduct tests for pyriproxyfen content in processed mushrooms (cooked, fried, canned, in brine)		
Body fluids, air,.... (Exposure)	Primary Confirmatory (if required)	NOT APPLICABLE No new methods submitted. When using dedicated protective measures: eye protection, face protection, gloves, there is no possibility of operator exposure.There is no possibility of pyriproxyfen being released into the air. No need to carry out tests for pyriproxyfen residues in body fluids. Please see the appendix attached to this document: *Expert Statement on the Classification of Indoor Mushroom Cultivation [Author: Marielle Fortin-McCuaig / EUROFINS Agroscience regulatory/ Completion date: 26 June 2025]*		
Soil, water,... (Ecotoxicology)	Primary Confirmatory (if required)	NOT APPLICABLE No new methods submitted. There is no possibility of pyriproxyfen being released into the environment and having an impact on the environment and animals. The specificity of spraying mushrooms excludes the effect of pyriproxyfen on birds and other terrestrial vertebrates, aquatic organisms, bees, arthropods, non-target soil meso- and macrofauna, microbial organisms, and non-target terrestrial plants. There is no need for a risk assessment. Mushrooms (champignons) are not intended for animal feed. There is no need for a risk assessment for drinking water exposure, for secondary exposure, for baits, pellets, granules, prills or treated. Please see the appendix attached to this document: *Expert Statement on the Classification of Indoor Mushroom Cultivation [Author: Marielle Fortin-McCuaig / EUROFINS Agroscience regulatory/ Completion date: 26 June 2025]*		
Water, buffer solutions,... (Properties)	Primary Confirmatory (if required)	NOT APPLICABLE No new methods submitted.		

Component of residue definition: metabolite

NOT APPLICABLE

Pyriproxyfen residues in mushrooms were below the detection limit. Since there are no residues in the mushrooms, there are also no his metabolites.

There is no possibility of metabolites of pyriproxyfen being released into the environment and having an impact on the environment and animals. The specificity of spraying mushrooms excludes the effect of pyriproxyfen metabolites on birds and other terrestrial vertebrates, aquatic organisms, bees, arthropods, non-target soil meso- and macrofauna, microbial organisms, and non-target terrestrial plants. No need for a risk assessment.

Mushrooms (champignons) are not intended for animal feed. There is no need for a risk assessment for drinking water exposure, for secondary exposure, for baits, pellets, granules, prills or treated.

When using dedicated protective measures: eye protection, face protection, gloves, there is no possibility of operator exposure.There is no possibility of pyriproxyfen metabolites being released into the air. No need to carry out tests for metabolites of pyriproxyfen residues in body fluids.

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

Residue definitions for monitoring
Food/feed of plant origin **Pyriproxyfen**

Method for the determination of pyriproxyfen (component included in the monitoring residue definition) residues in plant matrices:

Method (Eu evaluated)	Title	Analytes	Matrix (LOQ)	Technique	Year
Multi-method, monitoring (EU evaluated)	Guidance on pesticide Residue Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Puposes	Pyriproxyfen	LOQ 0.01 mg/kg	HPLC-MS/MS	2020 SANTE/2020/12830, Rev.2
Multi-method, monitoring (EU evaluated)	Food of plant origin-Multimethod for determination of pesticide residue using GC- and LC-based analysis following acetronitrile extration/partitioning and clean-up by dispersive SPE- Modular QuEChERS0method".	Pyriproxyfen	LOQ 0.01 mg/kg	HPLC-MS/MS	2018 European Commite for Standarization (CEN): EN 15662:2018

5.3.1 Analysis of the plant protection product (KCP 5.2)

The analytical method for the determination of the active substance pyriproxyfen in the product PYRIX 20 SL is presented in point 5.2

5.3.2 Description of analytical methods for the determination of residues of active substance pyriproxyfen (KCP 5.2)

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

Compared to the residue definition from EU level proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical.
Residue definitions for monitoring

Food- cultivated mushroom : Pyriproxyfen

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
Mushrooms (cultivated)	PYRIPROXYFEN	LOQ 0.01 mg/kg	EU MRL 0.01mg/kg
Muscle	not applicable	not applicable	-
Milk		not applicable	-
Eggs		not applicable	-
Fat		not applicable	-
Liver, kidney		not applicable	-
Soil (Ecotoxicology)	not applicable	not applicable	-
Drinking water (Human toxicology)	not applicable	not applicable	-
Surface water (Ecotoxicology)	not applicable	not applicable	-

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Air	not applicable	not applicable	-
Tissue (meat or liver)	not applicable	not applicable	-
Body fluids		not applicable	-

Matrix	**Residue definition**	**MRL/limit**	**Reference for MRL/level Remarks**
Plant, high water content	Pyriproxyfen	0.01 mg/kg	Reg. (EU) 2023/1753
Plant, high acid content		0.01 mg/kg	Reg. (EU) 2023/1753
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Reg. (EU) 2023/1753
Plant, high oil content		0.01 mg/kg	Reg. (EU) 2023/1753
Plant, difficult matrices (black tea)		0.01 mg/kg	Reg. (EU) 2023/1753
Muscle	Pyriproxyfen	0.01 mg/kg	Reg. (EU) 2023/1753
Milk		0.01 mg/kg	Reg. (EU) 2023/1753
Eggs		0.01 mg/kg	Reg. (EU) 2023/1753
Fat		0.01 mg/kg	Reg. (EU) 2023/1753
Liver		0.01 mg/kg	Reg. (EU) 2023/1753
Soil (Ecotoxicology)	Pyriproxyfen	0.01 mg/kg	common limit
Drinking water (Human toxicology)	Pyriproxyfen	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Pyriproxyfen	0.01 µg/L	NOEC *Daphnia pulex* 0.01 µg/L
Air	Pyriproxyfen	1 µg/m^3	AOEL: 0.04 mg/kg bw/d
Tissue (meat or liver)	Pyriproxyfen	0.01 mg/kg	SANTE/2020/12830, Rev. 2
Body fluids (blood and urine)		0.01 mg/L	SANTE/2020/12830, Rev. 2

EXPLANATION :

According the specific nature of mushroom cultivation:
buildings that are isolated from the external environment. Such buildings are fully closed with non-transparent walls and a non-transparent roof, with a tightly sealed, smooth concrete floor most often covered with ceramic tiles. The growing rooms are equipped with the necessary air conditioning and irrigation systems with a microclimate control system. The solid, hole-free shelves as well as the computerised low-water systems prevent nutrients from being washed away, thus also preventing pyriproxyfen residues from flowing onto the lower shelves or onto the sealed floors. These differences make mushroom cultivation chambers functionally and structurally distinct from greenhouses. This all points more towards CLOSED BUILDING.
There is no possibility of pyriproxyfen being released into the environment and therefore there is no need to carry out tests for pyriproxyfen residues in soil, water, surface waters, ground waters and air.

There is no possibility of pyriproxyfen being released into the environment and having an impact on the environment and animals. The specificity of spraying mushrooms excludes the effect of pyriproxyfen on birds and other terrestrial vertebrates, aquatic organisms, bees, arthropods, non-target soil meso- and macrofauna, microbial organisms, and non-target terrestrial plants. There is no need for a risk assessment.
Mushrooms (champignons) are not intended for animal feed. There is no need for a risk assessment for drinking water exposure, for secondary exposure, for baits, pellets, granules, prills or treated.
We are convinced that there is no possibility of pyriproxyfen being released into the air and therefore there is no need to carry out tests for pyriproxyfen residues in body fluids.
Mushrooms are not intended for animal feeding, there is no need to carry out residue testing.

Please see the appendix attached to this document: *Expert Statement on the Classification of Indoor Mushroom Cultivation [Author: Marielle Fortin-McCuaig / EUROFINS Agroscience regulatory/ Completion date: 26 June 2025]*

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 active substance pyriproxyfen in plant matrices is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

STUDY TITLE -FIELD PART:
Magnitude of the residue of pyriproxyfen (CAS 95737-68-1) in Mushrooms *Agaricus bisporus* (Raw Agricultural Commodity - RAC) grown in closed buildings conditions after one application of a formulated product of PYRIX 20 SL - one harvest and two decline curve trials in Northern Europe. Poland – 2025
Study number: LLP25-2
Harvest trial number: LLP25-2-01
Decline trial number: LLP25-2-02
Decline trial number: LLP25-2-03
Test Site : LymeLab Pharma sp. z o. o. sp. k. ;Cebulki 19 ; 13-124 Kozłowo, POLAND;
Test Facility : LymeLab Pharma sp. z o. o. sp. k. ;Trylogii 2/16 ; 01-982 Warsaw, POLAND
GAP-YES
Unbuplished

STUDY TITLE - ANALITYCAL PART:
1.Determination of the residue of pyriproxyfen in mushrooms after application of “PYRIX 20 SL” in northen Europe- Poland 2025.
Study code: 25/FSL/07
Year: 2025
Test Facility: Food Safety Laboratory.The National Institute of Horticultural Research; 13B Pomologiczna Street; 96-100 Skierniewice Poland
GLP- YES
Unpublished

2.Determination of the residue of pyriproxyfen in mushrooms after application of “PYRIX 20 SL” in northen Europe- Poland 2025.
Study code: 25/FSL/16
Year: 2025
Test Facility: Food Safety Laboratory.The National Institute of Horticultural Research; 13B Pomologiczna Street; 96-100 Skierniewice Poland
GLP- YES
Unpublished

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)

Analyte	Matrix	Fortification Level (mg/kg)	Analyte Residue Found** (mg/kg)	Mean Recovery (%)	RSD (%)	n	Overall Mean Recovery (%)	Overall RSD (%)
Pyriproxyfen	Mushrooms	Ion Mass Transition 322.1→96.0(Quantification)						
		0.01*	0.0101	101	1.3	5	99	2.6
		0.1	0.098	98	2.8	5		
		Ion Mass Transition 322.1→185.0 (Confirmation)						
		0.01*	0.0100	100	1.0	5	99	2.4
		0.1	0.097	97	2.6	5		

* Limit of quantification, defined by the lowest validated fortification level
** Residues in duplicate control (unfortified) specimen samples were below LOD

Component of residue definition: Pyriproxyfen				
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	LC-MS/MS	Lindner, M., 2014/EU agreed protected
	ILV	0.01 mg/kg	LC-MS/MS	-
	Confirmatory (if required)	-	Not required	-
High acid content	Primary	0.01 mg/kg	LC-MS/MS	Lindner, M., 2014/EU agreed protected
	ILV	-	Not required	-
	Confirmatory (if required)	-	Not required	-
High oil content	Primary	0.01 mg/kg	LC-MS/MS	Lindner, M., 2014/EU agreed protected
	ILV	-	Not required	-
	Confirmatory (if required)	-	Not required	-
High protein/high starch content (dry)	Primary	0.01 mg/kg	LC-MS/MS	Lindner, M., 2014/EU agreed protected
	ILV	0.01 mg/kg	LC-MS/MS	-
	Confirmatory (if required)	-	Not required	-
Difficult (black tea)	Primary	0.01 mg/kg	LC-MS/MS	Lindner, M., 2014/EU agreed protected
	ILV	0.01 mg/kg	LC-MS/MS	-

Component of residue definition: Pyriproxyfen				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year/missing/EU agreed**
	Confirmatory (if required)	-	Not required	-

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Not required, because:	Results of pyriproxyfen residue in Mushrooms samples from field trials are below Limit of Detection (LOD) 0.0025 mg/kg . ~~LOQ ustalono na poziomie 0.01 mg/kg.~~

zRMS comments:
Methods previously submitted for monitoring purposes and already EU evaluated in the DAR (November 2005) have been updated to meet current data requirements where appropriate (RAR, 2018). Some methods previously evaluated do not meet current guidance and are not accepted.

New method of Lindner, M., presented in RAR (2018) is available and evaluated as suitable post-registration.
This method is protected according to the List of information, tests and studies on which the RMS relies in its assessment for renewal of an active substance (August 2019) and cannot be used in this application to support the use of Pyrix 20 SL.

According to the RAR (2018):
There are published multi-methods available that have been shown to provide satisfactory accuracy and precision in determining residues of pyriproxyfen in foods. Of these, method EN 15662 (QuEChERS) is considered to be the most appropriate for current use by EU Reference Laboratories for MRL enforcement and it provides evidence that routine monitoring methods are compatible with those proposed by the Notifier.
European Standard method EN 15662 (Foods of plant origin – Determination of pesticide residues using LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS method) is widely used in EU Reference Laboratories (EURL) used for analysis of multiple pesticide residues. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) citrate multi residue method has been used for the analysis of pyriproxyfen in high water, dry, high acid and high oil crops. This method consists of liquid-liquid micro-extraction. This is followed by sample clean-up to remove unwanted matrix materials that interfere with chromatographic analysis. Samples are shaken with acetonitrile buffered with magnesium sulphate and then determined by LC-MS/MS. Dry and high acid commodities may be analysed using this method following the addition of water prior to extraction to weaken interactions of pesticides with the matrix to ensure adequate partitioning. The EN standard 15562 document indicates the applicability of the QuEChERS method for analysis of pyriproxyfen, summarising the observed recoveries following analysis using GC determination (mainly GC-MS) or LC determination (LC-MS/MS). This recovery data published on the EURL is summarised on the EURL website under the data pool (www.eurl-pesticides-datapool.eu/). This DataPool has been created by the EURL for Residues of Pesticides with the aim to provide pesticide residue analysts with a convenient and efficient access to information needed for proper decision-making in pesticide residue analysis. Within the data pool method validation data generated within the aim of method validation experiments from various laboratories using different methods of analysis is collated.
The existence of many reported and published validations of the established EU QuEChERS multi-method for pyriproxyfen with LOQ levels as low as 0.002 mg/kg reinforces the Notifier proposed monitoring methods and that the proposed residue definition for monitoring in food of plant origin can be enforced with an LOQ of at least 0.01 mg/kg for pyriproxyfen.

In the EURL database, for pyriproxyfen an abundant amount of data is included for QuEChERS. According to SANCO/825/00 rev 8.1 guidance, an ILV is not required if a published method is used.

The citrate buffered QuEChERS method is published in EN norm 15662:2008. The norm is currently being revised. The method validation provided under CA 4.2/03 employs a slightly different mobile phase than the published method, but considering the extraction technique is comparable, the RMS accepts that the QuEChERS method is acceptable for monitoring of the four major crop groups at an LOQ of 0.01 mg/kg. An additional ILV is not

considered required.

No additional method is required.

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

No new or additional studies have been submitted.

Please see the appendix attached to this document: *Expert Statement on the Classification of Indoor Mushroom Cultivation [Author: Marielle Fortin-McCuaig / EUROFINS Agroscience regulatory/ Completion date: 26 June 2025*

~~Since mushrooms are not normally fed to livestock, analythical methods for determination of pyriproxyfen residues in animal matrices not assessed in the framework of this application.~~

At this point, Agrofarm refers to non-protected study and data already evaluated at EU level -see point A2.1.2.2

Table 5.3-4: Validated methods for food and feed of animal origin (if appropriate)
~~Not required when using Pyrix 20SL as indicated in GAP~~

Component of residue definition: Pyriproxyfen				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year/missing/EU agreed**
Milk	Primary	0.01 mg/kg	LC-MS/MS	Lindner, M. Grewe, D.,2015/EU agreed protected
	ILV	0.01 mg/kg	LC-MS/MS	Mass, X., 2015/EU agreed protected
	Confirmatory (if required)	-	Not required	-
Eggs	Primary	0.01 mg/kg	LC-MS/MS	Lindner, M. Grewe, D.,2015/EU agreed protected
	ILV	-	Not required	-
	Confirmatory (if required)	-	Not required	-
Muscle	Primary	0.01 mg/kg	LC-MS/MS	Lindner, M. Grewe, D.,2015/EU agreed protected
	ILV	0.01 mg/kg	LC-MS/MS	Mass, X., 2015/EU agreed protected
	Confirmatory (if required)	-	Not required	-

Component of residue definition: Pyriproxyfen				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year/missing/EU agreed**
Fat	Primary	0.01 mg/kg	LC-MS/MS	Lindner, M. Grewe, D.,2015/EU agreed protected
	ILV	0.01 mg/kg	LC-MS/MS	Mass, X., 2015/EU agreed protected
	Confirmatory (if required)	-	Not required	-
Kidney	Primary	0.01 mg/kg	LC-MS/MS	Lindner, M. Grewe, D.,2015/EU agreed protected
	ILV	-	Not required	-
	Confirmatory (if required)	-	Not required	-

Table 5.3-5: Statement on extraction efficiency

~~Not required when using Pyrix 20SL as indicated in GAP~~

zRMS comments:
No methods were previously submitted for monitoring purposes of animal matrices in the DAR (November 2005). New methods presented in RAR (2018) are available and evaluated as suitable post-registration.
The methods of Lindner, M. Grewe, D., 2015 and Mass, X., 2015 are protected according to the List of information, tests and studies on which the RMS relies in its assessment for renewal of an active substance (August 2019) and cannot be used in this application to support the use of Pyrix 20 SL.

According to SANTE/2020/12830, an additional analytical method and its independent laboratory validation (ILV) for the determination of residues of pyriproxyfen in animal matrices are required (data gap).

September 2026 (commenting period):
The applicant provided additional clarification regarding the data gap.

Agrofarm takes the position that for the use of the plant protection product Pyrix 20 SL (active substance: pyriproxyfen) exclusively in the cultivation of mushrooms (Agaricus bisporus) there is no need to submit residue studies in animal tissues and products (the so-called "residue feeding studies"), and consequently no need to develop an analytical method and its validation for matrices of animal origin.
Our argumentation will be based on:
- a formal analysis of the scope of application of the standard dietary burden calculator recommended by EFSA/OECD
- a supplementary quantitative calculation. Worst-case scenario variant – result obtained under the least favourable conditions.

1. Lack of formal grounds for applying the standard dietary burden model:
The Animal model 2016.xls calculator, which serves as the EFSA/OECD reference tool for calculating the dietary burden of livestock, contains a closed catalogue of feed items grouped into categories. None of the approximately 106 feed items included in the calculator covers cultivated mushrooms or spent mushroom substrate. This means that the model does not have assigned consumption parameters (% share of the diet) for this type of plant material – because cultivated mushrooms are not, and have never been, treated as a component of the livestock diet under the EU OECD/EFSA methodology. Mushrooms are not, and should not be, used as feed for livestock (cattle, pigs, poultry). These animals are unable to digest mushrooms, and feeding them mushrooms may lead to serious digestive disorders or poisoning. As a result, the potential exposure of livestock to pyriproxyfen residues from this

use is zero.

2. Thresholds triggering the obligation to submit feeding studies

The EFSA guidelines (2015) specify two alternative thresholds, above which the submission of residue studies in animal products is required:

- Regulation (EC) 544/2011 (old data requirements): threshold of 0.1 mg/kg expressed on a dry matter (DM) basis.
- Regulation (EU) 283/2013 (new data requirements): threshold of 0.004 mg/kg body weight/day (bw/d).

„Under Regulation (EU) No 283/2013, animal intakes have to be expressed on a body weight basis and the threshold intake for the submission of animal studies is 0.004 mg/kg bw/d. If evidence is given from the metabolism studies that no residues are expected to be present in animal matrices above 0.01 mg/kg, the submission of livestock feeding studies is not required." — EFSA, September 2015.

3. Supplementary worst-case scenario calculation

Below we present a deliberately extremely unfavourable, traditional quantitative calculation intended to demonstrate that even under the hypothetical (and unrealistic) assumption that mushroom material would constitute 100% of an animal's daily feed ration, the resulting dietary burden remains below the threshold that triggers the obligation to conduct studies.

3.1 Input data

Parameter	Value	Source
HR (highest residue), mushrooms – fresh weight	0,0025 mg/kg (= LOD)	25/FSL/07, 25/FSL/16 (2025)
STMR, mushrooms – fresh weight	< 0,0025 mg/kg	25/FSL/07, 25/FSL/16 (2025)
LOD of the analytical method	0,0025 mg/kg	25/FSL/07, 25/FSL/16
LOQ of the analytical method	0,01 mg/kg	25/FSL/07, 25/FSL/16
EU MRL for pyriproxyfen	0,01 mg/kg	Regulation (EC) 396/2005
Dry matter content in mushrooms	7–14 %	Sadler, 2000 (Agaricus bisporus)
DM content used in the calculation (worst case)	7 %	worst-case value

Residues in all samples from both residue studies of the Pyrix 20SL product in mushrooms (25/FSL/07 and 25/FSL/16) were below the limit of detection (LOD = 0.0025 mg/kg). In accordance with standard regulatory practice, for results <LOD we adopted the value HR = LOD (0.0025 mg/kg) for the "worst case" calculation — an approach that is more conservative than assuming a zero value, while at the same time methodologically appropriate (adopting the LOQ would be excessively conservative, since no result even approached that level).

3.2 Concentration converted to a dry matter basis

The HR concentration expressed on a fresh weight basis was converted to a dry matter basis using the lowest dry matter content value in mushrooms (7%):

C(DM) = HR(fresh weight) / dry matter fraction = 0.0025 mg/kg / 0.07 = 0.0357 mg/kg DM

This value is more than 2.8 times lower than the threshold of 0.1 mg/kg DM set out in Regulation (EC) 544/2011.

3.3 Comparison with feeding parameters (Animal model 2016.xls – "Overview" tab)

For the risk assessment against the threshold expressed on a body weight basis (0.004 mg/kg bw/d, Regulation (EU) 283/2013), the standard body weight values and daily dry matter feed intake for individual livestock species/categories are compiled below:

Animal / category	Body weight (kg)	Feed intake, DM (kg/day)	DM intake / bw ratio (kg DM/kg bw)
Dairy cattle (Dairy)	650	25	0,0385
Beef cattle (Beef)	500	12	0,0240
Sheep – ewe/ram (Ram/Ewe)	75	2,5	0,0333
Lamb (Lamb)	40	1,7	0,0425
Pigs – breeding (Breeding)	260	6	0,0231
Pigs – finishing (Finishing)	100	3	0,0300
Poultry – broiler (Broiler)	1,7	0,12	0,0706
Poultry – layer (Layer)	1,9	0,13	0,0684
Turkey (Turkey) - worst-case scenario (worst case)	7,0	0,50	0,0714

The highest ratio of daily dry matter feed intake to body weight occurs in the poultry group (turkey: 0.0714 kg DM/kg bw), which makes this category the worst-case scenario for assessing exposure expressed on a body weight basis.

3.4 Result of the "worst case" calculation

Dose (mg/kg bw/day) = C(DM) × DM intake/bw ratio
Dose = 0.0357 mg/kg DM × 0.0714 kg DM/kg bw = 0.00255 mg/kg bw/day
The resulting value (0.00255 mg/kg bw/d) is lower than the threshold of 0.004 mg/kg bw/d set out in Regulation (EU) 283/2013 — even in a scenario in which 100% of an animal's daily feed ration would consist of mushroom material with the maximum observed residue level.

4. Conclusions

- Cultivated mushrooms and spent mushroom substrate are not included in any feed category of the OECD/EFSA catalogue (Animal model 2016.xls) and do not form part of the livestock diet in the EU — the actual exposure of animals to pyriproxyfen residues from this use is zero.
- Even assuming a hypothetical, extremely unfavourable scenario of a 100% share of mushroom material in an animal's diet, the calculated dietary burden (0.0357 mg/kg DM and 0.00255 mg/kg bw/d) remains below both regulatory thresholds that trigger the obligation to conduct animal feeding studies (0.1 mg/kg DM and 0.004 mg/kg bw/d, respectively).
- Consequently, in Agrofarm's opinion, there is no substantive basis for requiring the submission of pyriproxyfen residue studies in animal tissues/products (residue feeding studies), and thus no basis for requiring the submission of an analytical method together with its validation for the determination of pyriproxyfen residues in matrices of animal origin as part of the application for registration of the plant protection product Pyrix 20SL

data sources :

- Determination of the residue of \ in mushrooms after application of „PYRIX 20 SL" — Study code 25/FSL/07 (Polska, 2025).
- Determination of the residue of pyriproxyfen in mushrooms after application of „PYRIX 20 SL" — Study code 25/FSL/16 (Polska, 2025).
- Pyrix 20 SL — dRR Part B, Section 7 (7.2.2.5 Nature of residues in livestock), Agrofarm PL.
- EFSA (September 2015): Estimation of animal intakes and HR, STMR and MRL calculations for products of animal origin.
- OECD ENV/JM/MONO(2013)8, Series on Pesticides No. 73: Guidance Document on Residues in Livestock.
- animal model 2016.xls (EFSA/OECD calculator).
- Sadler M. (2000): Dry matter content in mushrooms (Agaricus bisporus) as an indicator for mushroom quality.

At the same time, Agrofarm informs that it is holding consultations with NATIONAL VETERINARY INSTITUTE – NATIONAL RESEARCH INSTITUTE IN PUŁAWY, POLAND with a view to the possible development of a method together with full validation for determining pyriproxyfen residues in animal matrices.

Reply zRMS:
In principle, according to the SANTE/2020/12830 guidance document, validation data must be submitted for methods for risk assessment and monitoring for all analytes included in the residue definitions and for all representative sample matrices to be analysed at relevant concentration levels. The validated LOQ of residue analytical methods should generally be at the default MRL of 0.01 mg/kg for plant and animal commodities. A validation of the primary monitoring method in an independent laboratory (ILV) is required for the determination of residues in food of plant and animal origin and in drinking water. Analysis of food of animal origin is required to check for compliance with MRLs. The selection of analytes for which methods for food of animal origin are required depends on the definition of the residue for which an MRL is set or applied for according to Regulation (EC) No 396/2005.
Therefore analytical method and its independent laboratory validation (ILV) for the determination of residues of pyriproxyfen in animal matrices are required.

EFSA in EFSA Journal 2019;17(7):5732 conlcuded that *default residue definition for monitoring and risk assessment is set as pyriproxyfen for animal matrices. MRLs are not required for products of animal origin.*

Based on a comprehensive evaluation of the argumentation submitted by the Applicant in this Reporting Table, the zRMS has reviewed the regulatory justification regarding the requirement for analytical monitoring methods in matrices of animal origin for the active substance pyriproxyfen, in the context of the proposed specific use of the plant protection product Pyrix 20 SL exclusively in the cultivation of cultivated mushrooms.
There is no expectation of measurable residues in animal tissues, milk, or eggs resulting from this specific use. Therefore, in this particular and exceptional case of mushroom cultivation, the zRMS considers the arguments valid and supports the lifting of the data gap for analytical monitoring methods for pyriproxyfen in matrices of animal origin.
In this case, **no additional analytical method or its ILV is required for matrices of animal origin.**

However, if applicant is still able to provide a monitoring method and its ILV for animal matrices, the zRMS will evaluate them, as this would be beneficial for your future registrations of pyriproxyfen.

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

No new or additional studies have been submitted.

Please see the appendix attached to this document:
Expert Statement on the Classification of Indoor Mushroom Cultivation [Author: Marielle Fortin-McCuaig / EUROFINS Agroscience regulatory/ Completion date: 26 June 2025

At this point, Agrofarm refers to non-protected study and data already evaluated at EU level -see point A 2.1.2.3

Table 5.3-6: Validated methods for soil (if appropriate)
~~Not required when using Pyrix 20SL as indicated in GAP~~

Component of residue definition: Pyriproxyfen			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	GC-NPD	Wolf, S. (2002) /EU agreed not protected
Confirmatory	-	GC-MS	Wolf, S., 2002/EU agreed not protected

zRMS comments:
The method (report No.: NNA-0091) was previously evaluated in the DAR (November 2005) and was successfully validated for the analysis of pyriproxyfen in soil. The method was fully re-evaluated and considered acceptable for monitoring of pyriproxyfen residues in soil with an LOQ of 0.01 mg/kg (RAR, 2018).
The method is not protected according to the List of information, tests and studies which are considered as relied

upon by the RMS for the evaluation with a view to the renewal of the active substance (August 2019) and can be used in this application to support the use of Pyrix 20 SL.
No additional method is required.

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

No new or additional studies have been submitted.

Please see the appendix attached to this document:
Expert Statement on the Classification of Indoor Mushroom Cultivation [Author: Marielle Fortin-McCuaig / EUROFINS Agroscience regulatory/ Completion date: 26 June 2025
At this point, Agrofarm refers to non-protected study and data already evaluated at EU level -see point A 2.1.2.4

Table 5.3-7: Validated methods for water (if appropriate)
~~Not required when using Pyrix 20SL as indicated in GAP~~

Component of residue definition: pyriproxyfen				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year/missing/EU agreed**
Drinking water	Primary	0.1 µg/L	GC-MS	Merdian, H., 2009/EU agreed protected
	ILV	0.1 µg/L	GC-MS	Lindner, M., Leischow, J., 2015b/EU agreed protected
	Confirmatory	-	Not required	-
Surface water	Primary	0.001 µg/L		Lindner, M., Leischow, J., 2015a/EU agreed protected
	Confirmatory	-	Not required	-

zRMS comments:
The methods (report No.: NNA-0084 and NNA-0092) was previously evaluated in the DAR (November 2005).
The methods included in the DAR are revalidated in RAR (2018) and considered to be obsolete.

The methods of Merdian H., 2009, Lindner, M., Leischow, J., 2015a and 2015b are protected according to the List of information, tests and studies on which the RMS relies in its assessment for renewal of an active substance (August 2019) and cannot be used in this application to support the use of Pyrix 20 SL.
According to SANTE/2020/12830, an additional analytical method and its independent laboratory validation (ILV) for the determination of residues of pyriproxyfen in water are required (data gap).

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

No new or additional studies have been submitted.

Please see the appendix attached to this document:
Expert Statement on the Classification of Indoor Mushroom Cultivation [Author: Marielle Fortin-McCuaig / EUROFINS Agroscience regulatory/ Completion date: 26 June 2025

At this point, Agrofarm refers to non-protected study and data already evaluated at EU level -see point A

2.1.2.5

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

Not required when using Pyrix 20SL as indicated in GAP

Component of residue definition: Pyriproxyfen			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	1 µg/m^3	GC-NPD	Wais, A., 2001/EU agreed not protected
Confirmatory	-	Not required	-

zRMS comments:
The method (report No.: NNA-0085) was previously evaluated in the DAR (November 2005) and was successfully validated for the analysis of pyriproxyfen in air. The method was fully re-evaluated and considered acceptable for monitoring of pyriproxyfen residues in air with an LOQ of 1 µg/m^3 (RAR, 2018).
The method is not protected according to the List of information, tests and studies which are considered as relied upon by the RMS for the evaluation with a view to the renewal of the active substance (August 2019) and can be used in this application to support the use of Pyrix 20 SL.
No additional method is required.

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

No new or additional studies have been submitted.
Not required, not a toxic or very toxic compound. Pyriproxyfen is not classified with regard to health hazards.

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

NOT APPLICABLE

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

zRMS comments:
Pyriproxyfen residues in body tissues can be determined by using the monitoring method for residues in food of animal origin as stated in the EU Review of pyriproxyfen (EFSA Journal 2019;17(7):5732). An analytical method for monitoring of pyriproxyfen residues in body fluids was not provided during the EU Review of pyriproxyfen (EFSA Journal 2019;17(7):5732) leading to a data GAP.

In 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 the active substance and relevant metabolites*" and this is a new requirement of SANTE/2020/12830. According to the SANTE/2020/12830: "*Analytical methods for monitoring residues in body fluids and tissues are required for detection of active substances and/or metabolites in humans and animals after possible intoxications or for biomonitoring purposes, regardless of their toxicological classification*."
Therefore, an additional analytical method for the determination of residues of pyriproxyfen body fluids and tissues are required (data gap).

5.3.2.8 Other studies/ information

NOT APPLICABLE.

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	Agrofarm	March 2025	Analytical method for determining the content of pyriproxyfen in the product Pyrix 20 SL. Agrofarm A310322. (Procedure for determining pyriproxyfen in the product) source : Agrofarm Sp. z o.o. GLP Unpublished	No	Agrofarm Sp. z o.o.
KCP 5.1.1	B.Ciorga, M.Włodarczyk	May 2025	Final report. Validation of analytical method for the determination of active substance (pyriproxyfen) content in the test item PYRIX 20SL Test item :PYRIX 20 SL Study code :0012/0003/FA source: SORBOLAB Research Laboratory LLC GLP: YES Unpublished	No	Agrofarm Sp. z o.o.
KCP 5.1.2	Marielle Fortin-McCuaig / EUROFINS Agroscience regulatory/	2025	Expert Statement on the Classification of Indoor Mushroom Cultivation GLP- NO Unpublished	No	Agrofarm Sp. z o.o.
KCP 5.2	Marielle Fortin-McCuaig / EUROFINS Agroscience regulatory/	2025	Expert Statement on the Classification of Indoor Mushroom Cultivation GLP- NO Unpublished	No	Agrofarm Sp. z o.o.
KCP 5.2	Damian Gorczyca, PhD B.Stepien K.Baranowski N.Witaszak LymeLab Pharma sp. z o. o. sp. k	2025	**STUDY TITLE -FIELD PART:** Magnitude of the residue of pyriproxyfen (CAS 95737-68-1) in Mushrooms *Agaricus bisporus* (Raw Agricultural Commodity - RAC) grown in closed buildings conditions after one application of a formulated product of PYRIX 20 SL - one harvest and two decline curve trials in Northern Europe. Poland – 2025 Study number: LLP25-2 Harvest trial number: LLP25-2-01 Decline trial number: LLP25-2-02 Decline trial number: LLP25-2-03 Test Site : LymeLab Pharma sp. z o. o. sp. k. ;Cebulki 19 ; 13-124 Kozłowo, POLAND; Test Facility : LymeLab Pharma sp. z o. o. sp. k. ;Trylogii 2/16 ; 01-982 Warsaw, POLAND GAP-YES Unbuplished	No	Agrofarm Sp. z o.o.

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 5.2	J.Kicinska; A.Miszczak, Dr Food Safety Laboratory.The National Institute of Horticultural Research -Poland	2025	**STUDY TITLE - ANALITYCAL PART:** **1.**Determination of the residue of pyriproxyfen in mushrooms after application of "PYRIX 20 SL" in northen Europe-Poland 2025. Study code: 25/FSL/07 Year: 2025 Test Facility: Food Safety Laboratory.The National Institute of Horticultural Research; 13B Pomologiczna Street; 96-100 Skierniewice Poland GLP- YES Unpublished	No	Agrofarm Sp. z o.o.
KCP 5.2	J.Kicinska; A.Miszczak, Dr Food Safety Laboratory.The National Institute of Horticultural Research -Poland	2025	**STUDY TITLE - ANALITYCAL PART:** **2.**Determination of the residue of pyriproxyfen in mushrooms after application of "PYRIX 20 SL" in northen Europe-Poland 2025. Study code: 25/FSL/16 Year: 2025 Test Facility: Food Safety Laboratory.The National Institute of Horticultural Research; 13B Pomologiczna Street; 96-100 Skierniewice Poland GLP- YES Unpublished	No	Agrofarm Sp. z o.o.
~~A2.1.2.1.1a; A2.1.2.1.1.1a~~	~~J.Kicinska; A.Miszczak, Dr Food Safety Laboratory.The National Institute of Horticultural Research -Poland~~	~~2025~~	~~Determination of the residue of pyriproxyfen in mushrooms after application of "PYRIX 20 SL" in northen Europe-Poland 2025. Study code: 25/FSL/07 Year: 2025 Test Facility: Food Safety Laboratory.The National Institute of Horticultural Research; 13B Pomologiczna Street; 96-100 Skierniewice Poland GLP- YES Unpublished~~	~~No~~	~~Agrofarm Sp. z o.o.~~
~~A2.1.2.1.1b; A2.1.2.1.1.1b~~	~~J.Kicinska; A.Miszczak, Dr Food Safety Laboratory.The National Institute of Horticultural Research~~	~~2025~~	~~Determination of the residue of pyriproxyfen in mushrooms after application of "PYRIX 20 SL" in northen Europe-Poland 2025. Study code: 25/FSL/16 Year: 2025 Test Facility: Food Safety Laboratory.The National Institute of Horticultural Research; 13B Pomologiczna Street; 96-100 Skierniewice Poland GLP- YES~~	~~No~~	~~Agrofarm Sp. z o.o.~~

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
	~~Poland~~		~~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
KCP 5.1.2	SANTE/2020/12830, Rev.2 European Commission	2020	Guidance on pesticide Residue Analytical Methods for Risdk Assessment and Post-approval Control and Monitoring Puposes. Published	No	European Commission's Directorate-General for Health and Food Safety-
KCP 5.1.2	European Commite for Standarization (CEN): EN 15662:2018	2018	" Food of plant origin-Multimethod for determination of pesticide residue using GC- and LC-based analysis following acetronitrile extration/partitioning and clean-up by dispersive SPE- Modular QuEChERS0method". Published	No	European Commision
A2.1.2.1.1.4	SANTE/2020/12830, Rev.2	14. February 2023	Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes. Supersedes Guidance Documents SANCO/3029/992 and SANCO/825/003 Published	-	European Commision
A2.1.2.2 (5.3.2.3)	RMS Netherland	February 2009	Final addendum to the Draft Assessment Report (DAR) - public version -PYRIPROXYFEN. Addendum to Volume 3,B.7 Residue data (revision). Published	-	European Commision-RMS Netherland
A2.1.2.3 (5.3.2.4)	RMS Netherland	February 2009	Final addendum to the Draft Assessment Report (DAR) - public version -PYRIPROXYFEN. Addendum to Volume 3,B.7 Residue data (revision). Published		European Commision-RMS Netherland
A2.1.2.4 (5.3.2.5)	RMS Netherland	February 2009	Final addendum to the Draft Assessment Report (DAR) - public version -PYRIPROXYFEN. Addendum to Volume 3,B.7 Residue data (revision). Published		European Commision-RMS Netherland

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 the active substance pyriproxyfen

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

New studies have been submitted.

An overview on the acceptable method and possible data gaps for analysis of active substance pyriproxyfen plant protection product PYRIX 20 SL is provided as follows:

- Analytical method for determining the content of pyriproxyfen in the product Pyrix 20 SL. Agrofarm A310322. (Procedure for determining pyriproxyfen in the product)

author(s) : Agrofarm Sp. z o.o.
Issue no.: A030625 ; 23.06.2025
GLP : YES

- Final report. Validation of analytical method for the determination of active substance (pyriproxyfen) content in the test item PYRIX 20SL

Test item : PYRIX 20 SL
Study code : 0012/0003/FA
Author(s) : SORBOLAB Research Laboratory LLC ; May 2025
GLP: YES

Comments of zRMS:	The method has been successfully validated according to the guidance document SANTE/2020/12830 rev. 2 for the determination of pyriproxyfen in mushrooms with the LOQ of 0.01 mg/kg. Mean recoveries were in the range of 70 – 110% with relative standard deviations of ≤20% for mushrooms at each level (0.01 and 0.1 mg/kg). The method is acceptable.

Reference:	KCP 5.2
Report	Determination of the residue of pyriproxyfen in mushrooms after application of "PYRIX 20 SL" in northen Europe- Poland 2025 Author: Joanna Kicińska, 2025 Food Safety Laboratory.The National Institute of Horticultural Research Poland. Study code: 25/FSL/07
Guideline(s):	1.Guidance on pesticide Residue Analytical Methods for Risdk Assessment and Post-approval Control and Monitoring Puposes; SANTE/2020/12830, rev.2 Final determination by HPLC-MS/MS 2." Food of plant origin-Multimethod for determination of pesticide residue using GC- and LC-based analysis following acetronitrile extration/partitioning and clean-up by dispersive SPE- Modular QuEChERS0method".
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
Selectivity and Confirmation of Residue Identity

Final determination by HPLC-MS/MS was conducted by monitoring two MS/MS ion mass transitions for this analyte.

Analyte	Ion mass transition
Pyriproxyfen	322.1→96.0 (Quantitation)
	322.1→185.0 (Confirmation)

Transition proposed for quantification was chosen due to their enhanced sensitivity, but both ion mass transitions were applicable and interchangeably for quantification and confirmation.

The MS/MS ion spectra are included in the figures section (Appendix B) of Report.
Exemplary chromatograms for pyriproxyfen in mushrooms metrices are presented in Appendix D of the Report.

Matrix Effects

The matrix effects that the mushrooms extract has upon Pyriproxyfen during analysis were determined. Matrix effects were evaluated by comparing peak areas of matrix-matched standard with standard at equivalent concentrations prepared in a neat solvent. A negative value of the matrix effects indicates matrix suppression and a positive value for matrix effects indicate matrix enhancement. Matrix effects for **the quantitative and confirmatory transition were calculated as descibed in Table below :**

Table. Matrix effects calculation for mushrooms matrix

Matrix effect (%)	$= [(100 \cdot A_{Matrix\text{-}Std} \cdot C_{Solv\text{-}Std}) / (A_{Solv\text{-}Std} \cdot C_{Matrix\text{-}Std})] - 100$		
$A_{Solv\text{-}Std}$	Peak area of solvent standard		
$A_{Matrix\text{-}Std}$	Peak area of matrix-matched standard		
$C_{Solv\text{-}Std}$	Nominal Concentration of solvent standard in µg/mL		
$C_{Matrix\text{-}Std}$	Nominal Concentration of matrix-matched standard in µg/mL		
Matrix / Commodity	**Standard Concentration (µg/mL)**	**Matrix Effect for Pyriproxyfen (%)**	
		Quantification (322.1→96.0)	**Confirmation (322.1→185.0)**
Mushrooms	0.0005	-2	0
	0.001	-2	-3
	0.002	1	1
	0.005	2	1
	0.01	1	1
	0.02	0	0
	0.05	2	2
	Mean:	**0.2**	**0.4**

Matrix effects on the detection of Pyriproxyfen in extracts of mushrooms were lower than 20% and thus considered insignificant, according to SANTE/2020/12830, Rev.2 [2] guideline. Nevertheless, determination of analyte was performed using matrix-matched calibration standards.

Calibration

The linearity of the detector response for Pyriproxyfen was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels ranging from 0.0005 µg/mL to 0.05 µg/mL for mushrooms. This range correspond from 0.0025 mg/kg to 0.25 mg/kg for mushrooms thus covering the range from no more than 30% of the LOQ and at least + 20% of the highest analyte concentration level detected in samples.

The calibration curves obtained for both ion mass transitions of Pyriproxyfen for mushrooms were linear with the coefficients of correlation (R) greater than 0.99. Linear regression was performed with 1/x weighting.
Representative linear regression curves are included in the figures section (Appendix C) of the Report.

Quantification

Quantification was performed by using weighted (1/x) linear regression as described in the section "Calibration".

Limit of Quantification (LOQ) and Limit of Detection (LOD)

The LOQ of the method was defined as the lowest analyte concentration at which the methodology had been successfully validated. Thus, an LOQ of 0.01 mg/kg was confirmed for Pyriproxyfen in mushrooms matrices.

The limit of detection (LOD) for analyte were set at 0.0025 mg/kg for mushrooms which is < 30% of the LOQ. As can be seen from representative chromatograms in Appendix D the chromatographic peaks at the LOD were more than three times the background noise.

Recovery and repeatability

Accuracy was determined by fortification of control samples with known amount of the reference items and subsequent determination of the recoveries when applying the extraction procedure. Precision was determined by repeatability (relative standard deviation – RSD).

Five recovery determinations were performed at the LOQ (0.01 mg/kg) and at the 10 x LOQ (0.1 mg/kg) for mushrooms. Analysis was performed by extraction and single injection.

The mean recovery values of Pyriproxyfen at the fortification levels of 0.01 mg/kg and 0.1 mg/kg for both ion mass transitions were all in the range 60 - 120% and 70 - 120%, respectively, thus comply with the standard acceptance criteria of the guidance document SANTE/2020/12830, Rev.2 [2]. All precision values at the fortification levels of 0.01 mg/kg and 0.1 mg/kg for both ion mass transitions were < 20%. Detailed Recovery and procision results for Pyriproxyfen in mushrooms specimens are shown in Table below.

Table. Detailed accuracy and precision for Pyriproxyfen in mushrooms

Analyte	Matrix	Fortification Level (mg/kg)	Analyte Residue Found** (mg/kg)	Recovery (%)	Mean Rec. (%)	RSD (%)	n	Overall Mean Rec. (%)	Overall RSD (%)
Pyriproxyfen	Mushrooms	Ion Mass Transition 322.1→ 96.0 (Quantification)							
		0.01*	0.0101 0.0102 0.0099 0.0100 0.0102	101 102 99 100 102	101	1.3	5	99	2.6
		0.1	0.094 0.100 0.100 0.095 0.098	94 100 100 95 98	98	2.8	5		
		Ion Mass Transition 322.1→ 185.0 (Confirmation)							
		0.01*	0.0101 0.0101 0.0100 0.0099 0.0101	101 101 100 99 101	100	1.0	5	99	2.4
		0.1	0.094 0.100 0.100 0.095 0.098	94 100 100 95 98	97	2.6	5		

* Limit of quantification, defined by the lowest validated fortification level
** Residues in duplicate control (unfortified) specimen samples were below LOD

Stability check in analytical sequence

Recoveries of the fortified samples within the acceptable range obtained with calibration solutions and the use of bracketing standards at LOQ level to ensure the integrity of the analytical sequence sufficiently demonstrated the stability of the analyte in the final dilution. Every 10 sample a calibration standard was analyzed. Maximum the drift between two bracketing injections of the same calibration standard at the start and end of a sample sequence did not exceed 30% (taking the higher response as 100%). Detailed results of stability of the analytical sequences for Pyriproxyfen in mushrooms specimens are shown in Table below.

Table. Tesults of stability of the analytical sequence at LOQ level for Pyriproxyfen in mushrooms specimens

Analyte	Matrix	Response at the start of the analytical sequence (LOQ level)	Response at the end of the analytical sequence (LOQ level)	Difference (%)
Pyriproxyfen	Mushrooms	Ion Mass Transition 322.1→96.0 (Quantification)		
		26241	27531	4.9
		Ion Mass Transition 322.1→185.0 (Confirmation)		
		6068	6310	4.0

System Suitability Test

System suitability test (SST) was performed to verify the performance of HPLC-MS/MS system and to ensure its adequacy for Pyriproxyfen determination in mushrooms. For the purpose, at the beginning of analytical sequence the matrix-matched calibration at a concentration level corresponding to LOQ in the sample was injected in triplicate. %RSD of Pyriproxyfen peak area was calculated for the first and second transition. The results of system suitability test were found within the acceptable range (peak area %RSD ≤ 15%) indicating that the system was suitable for the intended analysis.

Table. Results of system suitability test for mushrooms sequence

Parameters		Pyriproxyfen (mushrooms)	
		Quantification (322.1→96.0)	Confirmation (322.1→185.0)
Mushrooms	Replicate #1	27555	6385
	Replicate #2	27456	6389
	Replicate #3	27660	6461
Average		27557	6412
Std. Dev.		102	43
%RSD		0.4	0.670
Parameter Limit %RSD		≤ 15%	≤ 15%
Conformity		YES	YES

Final extract stability

The final extracts were analysed within 24 hours. Therefore, testing the final extract stability is not required according to SANTE/2020/12830, Rev.2 [2].

Stability of Analyte in Working Solutions

Working solutions used for preparation of the calibration samples were stored in refrigerator at 5°C ± 4°C during study. At the end of experimental phase, the solution used for preparation of the calibration level at 0.002 µg/mL was reanalyzed by quintuple injection and compared to a freshly prepared working solution from a new stock standard solution. Two ion mass transitions were used for evaluation stability of the analyte in the working solutions. The mean value from the new mix solution is taken to be 100% and is also used as a basis for the calculation of the percentage difference.

Difference beetween stored and newly prepared solution is ± 10%. The results indocated that Pyriproxyfen working solution prepared in acetonitrile was stab;e durong 3 days under refrigerated conditions. Details are shown in table below.

Table.Stability of Pyriproxyfen in working solution

Calibration Level (µg/mL)	Date of Preparation (dd.mm.yy)	Working Solution ID	Response	Mean peak area	RSD (%)	Difference (%)
Pyriproxyfen Ion Mass Transition 322.1→96.0(Quantification)						
0.002 (LOQ)	28.03.2025	1S1W9	29297/29610/28952/29049/29926	29367	1.4	-0.93
	31.03.2025	1S2W4	29827/28901/29752/30141/29587	29642	1.6	
Pyriproxyfen Ion Mass Transition 322.1→ 185.0 (Confirmation)						
0.002 (LOQ)	28.03.2025	1S1W9	6744/6830/6703/6701/6902	6776	1.3	-0.47
	31.03.2025	1S2W4	6865/6644/6871/6878/6783	6808	1.5	

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Mushrooms	pyriproxyfen	Ion Mass transition 322.1→ 96.0 (Quantification)			
		0.01*	101	1.3	Overall mean recovery: 99 % Overall RSD : 2.6 %
		0.1	98	2.8	
		Ion Mass transition 322.1→ 185.0 (Confirmation)			
		0.01*	100	1.0	Overall mean recovery: 99 % Overall RSD : 2.4 %
		0.1	97	2.6	

Table A 2: Characteristics for the analytical method used for validation of pyriproxyfen residues in mushrooms

	pyriproxyfen
Specificity	MS/MS product ion product spectra are presented Solvent blank, residues of pyriproxyfen < LOD Reagent blank(procedural), residues of pyriproxyfen < LOD
Calibration (type, number of data points)	individual calibration data presented| calibration line equation presented
Calibration range	Accepted calibration range in concentration units ranging from 0.0005 µg/mL to 0.05 µg/mL. This range corresponde from 0.0025 mg/kg to 0.25 mg/kg form mushrooms samples.
Assessment of matrix effects is presented	yes
Limit of determination/quantification	limit of quantification representing the lowest validated level with sufficient recovery and precision 0.01 mg/kg

Conclusion

Analythical method for determination of Pyriproxyfen in mushroom matrix was sufficiently validated in

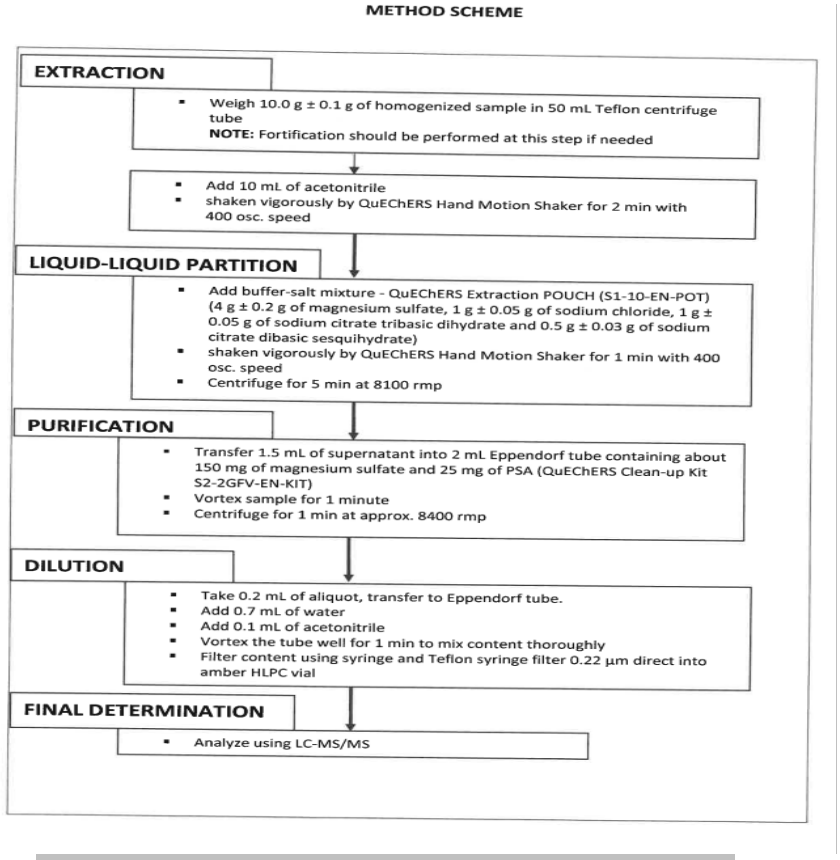
accordance to the guidance document SANTE/2020/12830, rev.2 of the European Commission. The validated limit of quantification for Pyriproxyfen is 0.01 mg/kg in mushrooms.

A 2.1.2.1.1.1b

Reference:	Determination of the residue of pyriproxyfen in mushrooms.
Report	Determination of the residue of pyriproxyfen in mushrooms after application of "PYRIX 20 SL" in northen Europe- Poland 2025. Study code: 25/FSL/16 Year: 2025 Test Facility: Food Safety Laboratory. The National Institute of Horticultural Research; 13B Pomologiczna Street; 96-100 Skierniewice Poland GLP- YES
Guideline(s):	YES 1.Guidance on pesticide Residue Analytical Methods for Risdk Assessment and Post-approval Control and Monitoring Puposes. Final determination by HPLC-MS/MS 2." Food of plant origin-Multimethod for determination of pesticide residue using GC- and LC-based analysis following acetronitrile extration/partitioning and clean-up by dispersive SPE- Modular QuEChERS0method".
Deviations:	No
GLP:	YES
Acceptability:	YES

Explonations:

The analytical method for the determination of pyriproxyfen used in this study was successfully validated in Mushrooms as a representative high-water context matrix. **The validation data were presented in *Final Report No. 25/FSL/05 at point 5 [Determination of the residue of pyriproxyfen in mushrooms after applica-tion of "PYRIX 20 SL" in northen Europe- Poland 2025,Point 5 METHOD VALIDATION)].***
Method validation description - see point A 2.1.2.1.1.1a.
The general principles of the analythical procedure are presented below:



A 2.1.1.1.1.1 Independent laboratory validation

NOT REQUIRED.

Analytical method for the determination of pyriproxyfen was successfully validated

A 2.1.1.1.1.2 Confirmatory method (if required)

NOT REQUIRED.
Analytical method for the determination of pyriproxyfen was successfully validated

A 2.1.1.1.1.3 Extraction efficiency

The final extracts were analysed within 24 hours. Therefore, testing the final extract stability is not required according to SANTE/2020/12830, Rev.2. *: Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes.Supersedes Guidance Documents SANCO/3029/992 and SANCO/825/003; 14. February 2023*

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)

New No studies have been submitted

~~A 2.1.2.1.1 Analytical method~~

~~A 2.1.2.1.1a~~
~~Determination of the residue of pyriproxyfen in mushrooms after application of "PYRIX 20 SL" in~~

northen Europe- Poland 2025.
Study code: 25/FSL/07
Year: 2025
Test Facility: Food Safety Laboratory.The National Institute of Horticultural Research; 13B Pomologiczna Street; 96-100 Skierniewice Poland
GLP- YES

A 2.1.2.1.1b
Determination of the residue of pyriproxyfen in mushrooms after application of "PYRIX 20 SL" in northen Europe- Poland 2025.
Study code: 25/FSL/16
Year: 2025
Test Facility: Food Safety Laboratory. The National Institute of Horticultural Research; 13B Pomologiczna Street; 96-100 Skierniewice Poland
GLP- YES

A 2.1.2.1.1.1 Method validation

A 2.1.2.1.1.1a

Reference:	Determination of the residue of pyriproxyfen in mushrooms. The Method Validation is part of the report of study: Determination of the residue of pyriproxyfen in mushrooms after ap plication of "PYRIX 20 SL" in northen Europe- Poland 2025.
Report	Determination of the residue of pyriproxyfen in mushrooms after application of "PYRIX 20 SL" in northen Europe- Poland 2025. (Point 5 METHOD VALIDATION) Food Safety Laboratory.The National Institute of Horticultural Research Poland. Study code: 25/FSL/07 Year: 2025
Guideline(s):	YES 1.Guidance on pesticide Residue Analytical Methods for Risdk Assessment and Post approval Control and Monitoring Puposes. Final determination by HPLC-MS/MS 2." Food of plant origin Multimethod for determination of pesticide residue using GC and LC based analysis following acetronitrile extration/partitioning and clean-up by dispersive SPE- Modular QuEChERS0method".
Deviations:	No
GLP:	YES
Acceptability:	YES

Materials and methods
Selectivity and Confirmation of Residue Identity

Final determination by HPLC-MS/MS was conducted by monitoring two MS/MS ion mass transitions for this analyte.

Analyte	Ion mass transition
Pyriproxyfen	322.1→96.0 (Quantitation)
	322.1→185.0 (Confirmation)

Transition proposed for quantification was chosen due to their enhanced sensitivity, but both ion mass transitions were applicable and interchangeably for quantification and confirmation.

The MS/MS ion spectra are included in the figures section (Appendix B) of Report.
Exemplary chromatograms for pyriproxyfen in mushrooms metrices are presented in Appendix D of the

Report.

Matrix Effects

The matrix effects that the mushrooms extract has upon Pyriproxyfen during analysis were determined. Matrix effects were evaluated by comparing peak areas of matrix-matched standard with standard at equivalent concentrations prepared in a neat solvent. A negative value of the matrix effects indicates matrix suppression and a positive value for matrix effects indicate matrix enhancement. Matrix effects for the quantitative and confirmatory transition were calculated as descibed in Table below :

Table. Matrix effects calculation for mushrooms matrix

Matrix effect (%)	$= [(100 \cdot A_{Matrix\text{-}Std} \cdot C_{Solv\text{-}Std}) / (A_{Solv\text{-}Std} \cdot C_{Matrix\text{-}Std})] - 100$		
$A_{Solv\text{-}Std}$	Peak area of solvent standard		
$A_{Matrix\text{-}Std}$	Peak area of matrix-matched standard		
$C_{Solv\text{-}Std}$	Nominal Concentration of solvent standard in µg/mL		
$C_{Matrix\text{-}Std}$	Nominal Concentration of matrix-matched standard in µg/mL		
Matrix / Commodity	**Standard Concentration (µg/mL)**	**Matrix Effect for Pyriproxyfen (%)**	
		Quantification (322.1→96.0)	**Confirmation (322.1→185.0)**
Mushrooms	0.0005	-2	0
	0.001	-2	-3
	0.002	1	1
	0.005	2	1
	0.01	1	1
	0.02	0	0
	0.05	2	2
	Mean:	**0.2**	**0.4**

Matrix effects on the detection of Pyriproxyfen in extracts of mushrooms were lower than 20% and thus considered insignificant, according to SANTE/2020/12830, Rev.2 [2] guideline. Nevertheless, determination of analyte was performed using matrix-matched calibration standards.

Calibration

The linearity of the detector response for Pyriproxyfen was demonstrated by single determination of matrix-matched calibration standards at seven concentration levels ranging from 0.0005 µg/mL to 0.05 µg/mL for mushrooms. This range correspond from 0.0025 mg/kg to 0.25 mg/kg for mushrooms thus covering the range from no more than 30% of the LOQ and at least + 20% of the highest analyte concentration level detected in samples.

The calibration curves obtained for both ion mass transitions of Pyriproxyfen for mushrooms were linear with the coefficients of correlation (R) greater than 0.99. Linear regression was performed with 1/x weighting.

Representative linear regression curves are included in the figures section (Appendix C) of the Report.

Quantification

Quantification was performed by using weighted (1/x) linear regression as described in the section "Calibration".

Limit of Quantification (LOQ) and Limit of Detection (LOD)

The LOQ of the method was defined as the lowest analyte concentration at which the methodology had been successfully validated. Thus, an LOQ of 0.01 mg/kg was confirmed for Pyriproxyfen in mushrooms matrices.

The limit of detection (LOD) for analyte were set at 0.0025 mg/kg for mushrooms which is < 30% of the LOQ. As can be seen from representative chromatograms in Appendix D the chromatographic peaks at the LOD were more than three times the background noise.

Recovery and repeatability

Accuracy was determined by fortification of control samples with known amount of the reference items and subsequent determination of the recoveries when applying the extraction procedure. Precision was determined by repeatability (relative standard deviation – RSD).

Five recovery determinations were performed at the LOQ (0.01 mg/kg) and at the 10 x LOQ (0.1 mg/kg) for mushrooms. Analysis was performed by extraction and single injection.

The mean recovery values of Pyriproxyfen at the fortification levels of 0.01 mg/kg and 0.1 mg/kg for both ion mass transitions were all in the range 60 - 120% and 70 - 120%, respectively, thus comply with the standard acceptance criteria of the guidance document SANTE/2020/12830, Rev.2 [2]. All precision values at the fortification levels of 0.01 mg/kg and 0.1 mg/kg for both ion mass transitions were < 20%. Detailed Recovery and procision results for Pyriproxyfen in mushrooms specimens are shown in Table below.

Table. Detailed accuracy and precision for Pyriproxyfen in mushrooms

Analyte	Matrix	Fortification Level (mg/kg)	Analyte Residue Found** (mg/kg)	Recovery (%)	Mean Rec. (%)	RSD (%)	n	Overall Mean Rec. (%)	Overall RSD (%)
Pyriproxyfen	Mushrooms	Ion Mass Transition 322.1→ 96.0 (Quantification)							
		0.01*	0.0101 0.0102 0.0099 0.0100 0.0102	101 102 99 100 102	101	1.3	5	99	2.6
		0.1	0.094 0.100 0.100 0.095 0.098	94 100 100 95 98	98	2.8	5		
		Ion Mass Transition 322.1→ 185.0 (Confirmation)							
		0.01*	0.0101 0.0101 0.0100 0.0099 0.0101	101 101 100 99 101	100	1.0	5	99	2.4
		0.1	0.094 0.100 0.100 0.095 0.098	94 100 100 95 98	97	2.6	5		

* Limit of quantification, defined by the lowest validated fortification level
** Residues in duplicate control (unfortified) specimen samples were below LOD

Stability check in analytical sequence

Recoveries of the fortified samples within the acceptable range obtained with calibration solutions and the use of bracketing standards at LOQ level to ensure the integrity of the analytical sequence sufficiently demonstrated the stability of the analyte in the final dilution. Every 10 sample a calibration standard was analyzed. Maximum the drift between two bracketing injections of the same calibration standard at the start and end of a sample sequence did not exceed 30% (taking the higher response as 100%). Detailed results of stability of the analytical sequences for Pyriproxyfen in mushrooms specimens are shown in Table below.

Table. Results of stability of the analytical sequence at LOQ level for Pyriproxyfen in mushrooms specimens

Analyte	Matrix	Response at the start of the analytical sequence (LOQ level)	Response at the end of the analytical sequence (LOQ level)	Difference (%)
Pyriproxyfen	Mushrooms	Ion Mass Transition 322.1→96.0 (Quantification)		
		26241	27531	4.9
		Ion Mass Transition 322.1→185.0 (Confirmation)		
		6068	6310	4.0

System Suitability Test

System suitability test (SST) was performed to verify the performance of HPLC-MS/MS system and to ensure its adequacy for Pyriproxyfen determination in mushrooms. For the purpose, at the beginning of analytical sequence the matrix-matched calibration at a concentration level corresponding to LOQ in the sample was injected in triplicate. %RSD of Pyriproxyfen peak area was calculated for the first and second transition. The results of system suitability test were found within the acceptable range (peak area %RSD ≤ 15%) indicating that the system was suitable for the intended analysis.

Table. Results of system suitability test for mushrooms sequence

Parameters		Pyriproxyfen (mushrooms)	
		Quantification (322.1→96.0)	Confirmation (322.1→185.0)
Mushrooms	Replicate #1	27555	6385
	Replicate #2	27456	6389
	Replicate #3	27660	6461
Average		27557	6412
Std. Dev.		102	43
%RSD		0.4	0.670
Parameter Limit %RSD		≤ 15%	≤ 15%
Conformity		YES	YES

Final extract stability

The final extracts were analysed within 24 hours. Therefore, testing the final extract stability is not required according to SANTE/2020/12830, Rev.2 [2].

Stability of Analyte in Working Solutions

Working solutions used for preparation of the calibration samples were stored in refrigerator at 5°C ± 4°C during study. At the end of experimental phase, the solution used for preparation of the calibration level at 0.002 µg/mL was reanalyzed by quintuple injection and compared to a freshly prepared working solution from a new stock standard solution. Two ion mass transitions were used for evaluation stability of the analyte in the working solutions. The mean value from the new mix solution is taken to be 100% and is also used as a basis for the calculation of the percentage difference.

Difference beetween stored and newly prepared solution is ± 10%. The results indocated that Pyriproxyfen working solution prepared in acetonitrile was stab;e durong 3 days under refrigerated conditions. Details are shown in table below.

Table. Stability of Pyriproxyfen in working solution

Calibration Level (µg/mL)	Date of Preparation (dd.mm.yy)	Working Solution ID	Response	Mean peak area	RSD (%)	Difference (%)
Pyriproxyfen Ion Mass Transition 322.1→96.0(Quantification)						
0.002 (LOQ)	28.03.2025	1S1W9	29297/29610/28952/29049/29926	29367	1.4	-0.93
	31.03.2025	1S2W4	29827/28901/29752/30141/29587	29642	1.6	
Pyriproxyfen Ion Mass Transition 322.1→ 185.0 (Confirmation)						
0.002 (LOQ)	28.03.2025	1S1W9	6744/6830/6703/6701/6902	6776	1.3	-0.47
	31.03.2025	1S2W4	6865/6644/6871/6878/6783	6808	1.5	

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Mushrooms	pyriproxyfen	Ion Mass transition 322.1→ 96.0 (Quantification)			
		0.01*	101	1.3	Overall mean recovery: 99 % Overall RSD : 2.6 %
		0.1	98	2.8	
		Ion Mass transition 322.1→ 185.0 (Confirmation)			
		0.01*	100	1.0	Overall mean recovery: 99 % Overall RSD : 2.4 %
		0.1	97	2.6	

Table A 4: Characteristics for the analytical method used for validation of pyriproxyfen residues in mashrooms

	pyriproxyfen
Specificity	MS/MS product ion product spectra are presented Solvent blank, residues of pyriproxyfen < LOD Reagent blank(procedural), residues of pyriproxyfen < LOD
Calibration (type, number of data points)	individual calibration data presented| calibration line equation presented
Calibration range	Accepted calibration range in concentration units ranging from 0.0005 µg/mL to 0.05 µg/mL. This range corresponde from 0.0025 mg/kg to 0.25 mg/kg form mushrooms samples.
Assessment of matrix effects is presented	yes
Limit of determination/quantification	limit of quantification representing the lowest validated level with sufficient recovery and precision

Conclusion

Analythical method for determination of Pyriproxyfen in mushroom matrix was sufficiently validated in

accordance to the guidance document SANTE/2020/12830, rev.2 of the European Commission. The validated limit of quantification for Pyriproxyfen is 0.01 mg/kg in mushrooms.

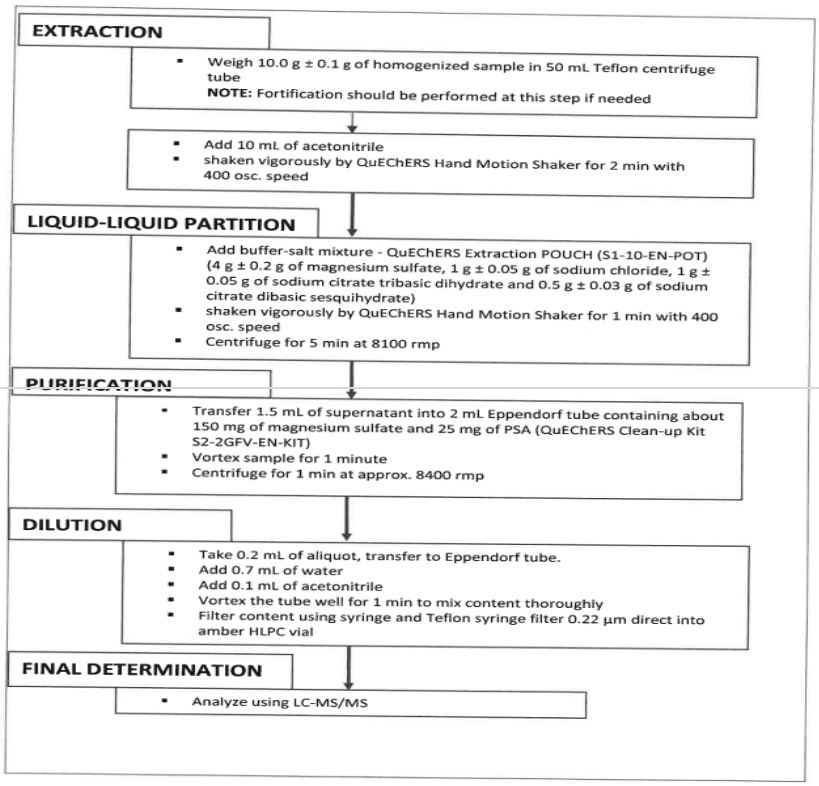
A 2.1.2.1.1.1b

Reference:	Determination of the residue of pyriproxyfen in mushrooms.
Report	Determination of the residue of pyriproxyfen in mushrooms after application of "PYRIX 20 SL" in northen Europe Poland 2025. Study code: 25/FSL/16 Year: 2025 Test Facility: Food Safety Laboratory. The National Institute of Horticultural Research; 13B Pomologiczna Street; 96-100 Skierniewice Poland GLP YES
Guideline(s):	YES 1.Guidance on pesticide Residue Analytical Methods for Risdk Assessment and Post approval Control and Monitoring Puposes. Final determination by HPLC-MS/MS 2." Food of plant origin-Multimethod for determination of pesticide residue using GC and LC based analysis following acetronitrile extration/partitioning and clean up by dispersive SPE Modular QuEChERS0method".
Deviations:	No
GLP:	YES
Acceptability:	YES

Explonations:

The analytical method for the determination of pyriproxyfen used in this study was successfully validated in Mushrooms as a representative high water context matrix. **The validation data were presented in *Final Report No. 25/FSL/05 at point 5 [Determination of the residue of pyriproxyfen in mushrooms after applica tion of "PYRIX 20 SL" in northen Europe Poland 2025,Point 5 METHOD VALIDATION)].***
Method validation descriptionSescription – see point A 2.1.2.1.1.1a.
The general principles of the analythical procedure are presented below:

METHOD SCHEME



A 2.1.2.1.1.2 Independent laboratory validation

NOT REQUIRED.

Analytical method for the determination of pyriproxyfen was successfully validated

A 2.1.2.1.1.3 Confirmatory method (if required)

NOT REQUIRED.
Analytical method for the determination of pyriproxyfen was successfully validated

A 2.1.2.1.1.4 Extraction efficiency

The final extracts were analysed within 24 hours. Therefore, testing the final extract stability is not required according to SANTE/2020/12830, Rev.2. *: Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes.Supersedes Guidance Documents SANCO/3029/992 and SANCO/825/003; 14. February 2023*

A 2.1.2.1.2 Analytical method 2

NOT APPLICABLE

A 2.1.2.1.3 Extraction efficiency

NOT APPLICABLE

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.

Since mushrooms are not normally fed to livestock, analythical methods for determination of pyriproxyfen residues in animal matrices not assessed in the framework of this application.

At this point, Agrofarm refers to non-protected study and data already evaluated at EU level.

Source: *Final addendum to the Draft Assessment Report (DAR) - public version -PYRIPROXYFEN, February 2009. Addendum to Volume 3,B.7 Residue data (revision).*

STUDY 1

Characteristics

Reference	:	Weber, H., Pelz, S., 2000 (IIA 4.2.1/01)	GLP statement	:	Yes
Type of study	:	Analytical methods for plants, animal tissues, milk and eggs	Guidelines	:	Directive 96/46/EC 1996 Draft working document 8064/VI/97 rev. 4 of 15 Dec '98
Year of execution	:	1999-2000	Acceptability	:	Acceptable for pre-registration
Test substance	:	Pyriproxyfen (Lot no. 981021G, purity 99.8%)	Method reference	:	DFG method S19 (extended revision, Modular Multi Method L00.00-34)

Description of the method

The method is based on multi-method S19 (DFG). Samples (cucumber fruit) were homogenized in acetone/water 2:1 (v/v, taking into account the water content of the sample). After addition of sodium chloride the mixture was partitioned using ethylacetate/cyclohexane 1:1 (v/v). The organic phase was partially evaporated and redissolved in ethyl acetate, after which sodium sulphate, sodium chloride and cyclohexane were added. The organic phase was subjected to gel permeation chromatography. The eluate was evaporated and redissolved in ethyl acetate before analysis by GC (Fused silica capillary column DB-5MS (J&W), methyl silicone with 5% phenyl groups, 30m x 0.25 mm i.d., film thickness 0.25 µm) with MS detection (m/z 136 (quantification) and 78, 226 (verification)). Reference standards prepared in solution were used as linearity standards (detector) and used for quantification in recovery samples (one point calibration at appropriate levels).

Conclusion
The QuEChERS method is validated for food/feed of animal origin. The primary validation was performed using bovine meat, fat and liver, poultry eggs and bovine milk. The LOQ is 0.01 mg/kg, using LC-MS/MS. An ILV was performed on milk, meat and fat. The method requires the use of matrix-matched standards. Analytical method 1
No new studies have been submitted

A 2.1.2.2.1.1 Method validation

No new studies have been submitted.

A 2.1.2.2.1.2 Independent laboratory validation

NONE /NOT APPLICABLE.
No new studies have been submitted.

A 2.1.2.2.1.3 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.2.2.1.4 Extraction efficiency

NONE /NOT APPLICABLE.
No new studies have been submitted.

A 2.1.2.2.2 Analytical method 2

NOT APPLICABLE.

A 2.1.2.2.2.1 Extraction efficiency

NOT APPLICABLE.

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

No new or additional studies have been submitted.
At this point, Agrofarm refers to non-protected study and data already evaluated at EU level

Source: *Final addendum to the Draft Assessment Report (DAR) - public version -PYRIPROXYFEN, February 2009. Addendum to Volume 3,B.7 Residue data (revision).*

STUDY 1

Characteristics

Reference	:	Wolf, S., 2002a (IIA 4.2.2/01)	GLP statement	:	Yes
Type of study	:	Analytical methods for soil, air and water	Guidelines	:	Directive 96/46/EC SANCO/825/00 rev. 6, 20 June 2000 SANCO/3029/99 rev. 4, 11 July 2000 Nachrichtenbl. Deut. Pflanzenschutzd. 52, 2000, p 292
Year of execution	:	2002	Acceptability	:	Acceptable
Test substance	:	Pyriproxyfen (Lot no. 991222G, purity 100%)	Method reference	:	BBA multi method L 00.00-34 (§ 35 LMBG, Nov. 1999)

Description of the method

The method is a multi method (BBA multi method L 00.00-34). Soil (sandy loam, 10/32/58 % clay/silt/sand, pH 6.3, 50 g dry mass) was extracted with acetone/water 2:1 (v/v, taking into account the water content of the sample) and filtered over Celite. The extract was partitioned using ethylacetate/cyclohexane 1:1 (v/v). The organic phase was evaporated until dryness, redissolved in ethylacetate/cyclohexane and subjected to gel permeation chromatography. The eluate was evaporated until dryness and redissolved in ethyl acetate. After addition of iso-octane, the residue was partially evaporated and passed through a silica gel column using subsequently hexane/toluene 65/35 (v/v) (twice), toluene (twice) and toluene-acetone 95/5 (v/v) (twice). The toluene-acetone eluate was evaporated to dryness and redissolved in toluene before analysis by GC/NPD (quantification, DB-17 column 18 m x 0.18 mm (0.3 µm)) and GC/MSD (verification, DB-17 column 20 m x 0.18 mm (0.3 µm), m/z 226 and (qualification) m/z 186, 136, 96). Reference standards prepared in toluene were used as calibration standards (calibration curve $Y=AX^B$). Although r>0.99 (recalculated by RMS), linear regression yields deviations >20% for concentrations at the lower end of the calibration curve. Regression using $Y=AX^B$ covers the whole calibration range (0.01 – 1.0 mg/L), with r>0.997.

Conclusion
BBA multi method L 00.00-34 is considered suitable for the determination of pyriproxyfen in soil with an LOQ of 0.01 mg/kg.

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

No new or additional studies have been submitted.
At this point, Agrofarm refers to non-protected study and data already at EU level

Source: *Final addendum to the Draft Assessment Report (DAR) - public version -PYRIPROXYFEN,*

February 2009. Addendum to Volume 3,B.7 Residue data (revision).

STUDY -determination of pyriproxyfen in tap water

Characteristics

Reference	:	Wolf, S., 2002b (IIA 4.2.3/01)	GLP statement	:	Yes
Type of study	:	Analytical methods for soil, air and water	Guidelines	:	Directive 96/46/EC SANCO/825/00 rev. 6, 20 June 2000 SANCO/3029/99 rev. 4, 11 July 2000 Nachrichtenbl. Deut. Pflanzenschutzd. 52, 2000, p 292
Year of execution	:	2002	Acceptability	:	Acceptable
Test substance	:	Pyriproxyfen (Lot no. 991222G, purity 100%)	Method reference	:	F12 (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlamm-untersuchung)

Description of the method

The method is a multi-residue method (F12, Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung). Subsequently tap water (1L), distilled water, air (for drying) and acetone were passed through a SPE C18 column. The acetone eluate was evaporated to an aqueous residue, to which ethanol was added followed by evaporation until dryness. The residue was reconstituted in toluene and was analyzed by GC/NPD (quantification, DB-17 column 18 m x 0.18 mm (0.3 µm)) and verified by GC/MS (DB-17 column 20 m x 0.18 mm (0.3 µm), m/z 226 and (qualification) m/z 186, 136, 96). Reference standards prepared in solution were used as calibration standards (calibration curve $Y=AX^B$). Although r>0.99 (recalculated by RMS), linear regression yields deviations >20% for concentrations at the lower end of the calibration curve. Regression using $Y=AX^B$ covers the whole calibration range (0.01 – 1.0 mg/L), with r>0.997.

Conclusion

Multi residue method F12 (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung) is considered suitable for the **determination of pyriproxyfen in tap water (LOQ 0.1 µg/L).**

Source: *Final addendum to the Draft Assessment Report (DAR) - public version -PYRIPROXYFEN, February 2009. Addendum to Volume 3,B.7 Residue data (revision).*

STUDY -determination of pyriproxyfen in surface water

Characteristics

Reference	:	Wais, A., 2001a (IIA 4.2.3/02)	GLP statement	:	Yes
Type of study	:	Analytical methods for soil, air and water	Guidelines	:	Directive 96/46/EC EC guidance 8064/VI/97 rev 4, 15 Dec 1998 SANCO/3029/99 rev. 4, 11 July 2000
Year of execution	:	2000	Acceptability	:	Acceptable
Test substance	:	Pyriproxyfen (Lot no. 991222G, purity 100%)	Method reference	:	F12 (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlamm-untersuchung)

Description of the method

The method is a multi-residue method (F12, Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung). Subsequently surface water (1L, river water, TOC 32.8 mg/L, pH 7.85), distilled water, air (for drying) and hexane were passed through a SPE C18 column. The hexane was evaporated to an aqueous residue, to which methanol was added followed by evaporation until dryness. The residue was reconstituted in toluene and analyzed by GC/NPD (quantification, DB-17 column 20 m x 0.18 mm (0.3 µm)) and verified by GC/MS (DB-17 column 20 m x 0.18 mm (0.3 µm), m/z 226 and (qualification) m/z 186, 136, 96). Reference standards prepared in solution were used as calibration standards.

Conclusion
Multi residue method F12 (Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung) is considered suitable for the determination of pyriproxyfen in surface water (LOQ 0.01 µg/L) in pre-registration studies and for post-registration monitoring purposes.

Remarks
The LOQ of 0.01 µg/L is below the level of the lowest appropriate toxicity value for aquatic organisms (in this case the NOEC for daphnia (0.015 µg/L) (See B.9.2.3.1.2)). The established LOQ is >NOEC/10 (i.e. including the risk assessment safety factor of 10).

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

No new or additional studies have been submitted.
At this point, Agrofarm refers to non-protected study and data already at EU level.

Source: *Final addendum to the Draft Assessment Report (DAR) - public version -PYRIPROXYFEN, February 2009. Addendum to Volume 3,B.7 Residue data (revision).*

STUDY- analysis of pyriproxyfen in air
Characteristics

Reference	:	Wais, A., 2001b (IIA 4.2.4/01)	GLP statement	:	Yes
Type of study	:	Analytical methods for soil, air and water	Guidelines	:	Directive 96/46/EC EC guidance 8064/VI/97 rev 4, 15 Dec 1998 SANCO/3029/99 rev. 4, 11 July 2000
Year of execution	:	2000-2001	Acceptability	:	Acceptable
Test substance	:	Pyriproxyfen (Lot no. 991222G, purity 100%)	Method reference	:	Not indicated

Description of the method

The front sections of Tenax adsorption tubes (100 mg adsorbent in the front and 50 mg in the back section) were fortified with pyriproxyfen (0.36 and 3.6 µg). Air was passed through each tube at a rate of 1L/min for 6 hours (total 360 L, equivalent to 1.0 and 10 µg/m3 of pyriproxyfen, respectively). For both concentrations, air conditions were ~20°C, ~30% rH and ~35°C, ≥ 80% rH. Front and back sections were extracted separately in toluene. Extracts were analyzed by GC/NPD (DB-17 column, 20 m x 0.18 mm, 0.3 µm film thickness). Results were confirmed by GC/MS (DB-17 column, 20 m x 0.18 mm, 0.3 µm film thickness, m/z 226 (and m/z 186, 136, 96 and 77 for qualification)). Reference standards prepared in toluene were used as calibration standards (calibration curve $Y=AX^B$).

Conclusion
The method is considered suitable for the analysis of pyriproxyfen in air with an LOQ of 1.0 µg/m3 (sampling at a rate of 1L/min during 6 hours; conditions ~20°C, ~30% rH and ~35°C, ≥80% rH). The LOQ (1 µg/m3) is sufficient according to SANCO/825/00 as it is below the limit of 15 µg/m3 calculated using: limit = AOELsystemic*0.1*60/20.

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.
Not required, not a toxic or very toxic compound. Pyriproxyfen is not classified with regard to health hazards.

A 2.1.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted

A 2.2 Analytical methods for the active substance 2

NOT APPLICABLE.

Appendix 2. List of method required

Table A3. Completeness check of analythical method for post-approval control and monitoring purposes

Matrix group/crop group	Residue definition for monitoring	LOQ	Methods		
			Primary method	Confirmatory Method (EU evaluated)	Independent lab validation
Dry commodities (high protein/ high starch content) Wheat grain	pyriproxyfen	LOQ 0.01 mg/kg	-	Multi-method, monitoring (EU evaluated LC-MS/MS 2014	European Standard method EN 15662 MRM QuEChERS (LC-MS/MS) European Standard method EN 15662 MRM QuEChERS (GC-MS)
Commodities with high water content: 1.cucumber 2.apple	pyriproxyfen	LOQ 0.01 mg/kg	-	Multi-method, monitoring (EU evaluated) GC-MS 2002 2) Multi-method, monitoring (EU evaluated LC-MS/MS 2014	-
Commodities with high oil content 1) Olives and cotton seed 2) oil seed rape	pyriproxyfen	LOQ 0.01 mg/kg	-	1)Multi-method, monitoring (EU evaluated) GC-MS 2002 2) Multi-method, monitoring (EU evaluated LC-MS/MS 2014	European Standard method EN 15662 MRM QuEChERS (LC-MS/MS) European Standard method EN 15662 MRM QuEChERS (GC-MS)
Commodities with high acid content Oranges	pyriproxyfen	LOQ 0.01 mg/kg	-	Multi-method, monitoring (EU evaluated LC-MS/MS 2014	European Standard method EN 15662 MRM QuEChERS (LC-MS/MS)
Commodities which are difficult to analyse black tea	pyriproxyfen	LOQ 0.01 mg/kg	-	Multi-method, monitoring (EU evaluated LC-MS/MS 2014	-
Milk	pyriproxyfen	LOQ 0.01 mg/kg	-	Multi-method, monitoring (EU evaluated LC-MS/MS 2015 [NNA-0118 ; NNA-0122]	-
Poultry Eggs	pyriproxyfen	LOQ 0.01 mg/kg	-	Multi-method, monitoring (EU evaluated	-

Matrix group/crop group	Residue definition for monitoring	LOQ	Methods		
			Primary method	Confirmatory Method (EU evaluated)	Independent lab validation
				LC-MS/MS 2015 [NNA-0118]	
Bovine Meat	pyriproxyfen	LOQ 0.01 mg/kg	-	Multi-method, monitoring (EU evaluated LC-MS/MS 2015 [NNA-0118 ; NNA-0122]	-
Fat	pyriproxyfen	LOQ 0.01 mg/kg	-	Multi-method, monitoring (EU evaluated LC-MS/MS 2015 [NNA-0118 ; NNA-0122]	-
Kidney/liver	pyriproxyfen	LOQ 0.01 mg/kg	-	Multi-method, monitoring (EU evaluated LC-MS/MS 2015 [NNA-0118]	-
Honey					
Soil	pyriproxyfen	LOQ 0.01 mg/kg	-	Multi-method, monitoring (EU evaluated GC-NPD GC-MS 2002	-
Drinking water	pyriproxyfen	LOQ 0.01 mg/kg	-	Monitoring method (EU evaluated) GC-NPD GC/MSD 2002 Multi-method, monitoring (EU evaluated) GC/MS 2009 Multi-method, monitoring (EU evaluated) GC/MS 2015	-
Surface water	pyriproxyfen	LOQ 0.01 mg/kg	-	Monitoring method (EU evaluated) GC-NPD GC/MSD 2001 Multi-method, monitoring (EU evaluated) LC-MS/MS 2015	-
Air	pyriproxyfen	LOQ 0.01 mg/kg (~35ºC, ~30% rH and ~ 35ºC, ≥		Monitoring method (EU evaluated)	-

Matrix group/crop group	Residue definition for monitoring	LOQ		Methods	
			Primary method	Confirmatory Method (EU evaluated)	Independent lab validation
		80% rH)		GC-NPD GC-MS	
Body fluids	Not required, not a toxic or very toxic compound				
Body tissues	Not required, not a toxic or very toxic compound				