FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: FLD-HER 306 SE

Product name(s): -

Chemical active substances:

2,4 D 300 g/L

florasulam 6.25 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

v.2 - supplement

Applicant:

Pestila Spółka z ograniczoną odpowiedzialnością

Submission date: January 2021 v.2 - 18/03/2021

MS Finalisation date: 08/2021; 11/2021

Version history

When	What
2021/03/18	Supplement of Appendix 1 - List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review
August 2021	ZRMs evaluated the dRR submitted by Applicant.
November 2021	Final Registration Report

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5 Analytical methods

5.1 Conclusion and summary of assessment

Metabolism and residues section:

All analytical methods are active substances data and were evaluated during the EU review of 2,4-D and Florasulam. They were considered adequate. No additional studies have been performed.

2,4-D

EFSA Journal 2014;12(9):3812:

LC-MS/MS methods are available for the analysis of materials of plant and animal origin. However, the validation of these methods with regard to extraction efficiency and validation of the hydrolysis step are lacking, therefore a data gap has been identified. LC-MS/MS and GC-MS methods are available for soil and water, and an LC-MS/MS method is available for air. An LC-MS/MS method is available for blood and urine.

Noticed data gaps should be addressed at renewal of the Flod 306 SE.

Florasulam

EFSA Journal 2015; 13(1):3984

Residues of florasulam in food and feed of plant origin can be monitored with LC-MS/MS method with LOQs of 0.01 mg/kg in all commodity groups. Florasulam can be monitored in food of animal origin with LC-MS/MS with LOQs of 0.01 mg/kg in meat, liver, fat, milk and eggs. Residues of flo-rasulam in soil can be monitored by LC-MS/MS with a LOQ of 0.05 µg/kg. Appropriate LC-MS/MS method with a LOQ of 0.05 µg/L exists for monitoring florasulam in surface water and drinking water. Residues of florasulam in air can be monitored by LC-MS/MS with a LOQ of 1.3 mg/m3. LC-MS/MS method with LOQs of 0.05 mg/L exists for the determination of florasulam in body fluids.State whether submitted data are sufficient for evaluation. Data gaps and conditions for authorization should be listed, if appropriate.

Note:
The Świstak, 2019 methods (0005/0067/FA, 0005/0088/FA, 0005/0074/FA and 0005/0079/FA) were not evaluated as they were not described in the Appendix 2.

Commodity/crop	Supported/ Not supported
Spring wheat	Supported
Spring triticale	Supported
Spring barley	Supported
Oat	Supported
Winter wheat	Supported
Winter triticale	Supported
Winter barley	Supported
Rye	Supported

Commodity/crop	Supported/ Not supported
Maize	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

An overview on the acceptable methods and possible data gaps for analysis of florasulam and 2,4-D in plant protection product is provided as follows:

Reference:	5.1.1/01 5.1.1/02
Report	FLD-HER 306 SE. Determination of active substances content in preparation in COEX bottle. Stage 1: Determination of active substances content in initial preparation and Stage 3: Determination of active substances content in preparation stored at temperature 54±2°C for 14 days., Zając S., 2019, report no 007/DPL/2019
Guideline(s):	Yes, SANCO/3030/99 rev.5 (22/03/19)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Determination of florasulam and 2,4-D in FLD-HER 306 SE was performed with high performance liquid chromatography technique (HPLC) with DAD detection wavelength 270 nm and external standard.

Equipment and chromatographic conditions for florasulam analysis

- Test system: HPLC - Agilent Technologies 1260 Infinity (AKP/02)
- Detector: DAD: wavelength 270 nm
- Column: Kinetex C18, 150 mm x 4.6 mm x 5 µm (044/HPLC)
- Eluent: ACN/H_2O/MeOH/H_3PO_4 (250/500/250/0.5) ml
- Solvent: 5 ml THF (directly into a volumetric flask) + ACN/H_2O/MeOH/H_3PO_4 (250/500/250/0.5) ml (to the volume)
- Column temperature: 25°C
- Flow rate: 1.2 ml/min
- Sample injected volume: 5 µl
- Syringe filters: 0.22 µm

- Time of analysis: 15 min
- Retention time of florasulam: ~2.9 min
- Pressure: ~150 bar

The preparation of test solution for florasulam analysis

Weigh 0.0200±0.0040 g of florasulam standard with accuracy of 0.0002 g into the flask of 100 ml. Next, add 5 ml THF into the flask and mix until the standard is dissolved, make up the flask to the volume of the solvent and mix. Next, transfer 10 ml prepared standard solution into the flask of 100 ml, make up the flask to the volume of solvent and mix. Filter through a 0.22 µm PTFE syringe filter to the vial.

The preparation of samples for florasulam analysis

Weigh 0.3200±0.0640 g of preparation with accuracy of 0.0002 g into a flask of 100 ml. Next, add 5 ml THF into the flask and mix until the preparation is dispersed, make up the flask to the volume of the solvent and mix. Filter through a 0.22 µm PTFE syringe filter to the vial.

Equipment and chromatographic conditions for 2,4-D analysis

- Test system: HPLC - Agilent Technologies 1260 Infinity (AKP/02)
- Detector: DAD: wavelength 270 nm
- Column: Kinetex C18, 150 mm x 4.6 mm x 5 µm (044/HPLC)
- Eluent: ACN/H2O/MeOH/H3PO4 (250/500/250/0.5) ml
- Solvent: 5 ml THF (only for test item solution, directly into a volumetric flask) + ACN/H2O/MeOH/H3PO4 (250/500/250/0.5) ml (to the volume)
- Saponification solution: 2-propanol/H2O/KOH (700/300) ml/15 g
- Neutralization solution: 5% orthophosphoric acid solution
- Column temperature: 25°C
- Flow rate: 1.2 ml/min
- Sample injected volume: 5 µl
- Syringe filters: 0.22 µm
- Time of analysis: 20 min
- Retention time of 2,4-D: ~5.4 min
- Pressure: ~150 bar

The preparation of test solution for 2,4-D analysis

Weigh 0.0800±0.0160 g of 2,4-D standard with accuracy of 0.0002 g into the flask of 100 ml. Next, make up to ¾ of the volume of the solvent. Leave in an ultrasonic bath for 10 minutes and after this time cool the solution to room temperature (18÷25°C) in a water bath for 30 min. Then make up the flask to the volume of the solvent, mix and filter through a 0.22 µm PTFE syringe filter to the vial.

The preparation of samples for 2,4-D analysis

Weigh 0.2667±0.0533 g of preparation with accuracy of 0.0002 g into a flask of 100 ml. Next, add 5 ml THF into a flask and mix until the preparation is dispersed. Add 25 ml of saponification solution, mix and leave the flask in an ultrasonic bath for 60 minutes. After this time add 20 ml of neutralization solution, mix and cool the solution to room temperature (18÷25°C) in a water bath for 30 min. Then make up the flask to the volume of the solvent, mix and filter through a 0.22 µm PTFE syringe filter to the vial.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances florasulam and 2,4-D in plant protection product FLD-HER 306 SE

	florasulam	2,4-D
Author(s), year	Zając S., 2019	
Principle of method	SANCO/3030/99 rev.5, 22 March 2019	
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	The linearity of the analytical method was assessed using five florasulam standard solutions in the concentration range from 11.8 mg/L (3.5 g/kg) to 27.9 mg/L (8.2 g/kg). Correlation coefficient: R^2 = 0.9999 Required: $R^2 \geq 0.99$ y = 5,7304 x + 0,5522	The linearity of the analytical method was assessed using five 2,4-D standard solutions in the concentration range from 477.6 mg/L (168.0 g/kg) to 1117.6 mg/L (393.2 g/kg). Correlation coefficient: R^2 = 0.9996 Required: $R^2 \geq 0.99$ y = 0,927 x – 13,518
Precision – Repeatability Mean n = 6 (%RSD)	H_r = 0.33 Required: $H_r \leq 1$ RSD = 0.97 % Required: RSD ≤ 2.90%	H_r = 0.34 Reqiured: $H_r \leq 1$ RSD = 0.55% Required: RSD ≤ 1.62%
Accuracy n = 6 (% Recovery)	101.8% (range: 101,5% - 102%) Required: 90% ÷ 110%	100.5% (range: 99.3% - 102.1%) Required: 97% ÷ 103%
Interference/ Specificity	There are no any interferences coming from impurities for the peak of the target analyte – florasulam.	There are small interferences from impurities coming from test item solution, < 3% of the total peak measured for 2,4-D.
Comment	No comments.	The degree of interferences in specificity meets criteria specified in SANCO/3030/99 rev.5, 22 March 2019.

Conclusion

The HPLC method, used to quantify florasulam in FLD-HER 306 SE was fully validated. Method validation included linearity, non-analyte interference, precision, accuracy and specificity. All measured parameters meet the criteria given in SANCO/3030/99 rev.5, 22 March 2019.

The HPLC method, used to quantify 2,4-D in FLD-HER 306 SE was fully validated. Method validation included linearity, non-analyte interference, precision, accuracy and specificity. All measured parameters meet the criteria given in SANCO/3030/99 rev.5, 22 March 2019.

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

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

Reference:	5.1.2/01
Report	Determination of the content of the relevant impurities of 2,4-D (free phenols) and florasulam (2,6-difluoroaniline) in the preparation, Gutowska I., 2019, report no BA-21/19
Guideline(s):	Yes, SANCO/3030/99 rev. 5 (22/03/19)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods for 2,6-difluoroaniline (2,6-DFA) content

Determination of residues of 2,6-difluoroaniline (2,6-DFA) content in florasulam was performed with liquid chromatograph equipped with UV-DAD.

Equipment and chromatographic conditions for 2,6-DFA analysis

- Shimadzu liquid chromatograph equipped with UV-DAD
- Column: Luna C18(2), 5µm,250 x4.6mm (Phenomenex)
- Analytical balance Mettler Toledo XS205 DU/M, accuracy 0.01 mg
- Glass pipettes
- Glass graduated flasks
- Ultrasonic bath
- Disposable syringes Syringe filters Pureland PTFE 0.22µm
- Automatic pipette
- Typical laboratory equipment
- Acetonitrile for HPLC SUPER GRADIENT
- Deionized water, ultra-pure
- orto-phosphoric acid, analytical grade
- Analytical standards (3.2.)
- Oven temperature: 35 ºC
- Mobile phase flow: v = 1.0 ml/min
- Wavelength: λ = 227 nm
- Injection volume: 10 µl
- Mobile phase composition: acetonitrile (A) + 0.1% H_3PO_4 (aq) (B)
- Time of analysis: 35 min

The preparation of test solution for 2,6-DFA analysis

350 mg of FLD-HER 306 SE preparation was weighed into a 10 ml volumetric flask, then acetonitrile was added up to the mark. The content was mixed and the flask was put into the ultrasonic bath for 5 minutes. The solution was adjusted to room temperature and then passed through a syringe filter with a pore size 0.22 µm.

The preparation of samples for 2,6-DFA analysis

10 mg of 2,6-DFA standard was weighed into a 10 ml volumetric flask and acetonitrile was added up to the mark. The flask was put into the ultrasonic bath for 5 minutes. The solution was adjusted to the room temperature. The working solution was diluted.

Materials and methods for free phenols content

Determination of residues of free phenols content in 2,4-D was performed with UV spectrophotometer at wavelength 520 nm.

Equipment and chromatographic conditions for free phenols analysis

- Spectrophotometer UV -1700, Shimadzu
- Analytical balance Mettler AT261 DR, accuracy 0.01 mg
- Glass graduated pipettes
- Automatic pipette
- Pipette 5ml, 10 ml
- Beaker 25 ml
- Graduated measuring cylinders with stoppers
- Glass graduated flasks 100 ml
- Typical laboratory equipment
- 4-aminoantipyrine 98 %
- Potassium hexacyanoferrate (III), $K_3[Fe(CN)_6]$
- Ammonia
- Acetone a.g.
- Ethanol a.g. 96 %

The preparation of test solution for free phenols analysis

Solution A 10.17 mg of 2,4-dichlorophenol standard was weighed into a 25 ml beaker, dissolved in 1 mL of acetone, transferred quantitatively into 100 ml volumetric flask and then filled up to 100 mL with acetonitrile. The solution was diluted 10-times with acetonitrile (solution A1).
Solution B (for blank sample preparation) was prepared by weighing: 65.29 mg of 2,4-D 2-ethylhexyl ester, 1.11 mg of florasulam and 98.31 mg of FLD-HER 306 SE placebo into beakers, then quantitatively transferred into a 100 ml flask. Ethanol (5 ml), ammonia solution (9 ml) were added and the flask was replenished with acetonitrile up to the mark.
Specimen preparation About 150 mg of FLD-HER 306 SE preparation was weighed with an accuracy of 0.0001 g into the 50 ml beaker. The sample was transferred quantitatively into a 100 ml volumetric flask, ethanol (5 ml), ammonia solution (9 ml) were added and the flask was replenished with acetonitrile up to the mark.

Validation - Results and discussions

Table 5.2-2a: Methods suitable for the determination of the relevant impurities (2,6 DFA and free phenols) in plant protection product (PPP) FLD-HER 306 SE

	Relevant impurity 2,6-DFA max. content in PPP	**Relevant impurity free phenols max. content in PPP**
Author(s), year	Gutowska I., 2019	
Principle of method	SANCO/3030/99 rev.5, 22 March 2019	

	Relevant impurity 2,6-DFA max. content in PPP	**Relevant impurity free phenols max. content in PPP**
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	The linearity of detector response was examined in the range of 0.000097 – 0.000579 mg/ml of 2,6-DFA. Solutions on each level were injected twice (besides the solution at concentration 0.000097 mg/ml, which was injected five times). The detector response against the substance content was plotted Correlation coefficient: R^2 = 0.9996 Required: $R^2 \geq 0.99$ Y = 34351318,0607 x – 201.7627	The absorbance of prepared solutions of 2,4-dichlorophenol were measured. The calibration curve was plotted. Correlation coefficient: R^2 = 0.9990 Required: $R^2 \geq 0.99$ y = 3,2520 x – 0,00005
Precision – Repeatability Mean n = 6 (%RSD)	RSD 1.08% Required: $RSD_r \leq 9.17\%$	RSD= 5.06% $RSD_r \leq 6.30\%$
Accuracy n = 12 (% Recovery)	100.7% (range: 96.8% - 103%) Required: (70 – 130%)	103.1% (range: 95.3% - 108.3%) Required: (75-135%)
Interference/ Specificity	fulfilled	fulfilled
LOQ	0.097 µg/ml	0,5 µg
Comment	Study accepted	Study accepted

Reference:	5.1.2/02
Report	GC method for determination of dioxins and furans in FLD-HER 306 SE, Grodowska K., 2020, report no RVM/2020/53
Guideline(s):	Yes, SANCO/3030/99 rev.5, 22/03/19.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods for dioxins and furans content

Determination of residues of dioxins and furans content in 2,4-D was performed with GC system equipped with solvent vent injector and MS single-quad detector.

Equipment and chromatographic conditions for dioxins and fruans analysis

- GC system equipped with solvent vent injector (MMI) and MS single-quad detector, Agilent Technologies, internal no.: 03.HS-GC-MS.007, valid to : 01.10.2021;
- GC column: DB-DIOXIN, 60 m x 0.25 mm, film thickness 0.25 µm, Agilent Technologies, serial no.: USP638423H, internal no.: 01.CC.880;
- Liner, Agilent Technologies 5190-2296;
- Class A volumetric glassware and pipettes;
- Rotary evaporator G3 XL, Heidolph, internal no.: C17.WP.01;
- Vacuum pump system RZ-6, Vaccubrand GMBH+CO KG, serial no.: 38801913;
- VLM Metal Block Thermostat, EVA – EC1-S, serial no.: 1610004;

- Silica column, Intarchim, part no.: PF-50SIHP-JP-F0120;
- Supelco Dioxin Prep System (6.35mm MULTI-LAYER SILICA GEL DIOXIN COLUMN, part no. 28397-U; lot no. 123684, 6.35/10MM DUAL LAYER CARBON Reversible Tube (Micro-Column), part no. 28399-U, lot no. 115842),
- Laboratory vacuum dryer, Jeio Tech (SAN-LAB), type OV-11, internal no.: 02.SP.01, valid to: 07.02.2021;
- Laboratory vacuum dryer, Binder, type 9030-0030, internal no.: A28.SP.001, valid to: 07.05.2021;
- Magnetic stirrer, IKA RCT Classic, thermometer IKA ETS-D5;
- Eppendorf Multipette, internal no.: P3.LA.43, valid to: 02.03.2021;
- Vortex mixer, IKA VORTEX3, internal no.: C19.VT.04;
- Methanol gradient grade for liquid chromatography; Supelco; batch no. I1059907; purity 99.93%; expiry date: 30.09.2022;
- Dichloromethane anhydrous; ≥99.8%, Sigma - Aldrich; batch no. STBJ4104; purity 99.95%, expiry date: 14.10.2025;
- n-Hexane for HPLC ≥ 95%; Sigma - Aldrich; batch no. no STBJ4282; purity ≥95%; expiry date: 19.11.2025;
- n-Hexane for HPLC ≥ 95%; Sigma - Aldrich; batch no. STBH6785; purity ≥95%; expiry date: 08.04.2025;
- Toluene HPLC grade; 99.7% min; Alfa Aesar; batch no. 61701096; purity ≥ 99.9%; expiry date: 19.05.2022;
- Toluene for gas chromatography MS; Supelco; batch no. I1104549; purity ≥ 99.8%; expiry date: 31.07.2023;
- n-Nonane; 99%; Alfa Aesar; batch no. 10214578; purity 99.2%; expiry date: 25.10.2021;
- n-Nonane; 99%; Alfa Aesar; batch no. 10223919; purity 99.4%; expiry date: 17.09.2022;
- Nitrogen 5.0; Linde Gas; GA 221; batch no. 105154221; parameter: nitrogen ≥ 99.999; oxygen ≤ 2 ppm; water ≤ 3 ppm.

The preparation of test solution for dioxins and furans analysis

To the round bottom flask transfer 10 mL of sample add 100 µL of Labelled Compound Stock Solution EDF-8999 (containing 15 labelled analogues of congeners) and 1 mL of Clean-up Standard EDF-6999 (containing Cl37 labelled 2,3,7,8-TCDD) and 25 µL PAR Stock Solution. Use a rotary evaporator to eliminate the solvents from the sample at temperature 80oC and 2 mbar vacuum for 20 minutes. The next steps of sample preparation are described in points 2-10 (see paragraph below).

The preparation of samples for dioxins and furans analysis

The sample preparation was based on method 1613. In general during sample preparation sample needs to be concentrated and purified to improve the overall quantification level of the whole method.
At the beginning the solubility test was performed to find optimum solvent for sample preparation.
The sample was dissolved in dichloromethane, acetone, hexane and methanol, THF.
The THF was chosen as solvent which will be used in during sample preparation.
The non-spiked sample solution was prepared according the procedure below:

1. To the round bottom flask transfer 10 mL of sample add 100 µL of Labelled Compound Stock Solution EDF-8999 (containing 15 labelled analogues of congeners) and 1 mL of Clean-up Standard EDF-6999 (containing Cl37 labelled 2,3,7,8-TCDD). Use a rotary evaporator to eliminate the solvents from the sample at temperature 80°C and 2 mbar vacuum for 20 minutes.
2. After initial removal of solvents by rotary evaporator, perform a vacuum distillation of the sample at 80°C and ca. 0.05 mbar for 30 minutes.
3. Dissolve the dried sample in 10 mL of THF and transfer quantitatively sample with another 10 mL methanol to the preparative silica column (PF-50SIHP-JP-F0120, Intarchim). Dry the column using a vacuum dryer at 40°C and below 10 mbar pressure for 40 minutes.

4. Wash out all analytes of interest using hexane for 25 minutes at a flow rate of 60 mL (1500 mL of hexane). Collect all of the eluate and reduce the volume to 5 – 10 mL using a rotary evaporator (200 mbar and 40°C).
5. Prepare the Supelco Dioxin Prep System for purification and concentration of eluate: Precondition the multi-layer column: mount the column, vacuum adapter and round bottom flask with use of standard flow of 200 mL of hexane with use of slight vacuum to obtain a constant flow of eluent. The column is ready to use. Precondition of the dual layer carbon column: mount the column, vacuum adapter and round bottom flask with use of standard flow of 40 mL of toluene and then 100 mL of hexane with use of slight vacuum to obtain the constant flow of eluent. Leave the column wet. The column is ready to use.
6. Mount the Supelco Dioxin prep System with two columns and introduce the eluate from point (4) above. Wash the round bottom flask with 5 mL of hexane and add this solution to the column. Turn on the vacuum pump and allow the eluate to be almost fully absorbed. Then add the 250 mL of hexane to the top flask and perform the purification. After finishing the chromatography remove the multi-layer column and flush the dual layer carbon column with additional portion of 50 mL of hexane.
7. Reverse the dual layer carbon column and remove the analytes of interest under vacuum to the clean round bottom flask with use of 100 mL of toluene.
8. Add 2 mL of nonane to collected toluene eluate and reduce to about 2 mL using a rotary evaporator (50 mbar and 50°C). Transfer quantitively the condensate to the glass conical vial. Rinse the flask with two 0.5 mL portions of nonane and transfer washes to the vial.
9. Add 100 µL of nonane to the sample and reduce the volume of the solution to about 90 µL with use of nitrogen stream. Measure the final volume of the sample by Hamilton syringe and adjust it up to 90 µL with use of nonane if necessary.
10. Transfer the whole solution to injection vial. Add 10 µL of Internal Standard Spiking Solution EDF-5999 (containing 1,2,3,4-TCDD-C13 and 1,2,3,7,8,9-HxCDD-C13 labelled internal standards). Mix gently with use of vortex. The sample is ready for injection to GC-MS system.

Validation - Results and discussions

Table 5.2-3b: Methods suitable for the determination of the relevant impurities (dioxins and furans) in plant protection product (PPP) FLD-HER 306 SE

	Relevant impurity - dioxins max. content in PPP	**Relevant impurity - furans max. content in PPP**
Author(s), year	Grodowska K.	
Principle of method	SANCO/3030/99 rev.5, 22 March 2019	
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	Correlation Coefficient R > 0.99	
Precision – Repeatability Mean n = 17 (%RSD)	H_r = 0.06 (range 0.04-0.11) Required: $H_r \leq 1$ RSD = 2 % (range 1.2-3.3) Required: % RSD < % RSDr	H_r = 0.061 (range 0.03-0.08) Reqiured: $H_r \leq 1$ RSD = 2.06% (range 1.1-2.8) Required: % RSD < % RSDr
Accuracy n = 17 (% Recovery)	113.07% (range: ~~99~~ 96% - 125%) Required: (70 – 130%)	110.75% (range: 96% - 119%) Required: (70-130%)
Interference/ Specificity	fulfilled	fulfilled
LOQ	2.58 µg (range 0.5-5.0)	2.52 µg (range 0.5-4.9)
Comment	Study accepted	Study accepted

Conclusion

Determination of residues of 2,6-DFA, free phenols and sum of dioxins and furans was was fully validated. The methods for determination are specific. The validation parameters for linearity, instrument precision, limit of quantification, repeatability and accuracy are within the acceptance range. There are not any interferences between relevant impurities and other ingredients of the samples.
The methods had good precision, accuracy and the linearity and fulfil requirements of SANCO/3030/99 rev.5.

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

Not relevant. FLD-HER 306 SE does not contain materials of toxicological, ecotoxicological or environmental concern.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

For 2,4-D salt aqueous solutions CIPAC Method 1.4/SL/M2/- (CIPAC Handbook 1C, page 2066) is suitable.
For florasulam no CIPAC method is available for the active substance in the preparation.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of 2,4-D for the generation of pre-authorization data is given in the following table. All studies have been previously evaluated at EU level and are described in detail in the RAR (Greece, 2013). New studies were not submitted.

Table 5.2-4: Validated methods for the generation of pre-authorization data

Component of residue definition: 2,4-D (sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
All four commodities (high water, dry, high acid and high oil)	Primary (ILV available)	0.01 mg/kg	LC-MS/MS	Gesell, J.T., 2013a (study no. 110357) / EU agreed (RAR Greece, 2014)
	Confirmatory (if required)	Not necessary	-	-
Food of animal origin (muscle, kidney, fat, milk and eggs) (Residues)	Primary (ILV available)	0.01 mg/kg	LC-MS/MS	Gesell, JT, 2013b (study no. 110468) / EU agreed (RAR Greece, 2014)
	Confirmatory (if required)	Not necessary	-	-
Soil (Residues)	Primary	0.05 mg/kg	LC-MS/MS and GC-MS (for 2,4-DCA)	Gesell, JT, 2012a (study no. 110503) / EU agreed (RAR Greece, 2014)
	Confirmatory (if required)	Not necessary	-	-

Component of residue definition: 2,4-D (sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Water (ground water, surface water and drinking water) (Residues)	Primary (ILV available)	0.1 µg/L	LC-MS/MS and GC-MS (for 2,4-DCA)	Gesell, JT, 2012b (study no. 110504) / EU agreed (RAR Greece, 2014)
	Confirmatory (if required)	Not necessary	-	-
Air (Residues)	Primary	4.5 µg/m^3 for 2,4-D in air or 1.8 µg of 2,4-D sampled with approx. 0.40 m^3 of air	LC-MS/MS	Class, T, 2011(study no. P 2166 G; Das Protocol No. 110026) / EU agreed (RAR Greece, 2014)
	Confirmatory (if required)	Not necessary	-	-
Body fluids and tissues (urine and blood (Residues)	Primary	0.05 mg/L (2,4-D)	LC-MS/MS	Senciuc, M, 2011 (study no. P 2167 G; Das Protocol No. 110027) /EU agreed (RAR Greece, 2014)
	Confirmatory (if required)	Not necessary	-	-
Water (*Daphnia magna)* (Ecotoxicology)	Primary	0.298483mg/L	HPLC-PAD	Świstak, M., 2019 Study code: 0005/0067/FA

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

The methods evaluated under point 5.2.1 can be applied.

5.3.2 Description of analytical methods for the determination of residues 2,4-D (KCP 5.2)

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

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Sum of 2,4-D, its salts, its	0.05 mg/kg	Regulation (EU) No 2019/1791

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high acid content	esters and its conjugates expressed as 2,4-D	0.05 mg/kg	Regulation (EU) No 2019/1791
Plant, high protein/high starch content (dry commodities)		0.05 mg/kg	Regulation (EU) No 2019/1791
Plant, high oil content		0.05 mg/kg	Regulation (EU) No 2019/1791
Plant, difficult matrices (hops, spices, tea)		0.1 mg/kg	Regulation (EU) No 2019/1791
Muscle	Sum of 2,4-D, its salts, its esters and its conjugates expressed as 2,4-D	0.05 mg/kg	Regulation (EU) No 2019/1791
Milk		0.01mg/kg	Regulation (EU) No 2019/1791
Eggs		0.01 mg/kg	Regulation (EU) No 2019/1791
Fat		0.05 mg/kg	Regulation (EU) No 2019/1791
Liver, kidney		0.05 mg/kg	Regulation (EU) No 2019/1791
Soil (Ecotoxicology)	2,4-D	0.05 mg/kg	common limit
Drinking water (Human toxicology)	2,4-D	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	2,4-D	12.5 mg/L (NOEC) *Daphnia magna*	Section B9 Świstak, M., 2019 Study code: 0005/0067/FA
Air	2,4-D	45 µg/m^3	AOEL sys: 0.15 mg/kg bw/d; EFSA Journal 2014;12(9):3812
Tissue (meat or liver)	Not residue relevant	Not required	not clasified as T / T+
Body fluids		Not required	not clasified as T / T+

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

An overview on the acceptable methods and possible data gaps for analysis of 2,4-D in plant matrices is given in the following tables. New studies were not provided.

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

Component of residue definition: 2,4-D (sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D)				
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	Gesell, J.T. and Li, Q., 2013/ EU agreed (RAR Greece, 2014)
	ILV	0.01 mg/kg	LC-MS/MS	Langridge, G., 2012 / EU agreed (RAR Greece, 2014)
	Confirmatory (if required)	-	Included in primary method	-

Component of residue definition: 2,4-D (sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High acid content	Primary	0.01 mg/kg	LC-MS/MS	Gesell, J.T. and Li, Q., 2013/ EU agreed (RAR Greece, 2014)
	ILV	-	Not required, ILV for 2 matrices available	-
	Confirmatory (if required)	-	Included in primary method	-
High oil content	Primary	0.01 mg/kg	LC-MS/MS	Gesell, J.T. and Li, Q., 2013/ EU agreed (RAR Greece, 2014)
	ILV	-	Not required, ILV for 2 matrices available	-
	Confirmatory (if required)	-	Included in primary method	-
High protein/high starch content (dry)	Primary	0.01 mg/kg	LC-MS/MS	Gesell, J.T. and Li, Q., 2013/ EU agreed (RAR Greece, 2014)
	ILV	0.01 mg/kg	LC-MS/MS	Langridge, G., 2012 / EU agreed (RAR Greece, 2014)
	Confirmatory (if required)	-	Included in primary method	-
Difficult (if required, depends on intended use)	Primary	-	Not required for intended uses	-
	ILV	-	Not required for intended uses	-
	Confirmatory (if required)	-	Not required for intended uses	-

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Renewal Assessment Report, Final Addendum, vol. 3, B.5.2.1, Greece, 2014
Not required, because:	-

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

An overview on the acceptable methods and possible data gaps for analysis of 2,4-D in animal matrices is given in the following tables. New studies were not provided.

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

Component of residue definition: 2,4-D (sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.01 mg/kg	LC-MS/MS	Gesell, J.T. and Li, Q., 2013/ EU agreed (RAR Greece, 2014)
	ILV	0.01 mg/kg	Not required, ILV for 3 matrices available	-
	Confirmatory (if required)	-	included in primary method	-
Eggs	Primary	0.01 mg/kg	LC-MS/MS	Gesell, J.T. and Li, Q., 2013/ EU agreed (RAR Greece, 2014)
	ILV	0.01 mg/kg	HPLC-MS/MS	Garcia-Alix, M., 2012 / EU agreed (RAR Greece, 2014)
	Confirmatory (if required)	-	included in primary method	-
Muscle	Primary	0.01 mg/kg	LC-MS/MS	Gesell, J.T. and Li, Q., 2013/ EU agreed (RAR Greece, 2014)
	ILV	0.01 mg/kg	Not required, ILV for 3 matrices available	-
	Confirmatory (if required)	-	included in primary method	-
Fat	Primary	0.01 mg/kg	LC-MS/MS	Gesell, J.T. and Li, Q., 2013/ EU agreed (RAR Greece, 2014)
	ILV	0.01 mg/kg	HPLC-MS/MS	Garcia-Alix, M., 2012 / EU agreed (RAR Greece, 2014)
	Confirmatory (if required)	-	included in primary method	-
Kidney, liver	Primary	0.01 mg/kg	LC-MS/MS	Gesell, J.T. and Li, Q., 2013/ EU agreed (RAR Greece, 2014)
	ILV	0.01 mg/kg	HPLC-MS/MS	Garcia-Alix, M., 2012 / EU agreed (RAR Greece, 2014)
	Confirmatory (if required)	-	included in primary method	-

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Renewal Assessment Report, Final Addendum, vol. 3, B.5.2.2, Greece, 2014
Not required, because:	-

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

An overview on the acceptable methods and possible data gaps for analysis of 2,4-D in soil is given in the following tables. New studies were not provided.

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

Component of residue definition: 2,4-D			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.05 mg/kg	LC-MS/MS	Gesell, J.T., 2012 / EU agreed (RAR Greece, 2014)
Confirmatory	-	included in primary method	-

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

An overview on the acceptable methods and possible data gaps for analysis of 2,4-D in surface and drinking water is given in the following tables. New studies were not provided.

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

Component of residue definition: 2,4-D				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.1 µg/L	LC-MS/MS	Gesell, J.T., 2012 / EU agreed (RAR Greece, 2014)
	ILV	0.1 µg/L	LC-MS/MS	Garcia-Alix, M., 2012 / EU agreed (RAR Greece, 2014)
	Confirmatory	-	included in primary method	-
Surface water	Primary	0.1 µg/L	LC-MS/MS	Gesell, J.T., 2012 / EU agreed (RAR Greece, 2014)
	Confirmatory	-	included in primary method	-

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

An overview on the acceptable methods and possible data gaps for analysis of 2,4-D in air is given in the following tables. New studies were not provided.

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

Component of residue definition: 2,4-D			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	4.5 µg/m^3	LC-MS/MS	Class, T., 2011 / EU agreed

Component of residue definition: 2,4-D			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
			(RAR Greece, 2014)
Confirmatory	-	included in primary method	-

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

An overview on the acceptable methods and possible data gaps for analysis of 2,4-D in body fluids and tissues is given in the following table. New studies were not provided. However, Analytical methods for body fluids and tissues are not necessary, because 2,4-D is not classified as toxic or very toxic.

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

Component of residue definition: 2,4-D			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.05 mg/L	LC-MS/MS	Senciuc, M., 2011 / EU agreed (RAR Greece, 2014)
Confirmatory	-	included in primary method	-

5.3.2.8 Other studies/ information

Not required.

5.3.3 Methods for the determination of residues of florasulam (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of florasulam for the generation of pre-authorization data is given in the following table. All studies have been previously evaluated at EU level and are described in detail in the RAR (Poland, 2013). New studies were not submitted.

Table 5.3-10: Validated methods for the generation of pre-authorization data

Component of residue definition: florasulam				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Apple, orange, corn, wheat, soybean, canola, potato (Residues)	Primary (ILV available)	0.01 mg/kg	LC-MS/MS	Rodrigues Junior, A., 2011 (DAS report no. 110535) / EU agreed (RAR Poland, 2013)
	Confirmatory (if required)	-	included in primary method	-

Component of residue definition: florasulam				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Food of animal origin (milk, eggs, liver, bovine meat, kidney and fat) (Residues)	Primary (ILV available)	0.01 mg/kg	LC-MS/MS	Bacher, R., 2011 (DAS report no. 110540) / EU agreed (RAR Poland, 2013)
	Confirmatory (if required)	-	included in primary method	-
Food of animal origin (bovine meat) (Residues)	Primary	0.01 mg/kg	LC-MS/MS	Robaugh David, A., JT, 2012 (DAS report no. 110541) / EU agreed (RAR Poland, 2013)
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Linder, M., 2011 (DAS report no. 110671) / EU agreed (RAR Poland, 2013)
Soil (Residues)	Primary	0.05 µg/kg	LC-MS/MS	Bacher, R., 2011 (DAS report no. 110537) / EU agreed (RAR Poland, 2013)
	Confirmatory (if required)	Not necessary	-	-
Water (ground water, surface water and drinking water) (Residues)	Primary (IV available)	0.05 µg/L	LC-MS/MS	Class, T., 2011 (DAS report no. 110538) / EU agreed (RAR Poland, 2013)
	Confirmatory (if required)	Not necessary	-	-
Air (Residues)	Primary	1.5 µg/m^3	LC-MS/MS	Class, T., 2011 (DAS report no. 110282) / EU agreed (RAR Poland, 2013)
	Confirmatory (if required)	Not necessary	-	-
Body fluids and tissues (urine and blood (Residues)	Primary	0.05 mg/L	LC-MS/MS	Class, T., Göcer, M., 2011 (DAS report no. 110283) / EU agreed (RAR Poland, 2013)
	Confirmatory (if required)	Not necessary	-	-
Water (*Daphnia magna)* (Ecotoxicology)	Primary	0.298483mg/L	HPLC-PAD	Świstak, M., 2019 Study code: 0005/0067/FA

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

5.4.1 Analysis of the plant protection product (KCP 5.2)

The methods evaluated under point 5.2.1 can be applied.

5.4.2 Description of analytical methods for the determination of residues

florasulam (KCP 5.2)

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

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

Table 5.4-1: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	florasulam	0.01 mg/kg	Regulation (EU) No 1317/2013
Plant, high acid content		0.01 mg/kg	Regulation (EU) No 1317/2013
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Regulation (EU) No 1317/2013
Plant, high oil content		0.01 mg/kg	Regulation (EU) No 1317/2013
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Regulation (EU) No 1317/2013
Muscle	florasulam	0.01 mg/kg	Regulation (EU) No 1317/2013
Milk		0.01 mg/kg	Regulation (EU) No 1317/2013
Eggs		0.01 mg/kg	Regulation (EU) No 1317/2013
Fat		0.01 mg/kg	Regulation (EU) No 1317/2013
Liver, kidney		0.01 mg/kg	Regulation (EU) No 1317/2013
Soil (Ecotoxicology)	florasulam	0.05 mg/kg	common limit
Drinking water (Human toxicology)	florasulam	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	florasulam	12.5 mg/L (NOEC) *Daphnia magna*	Section B9 Świstak, M., 2019 Study code: 0005/0067/FA
Air	florasulam	15 µg/m^3	AOEL sys: 0.05 mg/kg bw/d EFSA Journal 2015; 13(1):3984
Tissue (meat or liver)	florasulam	Not required	Not classified as T / T+
Body fluids		0.05 mg/L	Not classified as T / T+

5.4.2.2 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 florasulam in plant matrices is given in the following tables. New studies were not provided.

Table 5.4-2: Validated methods for food and feed of plant origin (required for all matrix types, "difficult" matrix only when indicated by intended GAP)

Component of residue definition: florasulam				
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	Rodrigues Junior, A., 2011 / EU agreed (RAR Poland, 2013)
	ILV	0.01 mg/kg	LC-MS/MS	Bacher, R., 2011 / EU agreed (RAR Poland, 2013)
	Confirmatory (if required)	-	included in primary method	-
High acid content	Primary	0.01 mg/kg	LC-MS/MS	Rodrigues Junior, A., 2011 / EU agreed (RAR Poland, 2013)
	ILV	0.01 mg/kg	LC-MS/MS	Bacher, R., 2011 / EU agreed (RAR Poland, 2013)
	Confirmatory (if required)	-	included in primary method	-
High oil content	Primary	0.01 mg/kg	LC-MS/MS	Rodrigues Junior, A., 2011 / EU agreed (RAR Poland, 2013)
	ILV	-	-	-
	Confirmatory (if required)	-	included in primary method	-
High protein/high starch content (dry)	Primary	-	Not required for intended uses	-
	ILV	-	Not required for intended uses	-
	Confirmatory (if required)	-	Not required for intended uses	-
Difficult (if required, depends on intended use)	Primary	-	Not required for intended uses	-
	ILV	-	Not required for intended uses	-
	Confirmatory (if required)	-	Not required for intended uses	-

Table 5.4-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Renewal Assessment Report, Final Addendum, vol. 3, B.5.2.1, Poland, 2013
Not required, because:	-

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

An overview on the acceptable methods and possible data gaps for analysis of florasulam in animal matrices is given in the following tables. New studies were not provided.

Table 5.4-4: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: florasulam				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.01 mg/kg	LC-MS/MS	Bacher, R., 2011 / EU agreed (RAR Poland, 2013)
	ILV	0.01 mg/kg	LC-MS/MS	Robaugh, David, A., 2012 / EU agreed (RAR Poland, 2013)
	Confirmatory (if required)	-	included in primary method	-
Eggs	Primary	0.01 mg/kg	LC-MS/MS	Bacher, R., 2011 / EU agreed (RAR Poland, 2013)
	ILV	0.01 mg/kg	LC-MS/MS	Robaugh, David, A., 2012 / EU agreed
	Confirmatory (if required)	-	included in primary method	-
Muscle	Primary	0.01 mg/kg	LC-MS/MS	Bacher, R., 2011 / EU agreed (RAR Poland, 2013)
	ILV	0.01 mg/kg	LC-MS/MS	Robaugh, David, A., 2012 / EU agreed (RAR Poland, 2013)
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Linder, M., 2011 / EU agreed (RAR Poland, 2013)
Fat	Primary	0.01 mg/kg	LC-MS/MS	Bacher, R., 2011 / EU agreed (RAR Poland, 2013)
	ILV	0.01 mg/kg	LC-MS/MS	Robaugh, David, A., 2012 / EU agreed (RAR Poland, 2013)
	Confirmatory (if required)	-	included in primary method	-
Kidney, liver	Primary	0.01 mg/kg	LC-MS/MS	Bacher, R., 2011 / EU agreed v
	ILV	0.01 mg/kg	LC-MS/MS	Robaugh, David, A., 2012 / EU agreed (RAR Poland, 2013)
	Confirmatory (if required)	-	included in primary method	-

Table 5.4-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Renewal Assessment Report, Final Addendum, vol. 3, B.5.2.2, Poland, 2013

	Method for products of animal origin
Not required, because:	-

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

An overview on the acceptable methods and possible data gaps for analysis of florasulam in soil is given in the following tables. New studies were not provided.

Table 5.4-6: Validated methods for soil (if appropriate)

Component of residue definition: florasulam			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.05 µg/kg	LC-MS/MS	Bacher, R., 2011 / EU agreed (RAR Poland, 2013)
Confirmatory	-	included in primary method	-

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

An overview on the acceptable methods and possible data gaps for analysis of florasulam in surface and drinking water is given in the following tables. New studies were not provided.

Table 5.4-7: Validated methods for water (if appropriate)

Component of residue definition: florasulam				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.05 µg/L	LC-MS/MS	Class, T., 2011 / EU agreed (RAR Poland, 2013)
	ILV	0.05 µg/L	LC-MS/MS	Souza, N., 2011 / EU agreed (RAR Poland, 2013)
	Confirmatory	-	included in primary method	-
Surface water	Primary	0.05 µg/L	LC-MS/MS	Class, T., 2011 / EU agreed (RAR Poland, 2013)
	Confirmatory	-	included in primary method	-

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

An overview on the acceptable methods and possible data gaps for analysis of florasulam in air is given in the following tables. New studies were not provided.

Table 5.4-8: Validated methods for air (if appropriate)

Component of residue definition: florasulam			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	1.5 µg/m^3	LC-MS/MS	Class, T., 2011 / EU agreed (RAR Poland, 2013)
Confirmatory	-	included in primary method	-

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

An overview on the acceptable methods and possible data gaps for analysis of florasulam in body fluids and tissues is given in the following table. New studies were not provided.

Table 5.4-9: Methods for body fluids and tissues (if appropriate)

Component of residue definition: florasulam			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.05 mg/L	LC-MS/MS	Class, T., Göcer, M., 2011/ EU agreed (RAR Poland, 2013)
Confirmatory	-	included in primary method	-

5.4.2.8 Other studies/ information

Not required.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

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

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
KCP 5.1.1/01	Zając S.	2019	FLD-HER 306 SE. Determination of active substances content in preparation in COEX bottle. Stage 1: Determination of active substances content in initial preparation Report No 007/DPL/2019 Pestila II Spółka z ograniczoną odpowiedzialnością Sp.k. GLP Yes Unpublished	N	Pestila*
KCP 5.1.1/02	Zając S.	2019	FLD-HER 306 SE. Determination of active substances content in preparation in COEX bottle. Stage 3: Determination of active substances content in preparation stored at temperature 54±2°C for 14 days. Report No 007/DPL/2019 Pestila II Spółka z ograniczoną odpowiedzialnością Sp.k. GLP Yes Unpublished	N	Pestila*
5.1.2/01	Gutowska I.	2019	Determination of the content of the relevant impurities of 2,4-D (free phenols) and florasulam (2,6-difluoroaniline) in the preparation. Report No BA-21/19 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry GLP Yes Unpublished	N	Pestila*
5.1.2/02	Gutowska I.	2020	FLD-HER 306 SE 2,4-D 300 g/L + Florasulam 6.25 g/L Determination Determination of the content of the relevant impurities of 2,4-D (free phenols) and florasulam (2,6-difluoroaniline) in the preparation after accelerate storage. Report No BA-09/20	N	Pestila*

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
			Łukasiewicz Research Network – Institute of Industrial Organic Chemistry GLP Yes Unpublished		
5.1.2/03	Grodowska K.	2020	GC method for determination of dioxins and furans in FLD-HER 306 SE Report No RVM/2020/53 Selvita Services Sp. z o.o. GLP: N Published: N	N	Pestila*
5.1.2/04	Pstuś J.	2020	Work Progress Report - Development of analytical method for determination of tetra-through octa-chlorinated dioxins and furans by isotope dilution for analysis in Florasulam/2,4-D formulation. Report No REP_20200311_SSV_MWU_Pestila_dioksyny_i_furany_R01v1 Selvita Services Sp. z o.o. GLP: Y Published: N	N	Pestila*
KCP 5.2-01a (filed as KCP 10.2-01a)	Świstak M.	2019	Validation of analytical method for the determination of test item FLD-HER 306 SE in media for breeding aquatic organisms and in deionized water. Study code: 0005/0067/FA SORBOLAB Research Laboratory LLC, Poznań, Poland GLP: Y Published: N	N	Pestila*
KCP 5.2-01b (filed as KCP 10.2-01b)	Świstak M.	2019	Validation of analytical method for the determination of test item FLD-HER 306 SE in media for breeding aquatic. Study code: 0005/0088/FA SORBOLAB Research Laboratory LLC, Poznań, Poland GLP: Y Published: N	N	Pestila*
KCP 5.2-02 (filed as KCP 10.3-02)	Świstak M.	2019	Validation of analytical method for the determination of test item FLD-HER 306 SE in 50% sucrose solution. Study code: 0005/0074/FA SORBOLAB Research Laboratory LLC, Poznań, Poland	N	Pestila*

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
			GLP: Y Published: N		
KCP 5.2-03 (filed as KCP 10.4-03)	Świstak M.	2019	Validation of analytical method for the determination of test item FLD-HER 306 SE in soil for breeding earthworms (*E. Fetida*). Study code: 0005/0079/FA SORBOLAB Research Laboratory LLC, Poznań, Poland GLP: Y Published: N	N	Pestila*

*Pestila Spółka z ograniczoną odpowiedzialnością.

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	Submitter or source
KCP 5.1.2/01	Gesell, J.T. Li, Q.	2013a	Revised Final Report – Method validation study for the determination of residues of (2,4-dichlorophenoxy)acetic acid in agricultural commodities using solid-phase extraction and liquid chromatography with tandem mass spectrometry detection. Dow AgroSciences LLC, Indianapolis, USA; Report No. 110357 GLP:Y Published: N	N	European Union 2,4-D Task Force 2012
KCP 5.1.2/02	Gesell, J.T. Li, Q.	2013b	Revised Final Report – Method validation study for the determination of residues of (2,4-dichlorophenoxy)acetic acid in bovine and poultry tissues using solid-phase extraction and liquid chromatography with tandem mass spectrometry detection. Dow AgroSciences LLC, Indianapolis, USA Report No. 110468 GLP:Y	N	European Union 2,4-D Task Force 2012

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	Submitter or source
			Published: N		
KCP 5.1.2/03	Gesell, J.T.	2012a	Method validation study for the determination of residues of (2,4-dichlorophenoxy) acetic acid and its metabolites in soil. Dow AgroSciences LLC, Indianapolis, USA DAS Protocol No. 110503 GLP: Y Published: N	N	European Union 2,4-D Task Force 2012
KCP 5.1.2/04	Gesell, J.T.	2012b	Method validation study for the determination of residues of (2,4-dichlorophenoxy) acetic acid and its metabolites in surface water, ground water and drinking water Dow AgroSciences LLC, Indianapolis, USA DAS Protocol No. 110504 GLP: Y Published: N	N	European Union 2,4-D Task Force 2012
KCP 5.1.2/05	Class, T.	2011	2,4-D: Development and validation of an analytical method for the determination of 2,4-D in air. PTRL Europe GmbH, Germany Study Code P 2166 G DAS Protocol No. 110026 GLP: Y Published: N	N	European Union 2,4-D Task Force 2012
KCP 5.1.2/06	Senciuc, M.	2011	2,4-D: Development and validation of an analytical method for the determination of 2,4-D in body fluid(s). PTRL Europe GmbH, Germany Study Code P 2167 G DAS Protocol No. 110027 GLP: Y Published: N	N	European Union 2,4-D Task Force 2012
KCP 5.1.2/07	Langridge, G.	2012	Independent laboratory validation of an analytical method for the determination of (2,4-dichlorophenoxy) acetic acid in crops. CEM Analytical Services, UK; Study Code CEMS-5229	N	European Union 2,4-D Task Force 2012

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	Submitter or source
			DAS Protocol No. 110762 GLP: Y Published: N		
KCP 5.1.2/08	Garcia-Alix, M.	2012a	Independent laboratory validation of an analytical method for the determination of (2,4-dichlorophenoxy) acetic acid in animal matrices. CEMS Analytical Services, UK; Study Code CEMS-5230 DAS Protocol No. 110763 GLP: Y Published: N	N	European Union 2,4-D Task Force 2012
KCP 5.1.2/09	Garcia-Alix, M.	2012b	Independent laboratory validation of an analytical method for the determination of (2,4-dichlorophenoxy)acetic acid, 2,4-dichlorophenol, 4-chlorophenol and 2,4-dichloroanisole in water. CEM Analytical Services, UK; Study Code CEMS-5324 DAS Protocol No.: 110821 GLP: Y Published: N	N	European Union 2,4-D Task Force 2012
KCP 5.1.2/10	Rodrigues Junior, A.	2011	Residue Method Validation for the Determination of Florasulam in Agricultural Commodities Dow AgroSciences DAS Report No.: 110535 (Accession Number) 2009969 GLP: Y Published: N	N	Dow AgroScience
KCP 5.1.2/11	Bacher, R.	2011c	Method Validation Study for the Determination of Residues of Florasulam in Foodstuffs of Animal Origin by Liquid Chromatography with Tandem Mass Spectrometry PTRL Europe GmbH, Helmholtzstr DAS Report No.: 110540 (Accession Number) 2009882 GLP: Y Published: N	N	Dow AgroScience

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	Submitter or source
KCP 5.1.2/12	Robaugh, D. A.	2011	Independent Laboratory Validation Study for the determination of Residues of Florasulam in Bovine and Poultry Tissues by Liquid Chromatography with Tandem Mass Spectrometry Pyxant Labs Inc DAS Report No.: 110541 (Accession Number) 2011453 GLP: Y Published: N	N	Dow AgroScience
KCP 5.1.2/13	Lindner, M.	2011	Examination of the Applicability of the Modular Analytical Method L 00.00-34 for the Determination of Residues of Florasulam Eurofins Agrosciences Services Chem GmbH DAS Report No.: 110671 (Accession Number) 2011133 GLP: Y Published: N	N	Dow AgroScience
KCP 5.1.2/14	Bacher, R.	2011b	Method Validation Study for the Determination of Residues of Florasulam and its 5-OH Metabolite in Soil by Liquid Chromatography with Tandem Mass Spectrometry PTRL Europe GmbH DAS Report No.: 110537 (Accession Number) 2011131 GLP: Y Published: N	N	Dow AgroScience
KCP 5.1.2/15	Class, T.	2011a	Method Validation Study for the Determination of Residues of Florasulam and its 5 OH Metabolite in Surface Water, Ground Water and Drinking Water by Liquid Chromatography with Tandem Mass Spectrometry PTRL Europe GmbH DAS Report No.: 110538 (Accession Number) 2011132 GLP: Y Published: N	N	Dow AgroScience
KCP 5.1.2/16	Class, T.	2011b	The Development and Validation of a Method for the Analysis of Florasulam in Air PTRL Europe GmbH	N	Dow AgroScience

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	Submitter or source
			DAS Report No.: 110282 (Accession Number) 2011198 GLP: Y Published: N		
KCP 5.1.2/17	Class, T. Göcer, M.	2011	Florasulam: Development of an Analytical Method for the Determination of Florasulam in Body Fluid(s) PTRL Europe GmbH DAS Report No.: 110283 (Accession Number) 2011127 GLP: Y Published: N	N	Dow AgroScience
KCP 5.1.2/18	Bacher, R.	2011a	Florasulam: Independent Laboratory Validation of a Residue Method for the Determination of Florasulam in Agricultural Commodities PTRL Europe GmbH DAS Report No.: 110536 (Accession Number) 2011200 GLP: Y Published: N	N	Dow AgroScience
KCP 5.1.2/19	Souza, N.	2011	Independent Laboratory Validation of Dow AgroSciences LLC Method - Determination of Residues of Florasulam and its 5 OH Metabolite in Drinking Water, Ground Water and Surface Water by Liquid Chromatography with Tandem Mass Spectrometric Detection Dow AgroSciences DAS Report No.: 110539 (Accession Number) 2010315 GLP: Y Published: N	N	Dow AgroScience

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for the florasulam

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

Please refer to the points 5.2.1.1 and 5.2.1.2.

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

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

A 2.2 Analytical methods for the 2,4-D

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

Please refer to the points 5.2.1.1 and 5.2.1.2.

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

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

A 2.2.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted.