# REGISTRATION REPORT

## Part B

## Section 5

## Analytical Methods

Detailed summary of the risk assessment

**Product code: F7B-39-30**

**Product name: RINPODE**

**Chemical active substance: Florpyrauxifen-benzyl 25 g**

Central Zone

Zonal Rapporteur Member State: Poland/zRMS

CORE ASSESSMENT

Applicant: Corteva Agriscience

Submission date: March 2023

zRMS Assessment date: 16/11/2023

Following commenting round: 11/04/2024

References correction: 31/07/2024

# Version history

| When | What |
| --- | --- |
| March 2023 | Submission to zRMS and concerned Member States |
| November 2023 | zRMS assessment |
| April 2024 | Following commenting round |
| July 2024 | References correction |

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# 5 Analytical methods

## 5.0 Introduction

This application was submitted by Corteva Agriscience in March 2023.
The application is for the first approval of the formulation F7B-39-30 (trademark: Rinpode) as new post-emergence herbicide developed by Corteva Agriscience. The formulation is an EC (emulsion concentrate) containing 25 g/L of florpyrauxifen-benzyl (19.870 g a.e./L) for use as an herbicide in sugar beets.
F7B-39-30 is submitted to Southern and Central zones with France and Poland acting as zRMS respectively. Concerned Member States are Spain, Italy, Portugal, Greece, Croatia in Southern zone and Belgium, The Netherlands, Luxembourg, Hungary, Germany, Austria, Romania, Czech Republic, Romania, Slovakia in Central zone.

Florpyrauxifen-benzyl (trademark: Rinskor® active) is a New Active Substance (NAS), developed by Corteva Agrisciences, approved in accordance with Regulation (EC) No 1107/2009 on July 3rd, 2019. Details of the approval Regulation, Commission Review Report and EFSA R.O. are provided in the below table:

| *Active Substance* | *Approval Regulation* | *SANCO/SANTE Review Report* | *EFSA Scientific Report* |
| --- | --- | --- | --- |
| Florpyrauxifen-benzyl (trademark: Rinskor® active) | Commission Implementing Regulation (EU) 2019/1138 of 3 July 2019 | SANTE/10658/2019 rev2 of 21 May 2019 | EFSA Journal 2018;16(8):5378. doi: 10.2903/j.efsa.2018.5378. |

The Regulation (EU) 2019/1138 for Florpyrauxifen-benzyl (trademark: Rinskor® active) provides specific provisions under Part B which need to be considered by the applicant in the preparation of their submission and by the MS prior to granting an authorisation: "*For the implementation of the uniform principles as referred to in Article 29(6) of Regulation (EC) No 1107/2009, the conclusions of the review report on 21 March 2019, and in particular Appendices I and II thereof, shall be taken into account. In this overall assessment Member States shall pay particular attention to: — the protection of aquatic and terrestrial non-target plants. Conditions of use shall include risk mitigation measures such as buffer zones and/or drift reduction nozzles, where appropriate*."
These concerns have been addressed within the current submission, where not otherwise stated.

Florpyrauxifen-benzyl (trademark: Rinskor® active) is a foliar post-emergence herbicide effective to control the most import weeds present in rice paddies; it is not yet authorized for sugar beets. Florpyrauxifen-benzyl is a member of the arylpicolinate family of chemistry, a new structural class of synthetic auxin herbicides, Group O (according to HRAC MOA classification). F7B-39-30 is active at low use rates in post-emergence applications against broadleaf weeds in sugar-beet.

F7B-39-30 is very similar to GF-3206 (trademark Loyant 25 Neo EC), with the addition of a food-grade dye, included in the composition at 0.0005% w/w. F7B-39-30 and GF-3206 are the same formulation type (emulsion concentrate) and contain equal amounts of active ingredient, antifoam, emulsifiers, solvents and adjuvant. The minimal difference in composition between F7B-39-30 and GF-3206 lead to toxicological and ecotoxicological properties that can be considered equivalent and in comparable performance on crop safety or efficacy. Based on comparability of both formulations, data generated with GF-3206 are used in support of the claim for F7B-39-30. GF-3206, which is authorized formulation since 2019 in all Southern Europe rice countries, is the representative formulation considered for the florpyrauxifen-benzyl (trademark: Rinskor® active) approval, so it was fully evaluated in the active substance European process.

Information on the detailed composition of F7B-39-30 or of the GF-3206 formulation used as read-across can be found in the CONFIDENTIAL dossier of this submission (draft Registration Report - Part C).

F7B-39-30 Rinpode critical and Country GAP within the Central zone is given in Part B, Section 0.

## 5.1 Conclusion and summary of assessment

State whether submitted data are sufficient for evaluation. Data gaps and conditions for authorization should be listed, if appropriate.

Sufficiently sensitive and selective analytical methods are available for the active substance(s) and relevant impurities in the plant protection product.
Noticed data gaps are:

- No data gaps

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

- No data gaps

| Commodity/crop | Supported/ Not supported |
| --- | --- |
| Sugar beet *Beta vulgaris* (BEAVA) and Fodder beet (BEAVC) | 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)

The plant protection product F7B-39-30 (Rinpode) is an EC formulation containing 25 g/L florpyrauxifen-benzyl (trademark: *Rinskor® active*) as active substance. F7B-39-30 is very similar to GF-3206 (the representative formulation for the EU approval of florpyrauxifen-benzyl), with the addition of a food-grade dye included in the composition at 0.0005% w/w. F7B-39-30 and GF-3206 (trademark Loyant 25 Neo EC) are the same formulation type (EC - emulsion concentrate) and contain equal amounts of active ingredient, antifoam, emulsifiers solvents and adjuvant. The fully validated analytical method developed for GF-3206 (DAS-AM-G-13-52) is considered applicable to the determination of florpyrauxifen-benzyl in formulation F7B-39-30 (Rinpote). The addition of the dye is not anticipated to impact the determination of the active ingredient. Suitability of analytical method DAS-AM-G-13-52 has been demonstrated by validation of absence of interferences with the F7B-39-30 formulation.

An overview on the acceptable methods and possible data gaps for analysis of florpyrauxifen-benzyl in plant protection product is provided as follows.

| | |
| --- | --- |
| Comments of zRMS: | The analytical method for the determination of florpyrauxifen-benzyl in the formulation GF- 3206 has been evaluated in the Draft Assessment Report of active substance.<br>The method has been considered acceptable and fully validated according to criteria of guidelines EPA OPPTS 830.1800 & EEC Guidelines SANCO/3030/99 rev. 4 |

| | |
| --- | --- |
| Reference: | CP 5.1.1 (a) |
| Report | Analytical Method and Validation for the Determination of XDE-848 BE in GF-3206; Sauer, J., 2014, DAS-AM-G-13-52 |
| Guideline(s): | Yes/ EPA OPPTS 830.1800 & EEC Guidelines SANCO/3030/99 rev. 4 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Description of the method | The method consists of a high pressure liquid chromatographic (HPLC) using a Supelco Ascentis Express C-18, 4.6 x 100 mm x 2.7 µm particle size column with an ultra-violet detector set at 244 nm. Concentrations were determined using internal standard calibration. |
| Validation | The method is applicable to the assay of XDE-848 BE in GF-3206 formulation over the range of 0.30 – 1.22 mg/mL (1.40 – 5.40 Wt.%) |
| Specificity | No significant interferences were detected between the solvent blank, formulation blank, and internal standard and technical grade active ingredient. |
| Interference by other substances | There are no interferences from any of the components present in the formulation. |
| Explanation of interferences contributing more than ±3% | Not relevant as no interferences were observed contributing at more than ± 3%. |
| Linearity and range, equation and $R^2$ | The detector response was shown to be linear for XDE-848 BE over a range of 1.40 – 5.40 Wt.% ($R^2$ = 0.9999). The detector response was shown to be linear for the internal standard from 1.00 – 4.01 mg/mL ($R^2$ = 0.9995).<br>(n=7)<br>y= 2931910x+ 19016 |
| Accuracy | The accuracy of the method was tested through the preparation of spiked samples at a range of concentrations. Recovery data were obtained over the range of 1.40 to 5.40% XDE-848 BE in GF-3206. The average recovery for XDE-848 BE over this range was 100.1%. |
| Repeatability | The relative standard deviation was 0.24% at an average concentration of 2.66% of XDE-848 BE. |
| Applicability of existing CIPAC methods | No CIPAC method is available |

| | |
| --- | --- |
| Comments of zRMS: | The analytical method HPLC; No 230138 for the Evaluation of Interferences when using DAS-AM- G-13-52 for the Determination of Florpyrauxifen-benzyl in F7B-39-30 Formulation confirmed no significant interferences detected and can be accepted. |

| | |
| --- | --- |
| Reference: | CP 5.1.1 |
| Report | Evaluation of Interferences when using DAS-AM- G-13-52 for the Determination of Florpyrauxifen-benzyl in F7B-39-30 Formulation; Emery, W. 2023; Corteva Report No 230138 |
| Guideline(s): | Yes/ EPA OPPTS 830.1800 & EEC Guidelines SANCO/3030/99 rev. 5 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |
| Description of the method | The analytical method for determination of the active substance in F7B-39-30 formulation is leveraged from the read-across formulation GF-3206. The analytical method DAS-AM-G-13-52 consists of a high pressure liquid chromatographic (HPLC) using a Supelco Ascentis Express C-18, 4.6 x 100 mm x 2.7 µm particle size column with an ultra-violet detector set at 244 nm. |

| | |
| --- | --- |
| | Concentrations are determined using internal standard calibration. |
| Validation | The method is applicable to the assay of Florpyrauxifen-benzyl in F7B-39-30 following evaluation of interferences. |
| Specificity | No significant interferences were detected between the solvent blank, F7B-39-30, formulation blank, internal standard and technical grade active ingredient. |
| Interference by other substances | There are no interferences from any of the components present in the F7B-39-30 formulation. |

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

| Comments of zRMS: | Justification was considered acceptable at EU level and no further data were required. |
| --- | --- |

Toluene is considered a relevant impurity in the florpyrauxifen-benzyl active ingredient and therefore could be present in the F7B-39-30 formulated product. The maximum concentration of toluene in the active ingredient is 3 g/kg. The maximum content of the technical active ingredient in the formulated product is 2.9% therefore the maximum content of toluene in the formulated product is 0.0087% w/w.

Toluene is a residual solvent from the active ingredient manufacturing process and therefore will not increase in concentration upon storage. The test substance was found to retain its level of active ingredient when stored at 54 $^{0}$C for 14 days (see CP Section 2 Study 15-010-G, Reference CP 2.4-1).

CIPAC MT 198 has been demonstrated to be suitable for the analysis of toluene in the read-across formulation GF-3206. Evaluation of the absence of interferences was determined in study DAS-AM-G-180734 and confirmed no issues. Given the similarities between the formulation compositions of GF-3206 and F7B-39-30, the CIPAC MT 198 method for determination of toluene is therefore leveraged for use with F7B-39-30 formulation.

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

No information is submitted. No methods are required as none of the co-formulants are considered as relevant for toxicity (environment, health).

### 5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There are no applicable CIPAC methods for determination of florpyrauxifen-benzyl in F7B-39-30 formulation.

### 5.2.2 Methods for the determination of residues (KCP 5.1.2)

The plant protection product F7B-39-30 (Rinpode) is an EC formulation containing 25 g/L florpyrauxifen-benzyl (trademark: *Rinskor® active*) as active substance. F7B-39-30 is very similar to GF-3206 (the representative formulation for the EU approval of florpyrauxifen-benzyl), with the addition of a food-grade dye included in the composition at 0.0005% w/w. F7B-39-30 and GF-3206 (trademark Loyant 25 Neo EC) are the same formulation type (EC - emulsion concentrate) and contain equal amounts of active ingredient, antifoam, emulsifiers solvents and adjuvant. Given the similarities between the formulation compositions of GF-3206 and F7B-39-30, the analytical methods are therefore leveraged for use with F7B-39-30 formulation. The addition of the dye is not anticipated to impact the determination of the active ingredient.

An overview on the acceptable methods and possible data gaps for analysis of residues of florpyrauxifen-benzyl for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new studies, it is referred to Appendix 2.

**Table 5.2-1: Validated methods for the generation of pre-authorization data**

| **Component of residue definition: Florpyrauxifen-benzyl** | | | | |
| --- | --- | --- | --- | --- |
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing / EU agreed** |
| Plants, plant products,... (Residues) | Primary | 0.01 mg/kg | LC-MS/MS | Devine, C. (2022), Residues of Florpyrauxifen-Benzyl in in Sugarbeet and Process Fractions at Intervals and at Harvest Following a Single or Multiple Applications of GF-3206 – Northern and Southern Europe – 2021, Report No. 210694 |

For all methods below, please, refer to DAR CA B5, Post-Authorization methods at the points indicated in brackets.

#### 5.2.2.1 Methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies

See Point 5.2 (c) (soil), Point 5.2 (d) (water, sediment) and Point 5.2 (e) (air).

#### 5.2.2.2 Methods in soil, water and any additional matrices used in support of efficacy studies

See Point 5.2 (c) (soil) and Point 5.2 (d) (water).

#### 5.2.2.3 Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

See Point 5.2 (a) (feed), Point 5.2 (b) (body fluids and tissues) and Point 5.2 (e) (air).

#### 5.2.2.4 Methods in body fluids, air and any additional matrices used in support of

**operator, worker, resident and bystander exposure studies**

See Point 5.2 (b) (body fluids) and Point 5.2 (e) (air).

#### 5.2.2.5 Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies

See Point 5.2 (a) (plants, plant products, processed food commodities, food of plant and animal origin, feed).

#### 5.2.2.6 Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

See Point 5.2 (c) (soil) and Point 5.2 (d) (water, sediment) and Point 5.2 (a) (plants, plant products, processed food commodities, food of plant and animal origin, feed).

#### 5.2.2.7 Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

Methods used in the studies are fully described in the corresponding physical and chemical properties test reports.

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

The plant protection product F7B-39-30 (Rinpode) is an EC formulation containing 25 g/L florpyrauxifen-benzyl (trademark: Rinskor® active) as active substance. F7B-39-30 is very similar to GF-3206 (the representative formulation for the EU approval of florpyrauxifen-benzyl), with the addition of a food-grade dye included in the composition at 0.0005% w/w. F7B-39-30 and GF-3206 (trademark Loyant 25 Neo EC) are the same formulation type (EC - emulsion concentrate) and contain equal amounts of active ingredient, antifoam, emulsifiers solvents and adjuvant. Given the similarities between the formulation compositions of GF-3206 and F7B-39-30, the analytical methods are therefore leveraged for use with F7B-39-30 formulation. The addition of the dye is not anticipated to impact the determination of the active ingredient.

### 5.3.1 Analysis of the plant protection product (KCP 5.2)

Analytical methods for the determination of the active substance and relevant impurities in the plant protection product were submitted in accordance with the requirements set out in point 5.2.1.

### 5.3.2 Description of analytical methods for the determination of residues of florpyrauxifen-benzyl ester (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 | florpyrauxifen-benzyl ester, florpyrauxifen acid and X11966341 | 0.01 mg/kg | EFSA 2018;16(8):5378 |
| Plant, high acid content | | 0.01 mg/kg | EFSA 2018;16(8):5378 |
| Plant, high protein/high starch content (dry commodities) | | 0.01 mg/kg | EFSA 2018;16(8):5378 |
| Plant, high oil content | | 0.01 mg/kg | EFSA 2018;16(8):5378 |
| Plant, difficult matrices (hops, spices, tea) | | 0.01 mg/kg | EFSA 2018;16(8):5378 |
| Muscle | florpyrauxifen-benzyl ester, florpyrauxifen acid and X11966341 | 0.01 mg/kg | EFSA 2018;16(8):5378 |
| Milk | | 0.01 mg/kg | EFSA 2018;16(8):5378 |
| Eggs | | 0.01 mg/kg | EFSA 2018;16(8):5378 |
| Fat | | 0.01 mg/kg | EFSA 2018;16(8):5378 |
| Liver, kidney | | 0.01 mg/kg | EFSA 2018;16(8):5378 |
| Soil (Ecotoxicology) | florpyrauxifen-benzyl ester, florpyrauxifen acid | 8.10 mg a.s/kg | NOEC derived from *Eisenia fetida* and *Hypoaspis aculeifer* for *GF-3206* |
| Drinking water (Human toxicology) | florpyrauxifen-benzyl ester, florpyrauxifen acid | 0.1 µg/L | general limit for drinking water |
| Surface water (Ecotoxicology) | florpyrauxifen-benzyl ester, florpyrauxifen acid | 0.00258 µg a.s./L | NOEC derived from *Myriophyllum spicatum* for *GF-3206* |
| Air | Florpyrauxifen-benzyl and florpyrauxifen (X11438848) | 15 µg/m³ | AOEL sys/AOEL inhal: 0.13 mg/kg bw/d Senciuc, M., 2023 |
| Tissue (meat or liver) | Florpyrauxifen-benzyl and florpyrauxifen (X11438848) | 0.01 mg/kg | EFSA 2018;16(8):5378 |
| Body fluids | | 0.01 mg/L | Lindner, M., Fiedler, S., 2022, Study ID 221184, new |

### 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 florpyrauxifen-benzyl ester in plant matrices is given in the following tables.

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

| Component of residue definition: florpyrauxifen-benzyl ester | | | | |
| --- | --- | --- | --- | --- |
| **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<br>High acid content<br>High oil content<br>High protein/high starch content (dry) | Primary Confirmatory | 0.01 mg/kg | LC-MS/MS | Huang, T. Y., Walter, M. J., 2015, EFSA 2018;16(8):5378 |
| | ILV | 0.01 mg/kg | LC-MS/MS | Senciuc, M. 2016, EFSA 2018;16(8):5378 |
| | Multi-Residue Method | 0.01 mg/kg | LC-MS/MS | Lindner, M., Grewe, D. 2015, EFSA 2018;16(8):5378 |
| | ILV | 0.01 mg/kg | LC-MS/MS | Austin, R., Turner, R., 2015, EFSA 2018;16(8):5378 |

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

**Table 5.3-3: Statement on extraction efficiency**

| | **Method for products of plant origin** |
| --- | --- |
| Required, available from: | Begley, K., 2017, EFSA 2018;16(8):5378 |
| Not required, because: | N/A |

### 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 florpyrauxifen-benzyl ester in animal matrices is given in the following tables.

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

| Component of residue definition: florpyrauxifen-benzyl ester | | | | |
| --- | --- | --- | --- | --- |
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (*i.e.* GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Milk<br>Eggs<br>Muscle<br>Fat<br>Kidney<br>Liver | Primary Confirmatory | 0.01 mg/kg | LC-MS/MS | Rawle, N. W., 2015, EFSA 2018;16(8):5378 |
| | ILV | 0.01 mg/kg | LC-MS/MS | Senciuc, M., 2015, EFSA 2018;16(8):5378 |
| | Multi-Residue Method | 0.01 mg/kg | LC-MS/MS | Lindner, M., Grewe, D. 2015, EFSA 2018;16(8):5378 |
| | ILV | 0.01 mg/kg | LC-MS/MS | Austin, R., Turner, R., 2015, EFSA 2018;16(8):5378 |

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

**Table 5.3-5: Statement on extraction efficiency**

| | **Method for products of animal origin** |
| --- | --- |
| Required, available from: | Begley, K., 2017, EFSA 2018;16(8):5378 |
| Not required, because: | N/A |

### 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 florpyrauxifen-benzyl ester in soil is given in the following tables.

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

| **Component of residue definition: florpyrauxifen-benzyl ester and florpyrauxifen acid** | | | |
| --- | --- | --- | --- |
| **Method type** | **Method LOQ** | **Principle of method (*i.e.* GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Primary<br>Confirmatory | 6.5 ng/kg (florpyrauxifen-benzyl ester)<br>12 ng/kg (florpyrauxifen acid) | LC-MS/MS | Tinnefeld, V. 2017, EFSA 2018;16(8):5378 |

### 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 florpyrauxifen-benzyl ester in surface and drinking water is given in the following tables.

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

| **Component of residue definition: florpyrauxifen-benzyl ester and florpyrauxifen acid** | | | | |
| --- | --- | --- | --- | --- |
| **Matrix type** | **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Drinking water<br>Surface water | Primary<br>Confirmatory | 0.0025 µg/L (florpyrauxifen-benzyl ester)<br>0.05 µg/L (florpyrauxifen acid) | LC-MS/MS | Lindner, M., 2017, EFSA 2018;16(8):5378 |
| | ILV | 0.0025 µg/L (florpyrauxifen-benzyl ester)<br>0.05 µg/L (florpyrauxifen acid) | LC-MS/MS | Kovacevic, E., 2018, EFSA 2018;16(8):5378 |

### 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 florpyrauxifen-benzyl ester in air is given in the following tables. For the detailed evaluation of new study please refer to Appendix 2.

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

| **Component of residue definition: Florpyrauxifen-benzyl and florpyrauxifen (X11438848** | | | |
| --- | --- | --- | --- |
| **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Primary<br>Confirmatory | 15 µg/m³ | LC-MS/MS | Senciuc, M., 2023, Study ID 221240, new |

| Component of residue definition: Florpyrauxifen-benzyl and florpyrauxifen (X11438848 | | | |
| --- | --- | --- | --- |
| **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Primary<br>Confirmatory | 150 µg/m³ | LC-MS/MS | Senciuc, M., Asekunowo, J., 2015, EFSA 2018;16(8):5378 |

### 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 florpyrauxifen-benzyl ester in body fluids and tissues is given in the following table. For the detailed evaluation of new study it is referred to Appendix 2.

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

| Component of residue definition: Florpyrauxifen-benzyl and florpyrauxifen (X11438848) | | | |
| --- | --- | --- | --- |
| **Method type** | **Method LOQ** | **Principle of method (i.e. GC-MS or HPLC-UV)** | **Author(s), year / missing** |
| Primary<br>Confirmatory | 0.01 mg/L | LC-MS/MS | Lindner, M., Fiedler, S., 2022, Study ID 221184, new |
| Primary<br>Confirmatory | 0.05 mg/L | LC-MS/MS | LaMonaca, S., 2017, EFSA 2018;16(8):5378 |
| Primary<br>Confirmatory | 0.01 mg/kg | LC-MS/MS | Rawle, N. W., 2015, EFSA 2018;16(8):5378 |

### 5.3.2.8 Other studies/ information

Not applicable.

# 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, NOT evaluated at EU peer review**

| Data point | Author(s) | Year | **Title**<br>**Company Report No.**<br>**Source (where different from company)**<br>**GLP or GEP status**<br>**Published or not** | **Vertebrate study Y/N** | Owner |
| --- | --- | --- | --- | --- | --- |
| KCP 5.1.1 | Emery, W. | 2023 | Evaluation of Interferences when using DAS-AM-G-13-52 for the Determination of Florpyrauxifen-benzyl in F7B-39-30 Formulation<br>Corteva Report No: 230138<br>Corteva Agrisciences LLC, 9330 Zionsville Road Indianapolis, Indiana 46268<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| KCP 5.1.2 | Devine, C. | 2022 | Residues of Florpyrauxifen-Benzyl in Sugarbeet and Process Fractions at Intervals and at Harvest Following a Single or Multiple Applications of GF-3206 – Northern and Southern Europe – 2021<br>DAS Report No: 210694<br>CEM Analytical Services Ltd (CEMAS), Wokingham, Berkshire, UK<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| KCP 5.2 | Seniuc, M. | 2023 | Development and Validation of an Analytical Method for the Determination of Florpyrauxifen-benzyl and its Metabolite X11438848 in Air<br>DAS Report No: 221240<br>Eurofins Agroscience Services EAG Laboratories, Ulm, Germany<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study<br>Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
| KCP 5.2 | Lindner, M., Fiedler, S. | 2022 | Validation of an Analytical Method for the Determination of Florpyrauxifen-benzyl and X11438848 in Body Fluids<br>DAS Report No: 221184<br>Eurofins Agroscience Services Chem GmbH, Hamburg, Germany<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |

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

| Data point | Author | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study<br>Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
| CP 5.1.1 (a) | Saur J. | 2014 | Analytical Method and Validation for the Determination of XDE-848 BE in GF-3206.<br>Dow AgroSciences LLC, Actives to Products R&D 9330 Zionsville Road Indianapolis, Indiana 46268<br>Report No. DAS-AM-G-13-52<br>GLP<br>Unpublished. | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.1.1 (a) | Kerbleski, H., Hamilton, T. | 2015 | Analytical Method and Validation for the Determination of Active Ingredient in XDE-848 BE Technical by Liquid Chromatography<br>DAS Report No. DAS AM-2015004<br>The Dow Chemical Company, Midland, MI USA<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.1.1 (b) | Kerbleski, H., Dixon, T. | 2015 | Analytical Method and Validation for the Determination Residual Solvents in XDE-848 BE Technical by Gas Chromatography | N | Corteva Agriscience (bringing together the |

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| --- | --- | --- | --- | --- | --- |
| | | | DAS Report No. DAS AM-2015003179<br>The Dow Chemical Company, Midland, MI USA<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | | global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.2 (a)/1 | Lindner, M., Grewe, D. | 2015 | Validation of a Multi-Residue Method following the QuEChERS Sample Preparation Technique for the Determination of XDE-848 BE and XDE-848 in Matrices of Plant and Animal Origin<br>DAS Report No. 130588<br>Eurofins Agroscience Services Chem GmbH, Hamburg, Germany<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.2 (a)/2 | Austin, R., Turner, R. | 2015 | Independent Laboratory Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of XDE-848 Benzyl Ester and its Metabolite X11438848 in Matrices of Plant and Animal Origin<br>DAS Report No. 140899<br>Battelle UK Ltd, Chelmsford, Essex, UK<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.2 (a)/3 | Huang, T. Y., Walter, M. J. | 2015 | Method Validation of the Determination of Residues of XDE-848 Benzyl Ester and its Metabolites in Rice Grain and Straw Using Liquid Chromatography with Tandem Mass Spectrometry<br>DAS Report No. 130794.01<br>Dow AgroSciences LLC, Indianapolis, Indiana, USA<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.2 (a)/4 | Huang, T. Y., Walter, M. J. | 2015 | Method Validation of the Determination of Residues of XDE-848 Benzyl Ester and its Metabolites in Rice Processed Fractions Using Liquid Chromatography with Tandem Mass Spectrometry<br>DAS Report No. 130794.02<br>Dow AgroSciences LLC, Indianapolis, Indiana, USA<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.2 (a)/5 | Austin, R., Turner, R. | 2015 | Independent Laboratory Validation of a Dow AgroSciences Method for the Determination of XDE-848 Benzyl Ester and Three Metabolites (X11438848, X12300837 and X11966341) in Agricultural Commodities<br>DAS Report No. 140963<br>Battelle UK Ltd, Chelmsford, Essex, UK<br>GLP/GEP (Y/N): Yes | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |

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| --- | --- | --- | --- | --- | --- |
| | | | Published (Y/N): No | | |
| CA 4.2 (a)/6 | Rawle, N. W. | 2015 | Validation of an Analytical Method for the Determination of XDE-848 Benzyl Ester, its Acid Metabolite (X11438848) and its Hydroxyl Acid Metabolite (X11966341) in Animal Matrices<br>DAS Report No. 140961<br>CEM Analytical Services Ltd (CEMAS), Wokingham, Berkshire, UK<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.2 (a)/7 | Senciuc, M. | 2015 | Independent Laboratory Validation (ILV) of the Determination of XDE-848 Benzyl Ester and two Metabolites X11438848 and X11966341 in Animal Matrices<br>DAS Report No. 140958<br>PTRL Europe GmbH, D-89081 Ulm, Germany<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.2 (a)/8 | Deziderio, L. Ap. G. | 2016 | Validation for the Determination of Residues of XDE-848 BE (Benzyl Ester) and its Metabolites XDE-848 acid (X11438848) and XDE-848 HA (X11966341) in Agricultural Commodities by Liquid Chromatography with Tandem Mass Spectrometry<br>DAS Report No. 150818<br>Dow AgroSciences Sementes e Biotecnologia Brasil Ltda, Cravinhos, SP, Brazil<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.2 (a)/9 | Senciuc, M. | 2016 | Independent Laboratory Validation (ILV) of the Determination of XDE-848 Benzyl Ester and Two Metabolites X11438848 and X11966341 in Crop Matrices<br>DAS Report No. 150105<br>PTRL Europe GmbH, D-89081 Ulm, Germany<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.2 (b)/1 | Huang, T. Y., Walter, M. J. | 2015 | Method Validation Study for the Determination of Residues of XDE-848 Benzyl Ester and Three Metabolites (X11438848, X12300837 and X11966341) in Soil by Liquid Chromatography with Tandem Mass Spectrometry<br>DAS Report No. 140956<br>Dow AgroSciences LLC, Indianapolis, Indiana, USA<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.2 (a)/10 | Begley, K. | 2017 | Determination of the Extraction Efficiency of XDE-848-BE and XDE-848-Acid Using Multiple Extraction Procedures Across NORs and Analytical Methods | N | Corteva Agriscience (bringing together the |

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| | | | DAR Report No. 160014<br>Charles River Laboratories Edinburg Ltd, Elphinstone Research Centre, Tranent, East Lothian, EH33 2NE, UK<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | | global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.2 (b)/2 | Huang, T. Y., Walter, M. J. | 2015 | Method Validation Study for the Determination of Residues of XDE-848 and Five Metabolites Metabolites (X11438848, X12300837, X11966341, X12131932 and X12393505) in Ground, Surface, and Drinking Water by Liquid Chromatography with Tandem Mass Spectrometry<br>DAS Report No. 140952<br>Dow AgroSciences LLC, Indianapolis, Indiana, USA<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.2 (b)/3 | Austin, R. | 2015 | Independent Laboratory Validation of a Dow AgroSciences Method for the Determination of XDE-848 Benzyl Ester and Five Metabolites (X11438848, X12300837, X11966341, X12131932 and X12393505) in Water<br>DAS Report No. 140962<br>Battelle UK Ltd, Chelmsford, Essex, UK<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.2 (b)/4 (CA 4.1.2 (a)-02) | Finger, N. | 2015 | Field dissipation study with one summer application of XDE-848 BE (GF 3162) at six sites in Southern Europe in 2013 - 2014<br>DAS Report No. 141185<br>Eurofins Agroscience Services Chem GmbH, Hamburg, Germany<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.2 (b)/5 | Tinnefeld V., Kovacevic, E. | 2017 | Validation Study for the Determination of XDE-848 Benzyl Ester and its Metabolite X11438848 in Soil<br>DAS Report No. 170588<br>Eurofins Agroscience Services EcoChem GmbH, Niefem-Öschelbronn, Germany<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.2 (b)/6 | Senciuc, M., Asekunowo, J. | 2015 | Validation of an Analytical Method for the Determination of XDE-848 BE in Ground-, Surface- and Drinking Water<br>DAS Report No. 170590<br>Eurofins Agroscience Services Chem GmbH, Hamburg, Germany<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |

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| CA 4.2 (c)/1 | Senciuc, M., Asekunowo, J. | 2015 | The Development and Validation of a Method for the Analysis of XDE848 Benzyl Ester in Air<br>DAS Report No. 140898<br>PTRL Europe GmbH, D-89081 Ulm, Germany<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 4.2 (g)/1 | LaMonaca, SM. | 2017 | Validation of Analytical Method for the Determination of XDE-848 BE in body fluids.<br>DAS Report No. 170551<br>JRF America, Inc., Audubon, Pennsylvania, USA<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 8.3.1.2 | Vergé, E. | 2017 | GF-3206 - Assessment of Effects on the Adult Honey Bee, *Apis mellifera* L., in a 10 Day Chronic Feeding Test under Laboratory Conditions.<br>DAS Report No. 170080<br>Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany<br>GLP/GEP (Y/N):Y<br>Published (Y/N):N | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |
| CA 8.3.1.3 | Vergé, E. | 2018 | Honey Bee (Apis mellifera L.) 22 Day Larval Toxicity Test (Repeated Exposure)<br>DAS Report No. 170081<br>Eurofins Agroscience Services , Niefern-Öschelbronn, Germany<br>GLP/GEP (Y/N): Yes<br>Published (Y/N): No | N | Corteva Agriscience (bringing together the global heritage businesses of Pioneer, DuPont Crop Protection, and Dow AgroSciences) |

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<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study<br>Y/N | Owner |
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**List of data relied on not submitted by the applicant but necessary for evaluation**

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study<br>Y/N | Owner |
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# Appendix 2 Detailed evaluation of submitted analytical methods

## A 2.1 Analytical methods for florpyrauxifen-benzyl ester

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

#### A 2.1.1.1 Analytical method 1

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| --- | --- |
| Comments of zRMS: | The analytical phase of residues study of the study 210694 (CEMS-9853) is acceptable and suitable for the determination of Florpyrauxifen-Benzyl in Sugarbeet.<br>The study has been performed in compliance with Good Laboratory Practice and SANTE/2020/12830 rev. 1.<br>The limits of quantitation (LOQ) was 0.01 mg/kg. |

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| Reference: | KCP 5.1.2 |
| Report author: | Devine, C. |
| Report year: | 2022 |
| Report title: | Residues of Florpyrauxifen-Benzyl in Sugarbeet and Process Fractions at Intervals and at Harvest Following a Single or Multiple Applications of GF-3206 – Northern and Southern Europe – 2021 |
| Report No.: | 210694 |
| Testing Facility Report No.: | CEMS-9853 |
| Method(s) used: | Validated within (see study plan amendment 6) |
| Guidelines followed in study: | SANTE/2020/12830 rev. 1 |
| Deviation from current test guidelines: | No |
| Previous evaluation: | No, not previously submitted |
| | |
| Analytical Performing Laboratory: | CEM Analytical Services Ltd (CEMAS) |
| GLP/Officially recognised testing facilities: | Wokingham, Berkshire, UK |
| Acceptability/Reliability: | Yes |

**MATERIALS AND METHODS**

**MATERIAL AND METHODS**

**Method Principle**

Residues of XDE-848 benzyl ester and metabolites were extracted from the sample matrices by shaking first with acetonitrile/water (90:10, v/v) followed by a separate extraction with

acetonitrile/0.2 N HCl (50:50, v/v). The sample was then rinsed with additional acetonitrile/0.2 N HCl (50:50, v/v). An aliquot of the extract was transferred along with internal standard. The sample was evaporated to dryness. Samples were then hydrolyzed at 80°C for 60 minutes in the presence of 1 N HCl. After cooling, ethyl acetate was added followed by mixing. An aliquot of the upper ethyl acetate layer was transferred to a plate and evaporated to dryness. The samples were reconstituted and analyzed for XDE-848 BE and metabolites by liquid chromatography with positive ion electrospray ionization tandem mass spectrometry (HPLC-MS/MS).

**RESULTS AND DISCUSSION**

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). The results obtained are summarised in the following tables.

**Table:1 Recovery results from method validation of XDE-848 BE (m/z441.0/91.0) using the analytical method**

| Matrix | Fortification level mg/kg | Mean Recovery (%) | RSD (%) | n | Comments |
| --- | --- | --- | --- | --- | --- |
| Sugarbeet Roots | 0.01 | 95 | 10.0 | 17 | |
| Sugarbeet Roots | 0.1 | 102 | 7.5 | 18 | |
| Sugarbeet Tops With Leaves | 0.01 | 97 | 16.4 | 16 | |
| Sugarbeet Tops With Leaves | 0.1 | 102 | 9.7 | 16 | |

**Table:2 Procedural recovery results of X11438848 (m/z 351.0/124.0) using the analytical method**

| Matrix | Fortification level mg/kg | Mean Recovery (%) | RSD (%) | n | Comments |
| --- | --- | --- | --- | --- | --- |
| Sugarbeet Roots | 0.01 | 109 | 6.5 | 17 | |
| Sugarbeet Roots | 0.1 | 107 | 9.5 | 18 | |
| Sugarbeet Tops With Leaves | 0.01 | 107 | 12.0 | 16 | |
| Sugarbeet Tops With Leaves | 0.1 | 110 | 9.6 | 16 | |

**Table:3 Procedural recovery results of X11966341 (m/z 335.0/254.0) using the analytical method**

| Matrix | Fortification level mg/kg | Mean Recovery (%) | RSD (%) | n | Comments |
| --- | --- | --- | --- | --- | --- |
| Sugarbeet Roots | 0.01 | 106 | 6.6 | 17 | |
| Sugarbeet Roots | 0.1 | 105 | 7.2 | 18 | |
| Sugarbeet Tops With Leaves | 0.01 | 98 | 11.0 | 16 | |
| Sugarbeet Tops With Leaves | 0.1 | 100 | 10.1 | 16 | |

**Table:4 Procedural recovery results of X12393505 (m/z 315.0/233.9) using the analytical method**

| Matrix | Fortification level mg/kg | Mean Recovery (%) | RSD (%) | n | Comments |
| --- | --- | --- | --- | --- | --- |
| Sugarbeet Roots | 0.01 | 110 | 7.6 | 17 | |
| Sugarbeet Roots | 0.1 | 108 | 7.5 | 18 | |
| Sugarbeet Tops With Leaves | 0.01 | 106 | 8.6 | 16 | |
| Sugarbeet Tops With Leaves | 0.1 | 110 | 7.4 | 16 | |

**Table:5 Procedural recovery results of X12568215 (m/z 301.0/220.0) using the analytical method**

| Matrix | Fortification level mg/kg | Mean Recovery (%) | RSD (%) | n | Comments |
| --- | --- | --- | --- | --- | --- |
| Sugarbeet Roots | 0.01 | 109 | 8.7 | 17 | |
| Sugarbeet Roots | 0.1 | 107 | 8.0 | 18 | |
| Sugarbeet Tops With Leaves | 0.01 | 103 | 13.3 | 16 | |
| Sugarbeet Tops With Leaves | 0.1 | 105 | 12.6 | 16 | |

**Table:6 Characteristics for the analytical method used for determination of residues of validation of XDE-848 BE, X11438848, X11966341, X12393505 and X12568215 in sugarbeet roots and tops with leaves**

| Analyte | XDE-848 BE | X11438848 | X11966341 | X12393505 | X12568215 |
| --- | --- | --- | --- | --- | --- |
| Matrix | Sugarbeet, roots and tops with leaves | Sugarbeet, roots and tops with leaves | Sugarbeet, roots and tops with leaves | Sugarbeet, roots and tops with leaves | Sugarbeet, roots and tops with leaves |
| Technique | LC-MS/MS | LC-MS/MS | LC-MS/MS | LC-MS/MS | LC-MS/MS |
| Specificity | *m/z* 441.0/91.0 blank value <30% LOQ | *m/z* 351.0/124.0 blank value <30% LOQ | *m/z* 335.0/254.0 blank value <30% LOQ | *m/z* 315.0/233.9 blank value <30% LOQ | *m/z* 301.0/220.0 blank value <30% LOQ |
| Calibration (type, number of data points) | linear regression analysis with 1/x weighting r≥0.999 8 data points | linear regression analysis with 1/x weighting r≥0.999 8 data points | linear regression analysis with 1/x weighting r≥0.999 8 data points | linear regression analysis with 1/x weighting r≥0.999 8 data points | linear regression analysis with 1/x weighting r≥0.999 8 data points |
| Calibration range | Concentration range of 0.075-7.5 ng/mL equivalent to 0.003-0.3 mg/kg | Concentration range of 0.075-7.5 ng/mL equivalent to 0.003-0.3 mg/kg | Concentration range of 0.075-7.5 ng/mL equivalent to 0.003-0.3 mg/kg | Concentration range of 0.075-7.5 ng/mL equivalent to 0.003-0.3 mg/kg | Concentration range of 0.075-7.5 ng/mL equivalent to 0.003-0.3 mg/kg |
| Limit of quantitation | 0.01 mg/kg | 0.01 mg/kg | 0.01 mg/kg | 0.01 mg/kg | 0.01 mg/kg |
| Validation Range | 0.01-0.1 mg/kg | 0.01-0.1 mg/kg | 0.01-0.1 mg/kg | 0.01-0.1 mg/kg | 0.01-0.1 mg/kg |

**CONCLUSION**

This method was successfully validated for the determination of XDE-848 BE, X11438848, X11966341, X12393505 and X12568215 in sugar beet roots and tops with leaves according to the guidelines set forth in SANTE/2020/12830 rev.1 and may be considered fit for purpose in supporting the residue data generated herein.

## 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)

| Comments of zRMS: | The method Determination of Florpyrauxifen-benzyl and its Metabolite X11438848 in Air is acceptable validated in accordance with SANTE/2020/12830 rev. 1. The limits of quantitation (LOQ) were at ≤ 15 µg/m$^3$. |
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| Reference: | KCP 5.2 |
| Report author: | Senciuc, M. |
| Report year: | 2023 |
| Report title: | Development and Validation of an Analytical Method for the Determination of Florpyrauxifen-benzyl and its Metabolite X11438848 in Air |
| Report No.: | 221240 |
| Testing Facility Report No.: | S22-08469 |
| Method(s) used: | 221240 |
| Guidelines followed in study: | SANTE/2020/12830 rev. 1 |
| Deviation from current test guidelines: | No |
| Previous evaluation: | No, not previously submitted |
| Analytical Performing Laboratory: | Eurofins Agroscience Services EAG Laboratories, Ulm, Germany |
| GLP/Officially recognised testing facilities: | Yes |
| Acceptability/Reliability: | Yes |

## MATERIALS AND METHODS

### Test Item(s)

| | |
| --- | --- |
| Test item (common name): | Florpyrauxifen-benzyl |
| Purity: | 99.2 % |
| Description (physical state): | White powder |
| Lot/batch no.: | JY-001-174-22 TSN305894 |

| | |
| --- | --- |
| Test item (common name): | X11438848 |
| Purity: | 100 % |
| Description (physical state): | White powder |
| Lot/batch no.: | XP3-124113-033 TSN304667 |

**Method Scope**

This method is applicable for the quantitative determination of residues of florpyrauxifen-benzyl and X11438848 in XAD adsorption air tubes. The method was independently validated in ambient air and warm, humid air over the concentration range of ≤ 15 - ≤ 150 µg/m$^3$ with a validated limit of quantitation of ≤ 15 µg/m$^3$.

**Method Principle**

Residues of florpyrauxifen-benzyl and X11438848 are extracted from samples by methanol. The final sample is analysed for florpyrauxifen-benzyl and X11438848 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

**Linearity**

For each analyte, the linearity of detector response was evaluated using ≥ 5 solvent standard solutions at different concentrations ranging from 1.08 to 108 ng/mL (equivalent to 3.0 to 300 µg/m$^3$. Calibration curves were calculated by linear regression analysis with 1/x weighting.

**Selectivity**

The LC-MS/MS method is highly selective for both the quantitation and confirmation of Florpyrauxifen-benzyl and X11438848. Significant peak response (>30% of the LOQ peak area) is not observed in reagent blank and extracts of untreated blank control samples at the expected retention times of the analytes. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte as follows in the table below.

**Table 1:Transitions monitored**

| | |
| --- | --- |
| Florpyrauxifen-benzyl | *m/z* Q1/Q3 441/91 (quantitative) |
| Florpyrauxifen-benzyl | *m/z* Q1/Q3 439/91 (confirmatory) |
| X11438848 | *m/z* Q1/Q3 351/270 (quantitative) |
| X11438848 | *m/z* Q1/Q3 349/268 (confirmatory) |

**Confirmation**

Confirmation of the presence of florpyrauxifen-benzyl and X11438848 was by comparison of retention times (liquid chromatography) of recovery samples with the retention times of the calibration standards as well as by monitoring two structurally characteristic MS/MS transitions for each analyte by tandem mass spectrometry. Validation data obtained using the confirmatory MS/MS transitions met the same acceptance criteria as the validation data generated using the quantitative MS/MS transitions, therefore demonstrating that the analyte signal of the quantitative MS/MS transition is selective and not affected by interferences.

## Limits of Detection and Quantitation

The limit of quantitation, defined as the lowest concentration of an analyte tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable relative standard deviation is obtained, is ≤ 15 µg/m$^3$ for both analytes in both tested matrices.

The limit of detection, defined as 20% of the LOQ, is ≤ 3.0 µg/m$^3$ for all analytes in all tested matrices. The LOD is equivalent to the lowest calibration point.

## RESULTS AND DISCUSSION

## Summary of Recovery

Results obtained were within guideline requirements (mean recovery 70-110%; RSD ≤ 20%). For each analyte, the two ion mass transitions could be used interchangeably for quantification and confirmation. The results obtained are summarised in the following tables.

**Table 2: Summary of quantitative recovery of Florpyrauxifen-benzyl (*m/z* 441/91)**

| Matrix group | Matrix | LOQ | Fortification level | Recovery (%) | | SD | RSD | n |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | µg/m3 | mean | range | (%) | (%) | |
| air | Ambient air | 14.3 | 14.3 | 84.6 | 70.6 - 101 | 14 | 16 | 5 |
| air | Ambient air | 14.3 | 144 | 91.3 | 74.6 - 104 | 12 | 13 | 5 |
| air | Warm humid air | 14.2 | 14.2 | 90.4 | 79.1 - 102 | 8.5 | 9.4 | 5 |
| air | Warm humid air | 14.2 | 141 | 86.6 | 80.6 – 90.6 | 4.4 | 5.1 | 5 |

**Table 3: Summary of confirmatory recovery of Florpyrauxifen-benzyl (*m/z* 439/91)**

| Matrix group | Matrix | LOQ | Fortification level | Recovery (%) | | SD | RSD | n |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | µg/m3 | mean | range | (%) | (%) | |
| air | Ambient air | 14.3 | 14.3 | 85.4 | 70.2 - 101 | 14 | 16 | 5 |
| air | Ambient air | 14.3 | 144 | 91.8 | 75.2 - 104 | 12 | 13 | 5 |
| air | Warm humid air | 14.2 | 14.2 | 91.3 | 80.2 - 104 | 9.7 | 11 | 5 |
| air | Warm humid air | 14.2 | 141 | 86.4 | 81.5 – 90.2 | 3.5 | 4.1 | 5 |

**Table 4: Summary of quantitative recovery of X11438848 (*m/z* 351/270)**

| Matrix group | Matrix | LOQ | Fortification level | Recovery (%) | | SD | RSD | n |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | µg/m3 | mean | range | (%) | (%) | |
| air | Ambient air | 14.3 | 14.3 | 81.7 | 70.2 – 89.8 | 9.2 | 11 | 5 |
| air | Abient air | 14.3 | 144 | 82.4 | 72.4 – 90.9 | 8.0 | 9.8 | 5 |
| air | Warm humid air | 14.2 | 14.2 | 90.1 | 83.1– 96.1 | 4.8 | 5.4 | 5 |
| air | Warm humid air | 14.2 | 141 | 83.9 | 77.0 – 87.2 | 4.3 | 5.1 | 5 |

**Table 5: Summary of confirmatory recovery of X11438848 (*m/z* 349/268)**

| Matrix group | Matrix | LOQ | Fortification level | Recovery (%) | | SD | RSD | n |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | µg/m3 | mean | range | (%) | (%) | |
| air | Ambient air | 14.3 | 14.3 | 83.6 | 71.1 - 91.9 | 9.8 | 12 | 5 |
| air | Ambient air | 14.3 | 144 | 81.6 | 72.8 – 89.4 | 7.4 | 9.1 | 5 |
| air | Warm humid air | 14.2 | 14.2 | 88.1 | 80.4 – 95.7 | 6.4 | 7.3 | 5 |
| air | Warm humid air | 14.2 | 141 | 85.0 | 77.4 – 89.1 | 4.6 | 5.4 | 5 |

**Repeatability**

Repeatability achieved by demonstrating acceptable recovery at multiple concentration levels, resulting in a relative standard deviation (RSD) < 20%.

**Working Solution Stability**

Stock solutions of florpyrauxifen-benzyl and X11438848 prepared in methanol were tested after 16 days of storage at 1 to 10 °C and were found to be stable.

Calibration standard solutions of florpyrauxifen-benzyl and X11438848 prepared in methanol/water (1/1, v/v) with 0.1 % formic acid were tested after 16 days of storage at 1 to 10 °C and were found to be stable.

**Sample Extract Stability**

Sample extracts of florpyrauxifen-benzyl and X11438848 in final extracts were tested after 12 days of storage at 1 to 10 °C and were found to be stable.

**Matrix Effects**

Matrix effects were evaluated by comparing the response of the analyte fortified in a control extract after processing (for each matrix type) to the response of the analyte fortified in neat solvent. The results demonstrate that matrix effects are within ±20%.

**Extraction Efficiency**

Extraction efficiency of florpyrauxifen-benzyl and X11438848 from XAD adsorption tubes with methanol was performed with average recoveries in the range of 107 to 119 %.

Extraction efficiency was not assessed as a part of this study.

**CONCLUSION**

Method is acceptable based on current guidelines: SANTE/2020/12830 Rev.1, as well as PMRA Regulatory Directive Dir98-02.

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

| | |
| --- | --- |
| Comments of zRMS: | The method Determination of Florpyrauxifen-benzyl and X11438848 in Body Fluids is acceptable validated in accordance with SANTE/2020/12830 rev. 1. The limits of quantitation (LOQ) were at 0.01 mg/L. |

| | |
| --- | --- |
| Reference: | KCP 5.2 |
| Report author: | Lindner, M., Fiedler, S. |
| Report year: | 2022 |
| Report title: | Validation of an Analytical Method for the Determination of Florpyrauxifen-benzyl and X11438848 in Body Fluids |
| Report No.: | 221184 |
| Testing Facility Report No.: | S22-08366 |
| Method(s) used: | 21184 |
| Guidelines followed in study: | SANTE/2020/12830, rev.1 (2021); OPPTS 860.1340 (1996) |
| Deviation from current test guidelines: | No |
| Previous evaluation: | No, not previously submitted |
| Analytical Performing Laboratory: | Eurofins Agroscience Services Chem GmbH, Hamburg, Germany |
| GLP/Officially recognised testing facilities: | Yes |
| Acceptability/Reliability: | Yes |

**MATERIALS AND METHODS**

**Test Item(s)**

| | |
| --- | --- |
| Test item (common name): | Florpyrauxifen-benzyl |
| Purity: | 99.2 % |
| Description (physical state): | - |
| Lot/batch no.: | JY-001-174-22 (TSN305894) |

| | |
| --- | --- |
| Test item (common name): | X11438848 |
| Purity: | 97 % |
| Description (physical state): | - |
| Lot/batch no.: | XP3-124113-033 (TSN304667) |

**Method Scope**

This method is applicable for the quantitative determination of residues of florpyrauxifen-benzyl and X11438848 in biofluids. The method was validated in porcine urine and bovine whole blood over the concentration range of 0.01-0.1 mg/L with a validated limit of quantitation of` 0.01 mg/L.

**Method Principle**

Residues of florpyrauxifen-benzyl and X11438848 are extracted from samples by acetonitrile. The final sample is analysed for florpyrauxifen-benzyl and X11438848 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

**Linearity**

For each analyte, the linearity of detector response was evaluated using at least 8 matrix matched standard solutions across the range of 0.03 – 2.0 ng/mL. Calibration curves were calculated by linear regression analysis with 1/x weighting.

**Selectivity**

The LC-MS/MS method is highly selective for both the quantitation and confirmation of Florpyrauxifen-benzyl and X11438848. Significant peak response (>30% of the LOQ peak area) is not observed in reagent blank and extracts of untreated blank control samples at the expected retention times of the analytes. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte as follows in the table below.

**Table 2:Transitions monitored**

| | |
| --- | --- |
| Florpyrauxifen-benzyl | *m/z* Q1/Q3 439/91 (quantitative) |
| Florpyrauxifen-benzyl | *m/z* Q1/Q3 441/65 (confirmatory) |
| X11438848 | *m/z* Q1/Q3 349/268 (quantitative) |
| X11438848 | *m/z* Q1/Q3 351/270 (confirmatory) |

**Confirmation**

Confirmation of the presence of Florpyrauxifen-benzyl and X11438848 was by comparison of retention times (liquid chromatography) of recovery samples with the retention times of the calibration standards as well as by monitoring two structurally characteristic MS/MS transitions for each analyte by tandem mass spectrometry. Validation data obtained using the confirmatory MS/MS transitions met the same acceptance criteria as the validation data generated using the quantitative MS/MS transitions, therefore demonstrating that the analyte signal of the quantitative MS/MS transition is selective and not affected by any interferences.

**Limits of Detection and Quantitation**

The limit of quantitation, defined as the lowest concentration of an analyte tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery

with an acceptable relative standard deviation is obtained, is 0.01 mg/L for all analytes in all tested matrices.

The limit of detection, defined as 30% of the LOQ is 0.003 mg/L (equivalent to the lowest calibration standard) for all analytes in all tested matrices.

## RESULTS AND DISCUSSION

### Summary of Recovery

Results obtained were within guideline requirements (mean recovery 70-110%; RSD ≤ 20%). For each analyte, the two ion mass transitions could be used interchangeably for quantification and confirmation. The results obtained are summarised in the following tables.

**Table 3: Summary of quantitative recovery of Florpyrauxifen-benzyl (*m/z* 439/91*)***

| Matrix group | Matrix | Fortification level | Recovery (%) | | RSD | n |
| --- | --- | --- | --- | --- | --- | --- |
| | | mg/L | mean | range | (%) | |
| Body Fluids | Porcine Urine | 0.01 | 93 | 90 - 96 | 3.2 | 5 |
| Body Fluids | Porcine Urine | 0.1 | 89 | 83 - 92 | 4.0 | 5 |
| Body Fluids | Bovine whole Blood | 0.01 | 98 | 95 - 102 | 2.5 | 5 |
| Body Fluids | Bovine whole Blood | 0.1 | 83 | 75 - 93 | 8.4 | 5 |

**Table 4: Summary of confirmatory recovery of Florpyrauxifen-benzyl (*m/z* 441/65)**

| Matrix group | Matrix | Fortification level | Recovery (%) | | RSD | n |
| --- | --- | --- | --- | --- | --- | --- |
| | | mg/L | mean | range | (%) | |
| Body Fluids | Porcine Urine | 0.01 | 93 | 90 - 98 | 3.8 | 5 |
| Body Fluids | Porcine Urine | 0.1 | 89 | 82 - 91 | 4.4 | 5 |
| Body Fluids | Bovine whole Blood | 0.01 | 95 | 94 - 96 | 1.1 | 5 |
| Body Fluids | Bovine whole Blood | 0.1 | 83 | 74 - 94 | 8.8 | 5 |

**Table 5: Summary of quantitative recovery of X11438848 (*m/z* 349/268)**

| Matrix group | Matrix | Fortification level | Recovery (%) | | RSD | n |
| --- | --- | --- | --- | --- | --- | --- |
| | | mg/L | mean | range | (%) | |
| Body Fluids | Porcine Urine | 0.01 | 79 | 75 - 85 | 5.2 | 5 |
| Body Fluids | Porcine Urine | 0.1 | 83 | 81 - 86 | 2.1 | 5 |
| Body Fluids | Bovine whole Blood | 0.01 | 86 | 79 - 95 | 6.8 | 5 |
| Body Fluids | Bovine whole Blood | 0.1 | 82 | 77 - 89 | 5.5 | 5 |

**Table 6: Summary of confirmatory recovery of X11438848 (*m/z* 351/270)**

| Matrix group | Matrix | Fortification level | Recovery (%) | | RSD | n |
| --- | --- | --- | --- | --- | --- | --- |
| | | mg/L | mean | range | (%) | |
| Body Fluids | Porcine Urine | 0.01 | 80 | 75 - 85 | 5.3 | 5 |
| Body Fluids | Porcine Urine | 0.1 | 84 | 83 - 86 | 1.2 | 5 |
| Body Fluids | Bovine whole Blood | 0.01 | 82 | 79 - 84 | 2.6 | 5 |
| Body Fluids | Bovine whole Blood | 0.1 | 83 | 76 - 90 | 6.4 | 5 |

## Repeatability

Repeatability was achieved by validating the method in different matrices, at different concentration levels, resulting in % RSD values <20%.

## Working Solution Stability

The stability of working solutions relied upon historical data (Study 130588), which demonstrated stock and fortification solutions in the same solvent, stored refrigerated for up to 195 days.

**Sample Extract Stability**

Porcine urine sample extracts of Florpyrauxifen-benzyl and X11438848 in methanol / water (1+1, v+v) containing 0.1 % formic acid were tested after 8 days of storage at typically 1 °C to 10 °C and were found to be stable.

Bovine whole blood sample extracts of Florpyrauxifen-benzyl and X11438848 in methanol / water (1+1, v+v) containing 0.1 % formic acid were tested after 9 days of storage at typically 1 °C to 10 °C and were found to be stable.

**Matrix Effects**

Matrix effects were evaluated by comparing the response of the analyte fortified in a control extract after processing (for each matrix type) to the response of the analyte fortified in neat solvent. For florpyrauxifen-benzyl in porcine urine and bovine whole blood, the results demonstrate that matrix effects are within ±20%. For {X11438848 in porcine urine and bovine whole blood, the results demonstrate that matrix effects exceed ±20%. Matrix matched standards were used for quantification for both analytes in all matrices for this study.

**Extraction Efficiency**

Extraction efficiency was not assessed as a part of this study.

**CONCLUSION**

Method is acceptable based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340 and SANTE/2020/12830 Rev.1

## A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted.