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| REGISTRATION REPORT  Part B  Section 5  Analytical Methods  Detailed summary of the risk assessment |
| Product code: ADM.09250.H.1.A  Product name(s): 2,4-D 95 SP  Chemical active substance:  2,4-dichlorophenoxy acetic acid, 80.4% or 804 g/Kg |
| Central Zone  Zonal Rapporteur Member State: Poland |
| CORE ASSESSMENT  (authorization) |
| Applicant: XXXX  Sponsor: XXXX  Submission date: March 2023 updated November 2023  Evaluation date: December 2023  MS Finalisation date: March 2024 |

Version history

|  |  |
| --- | --- |
| When | What |
| March 2023 | 1st applicant version |
| November 2023 | Update following reciept of final reports S23-100476 and S21-07464 and reports S23-101771, S23-101772 and S23-102036 |
| December 2023 | Initial RR |
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# Analytical methods

## Conclusion and summary of assessment

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: none.

~~data gap 1~~

~~data gap 2~~

~~data gap 3~~

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

The dRR was not rewritten by the zRMS. The added comments are on a grey background.

Noticed data gaps are: none

| Commodity/crop | Supported/ Not supported |
| --- | --- |
| Cereals | Supported |

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

### Analysis of the plant protection product (KCP 5.1.1)

#### 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 2,4-D in plant protection product is provided as follows:

|  |  |
| --- | --- |
| Comments of zRMS: | This method is validated and accepted. It can be used for analysing the 2,4-D in the PPP. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.1/01 |
| Report | Comb, T. 2022  2,4 D 95 SP: Method validation  Report No.: ACE-21-387  Sponsor reference No.: 000109838 |
| Guideline(s): | SANCO/3030/99 Rev. 5 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

The analysis of 2,4-D in ADM.09250.H.1.A was performed by high performance liquid chromatography (HPLC).

The formulation was prepared by weighing 50 mg of the test item into 10 mL volumetric flasks and diluting to volume with methanol:water:80% acetic acid solution (55:41:4 v/v/v). The sample was then further diluted by a factor of 25 with methanol:water:80% acetic acid solution (55:41:4 v/v/v). Analysis was performed using high performance liquid chromatography (HPLC) with UV detection employing an Ultrasphere ODS, 5µm column (150 x 4.6 mm) at 284 nm using external calibration standards.

Validation - Results and discussions

Table 5.2‑1: Methods suitable for the determination of 2,4-D in ADM.09250.H.1.A

|  | 2,4-D |
| --- | --- |
| Author(s), year | Comb, T. 2022 |
| Principle of method | HPLC-UV |
| Linearity  (linear between mg/L/% range of the declared content)  (correlation coefficient, expressed as r) | Linear range 60 – 300 mg/L, single determinations at 5 levels  Equivalent to approximately 38 – 190% of the nominal concentration of the formulation.  A = 12.29 x – 3.812, r = 1.0000 |
| Precision–Repeatability Mean  n=5  (%RSD) | RSD = 0.52% (n = 5, method precision) at 160 mg/L, 94.5% w/w  modified Horwitz value = 1.4%  ~~Horwitz~~ HoRRat value = 0.4 (acceptable) |
| Accuracy  n=5  (% Recovery) | Level 100%, (95% w/w) mean total recovery 99.7% |
| Interference/Specificity | Retention time for 2,4-D matches between reference item and test item, confirming the identity of the analyte.  No interference was observed in solvent blank, formulation blank, reference item and test item at the retention time of 2,4-D |
| Comment | Acceptable against SANCO/3030/99 rev.5 criteria |

Conclusion

The analytical method for the determination of 2,4-D in ADM.09250.H.1.A is fully validated according to SANCO/3030/99 rev. 5. The results obtained show that this method is suitable for the detection and quantitation of the active substance 2,4-D in the formulation ADM.09250.H.1.A.

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

**Free phenols**

CIPAC Method 69.1 is available for the determination of free phenols.

**Chlorophenols**

In addition to the CIPAC method, an overview on the acceptable methods and possible data gaps for analysis of chlorophenols in the plant protection product is provided as follows:

|  |  |
| --- | --- |
| Comments of zRMS: | The method is accepted for analysing chlorophenols in this PPP. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.1/02 |
| Report | Bacher, R. 2023  Method validation and analysis of impurities of chlorophenols in a batch of Pielik 95 SP  Report No.: S21-07464  Sponsor reference No.: 000109288  ~~Draft report only available. Not finalised~~ |
| Guideline(s): | SANCO/3030/99 Rev. 5 |
| Deviations: | None |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

The analysis of 12 chlorophenols in ADM.09250.H.1.A was performed by gas chromatography with mass selective detection (GC-MS).

Samples (20 mg) of the test item were diluted to 20 mL with acetone. Analysis was performed using gas chromatography (GC) with mass selective detection employing an Optima 17, column (30m x 0.25mm i.d x 0.5 µm) monitoring 3 fragment ions for each analyte using external calibration standards. Analytes and the corresponding fragment ions are shown below:

Table 5.2‑2: Analytes monitored and corresponding fragment ions

|  |  |
| --- | --- |
| Analyte monitored | Fragment ions monitored (m/z) |
| 2-Chlorophenol | 130 m/z, 128 m/z, 65 m/z |
| 3-Chlorophenol | 130 m/z, 128 m/z, 63 m/z |
| 4-Chlorophenol | 130 m/z, 128 m/z, 65 m/z |
| 2,3-Dichlorophenol | 164 m/z, 162 m/z, 126 m/z |
| 2,4-Dichlorophenol | 164 m/z, 162 m/z, 98 m/z |
| 2,5-Dichlorophenol | 164 m/z, 162 m/z, 63 m/z |
| 2,6-Dichlorophenol | 164 m/z, 162 m/z, 126 m/z |
| 2,3,4-Trichlorophenol | 198 m/z, 196 m/z, 160 m/z |
| 2,3,5-Trichlorophenol | 200 m/z, 198 m/z, 196 m/z |
| 2,4,5-Trichlorophenol | 200 m/z, 198 m/z, 196 m/z |
| 2,4,6-Trichlorophenol | 198 m/z, 196 m/z, 160 m/z |
| 3,4,5-Trichlorophenol | 200 m/z, 198 m/z, 133 m/z |

Validation - Results and discussions

Table 5.2‑3: Method used for the determination of chlorophenols in ADM.09250.H.1.A

|  | Chlorophenols |
| --- | --- |
| Author(s), year | Bacher, R. 2023 |
| Principle of method | GC-MS |
| Linearity  (linear between mg/L/% range of the declared content)  (correlation coefficient, expressed as r) | 0.050 µg/mL to 10 µg/mL for all analytes except:  2,4-dichlorophenol, 2,6-dichlorophenol and 3,4,5-trichlorophenol range 0.1 to 10 µg/mL  2,4-dichlorophenol and 2,5-dichlorophenol (sum) range 0.2 to 20 µg/mL  Calibration range corresponds to 0.050 to 10 g/kg, 0.1 to 10 g/kg or 0.2 to 20 g/kg  Single determinations at ≥5 levels  Equivalent to approximately ≤30 – ≥120% of the nominal concentration in the formulation.  r2 ≥ 0.99 for all analytes  linear 1/x weighting |
| Accuracy and Precision  n=5 for each level  (% Recovery)  (%RSD) | **2-chlorophenol**  Fragment ion 130 m/z  0.5 g/kg, recovery 98.5%, RSD = 1.67%  Horwitz value = 0.4 (acceptable)  5 g/kg, recovery 96.4%, RSD = 1.59%  Horwitz value = 0.53 (acceptable)  Fragment ion 128 m/z  0.5 g/kg, recovery 101%, RSD = 1.12%  Horwitz value = 0.26 (acceptable)  5 g/kg, recovery 97.1%, RSD = 1.39%  Horwitz value = 0.47 (acceptable)  Fragment ion 65 m/z  0.5 g/kg, recovery 95.7%, RSD = 1.89%  Horwitz value = 0.45 (acceptable)  5 g/kg, recovery 94.2%, RSD = 1.58%  Horwitz value = 0.53 (acceptable)  **3-chlorophenol**  Fragment ion 130 m/z  0.5 g/kg, recovery 90.2%, RSD = 1.68%  Horwitz value = 0.4 (acceptable)  5 g/kg, recovery 97.7%, RSD = 1.93%  Horwitz value = 0.65 (acceptable)  Fragment ion 128 m/z  0.5 g/kg, recovery 90.0%, RSD = 1.95%  Horwitz value = 0.46 (acceptable)  5 g/kg, recovery 95.8%, RSD = 1.73%  Horwitz value = 0.58 (acceptable)  Fragment ion 63 m/z  0.5 g/kg, recovery 92.3%, RSD = 1.92%  Horwitz value = 0.46 (acceptable)  5 g/kg, recovery 92.7%, RSD = 1.70%  Horwitz value = 0.57 (acceptable)  **4-chlorophenol**  Fragment ion 130 m/z  0.5 g/kg, recovery 92.5%, RSD = 1.38%  Horwitz value = 0.33 (acceptable)  5 g/kg, recovery 92.1%, RSD = 1.59%  Horwitz value = 0.54 (acceptable)  Fragment ion 128 m/z  0.5 g/kg, recovery 90.4%, RSD = 1.89%  Horwitz value = 0.45 (acceptable)  5 g/kg, recovery 95.1%, RSD = 1.60%  Horwitz value = 0.54 (acceptable)  Fragment ion 65 m/z  0.5 g/kg, recovery 88.3%, RSD = 2.42%  Horwitz value = 0.58 (acceptable)  5 g/kg, recovery 92.1%, RSD = 1.99%  Horwitz value = 0.67 (acceptable)  **2,3-dichlorophenol**  Fragment ion 164 m/z  0.5 g/kg, recovery 94.9%, RSD = 1.50%  Horwitz value = 0.36 (acceptable)  5 g/kg, recovery 98.8%, RSD = 1.52%  Horwitz value = 0.51 (acceptable)  Fragment ion 162 m/z  0.5 g/kg, recovery 95.5%, RSD = 1.39%  Horwitz value = 0.33 (acceptable)  5 g/kg, recovery 97.6%, RSD = 1.39%  Horwitz value = 0.47 (acceptable)  Fragment ion 126 m/z  0.5 g/kg, recovery 93.7%, RSD = 1.87%  Horwitz value = 0.44 (acceptable)  5 g/kg, recovery 96.7%, RSD = 1.72%  Horwitz value = 0.58 (acceptable)  **2,4-dichlorophenol**  Fragment ion 164 m/z  0.5 g/kg, recovery 112%, RSD = 0.691%  Horwitz value = 0.16 (acceptable)  5 g/kg, recovery 102%, RSD = 0.945%  Horwitz value = 0.32 (acceptable)  Fragment ion 162 m/z  0.5 g/kg, recovery 110%, RSD = 0.849%  Horwitz value = 0.20 (acceptable)  5 g/kg, recovery 98.1%, RSD = 1.96%  Horwitz value = 0.66 (acceptable)  Fragment ion 98 m/z  0.5 g/kg, recovery 104%, RSD = 0.828%  Horwitz value = 0.20 (acceptable)  5 g/kg, recovery 98.9%, RSD = 0.524%  Horwitz value = 0.18 (acceptable)  **2,4 + 2,5-dichlorophenol**  Fragment ion 164 m/z  1 g/kg, recovery 115%, RSD = 1.49%  Horwitz value = 0.39 (acceptable)  10 g/kg, recovery 103%, RSD = 1.99%  Horwitz value = 0.74 (acceptable)  Fragment ion 162 m/z  1 g/kg, recovery 115%, RSD = 0.78%  Horwitz value = 0.21 (acceptable)  10 g/kg, recovery 98.6%, RSD = 1.26%  Horwitz value = 0.47 (acceptable)  Fragment ion 98 + 63 m/z  1 g/kg, recovery 117%, RSD = 1.21%  Horwitz value = 0.32 (acceptable)  10 g/kg, recovery 101%, RSD = 1.63%  Horwitz value = 0.61 (acceptable)  **2,6-dichlorophenol**  Fragment ion 164 m/z  0.5 g/kg, recovery 113%, RSD = 1.19%  Horwitz value = 0.28 (acceptable)  5 g/kg, recovery 99.1%, RSD = 1.97%  Horwitz value = 0.66 (acceptable)  Fragment ion 162 m/z  0.5 g/kg, recovery 112%, RSD = 1.14%  Horwitz value = 0.27 (acceptable)  5 g/kg, recovery 99.4%, RSD = 1.63%  Horwitz value = 0.55 (acceptable)  Fragment ion 126 m/z  0.5 g/kg, recovery 117%, RSD = 1.35%  Horwitz value = 0.32 (acceptable)  5 g/kg, recovery 106%, RSD = 1.11%  Horwitz value = 0.37 (acceptable)  **2,3,4-trichlorophenol**  Fragment ion 198 m/z  0.5 g/kg, recovery 84.3%, RSD = 2.47%  Horwitz value = 0.59 (acceptable)  5 g/kg, recovery 98.0%, RSD = 1.49%  Horwitz value = 0.50 (acceptable)  Fragment ion 196 m/z  0.5 g/kg, recovery 86.3%, RSD = 1.16%  Horwitz value = 0.28 (acceptable)  5 g/kg, recovery 96.9%, RSD = 1.83%  Horwitz value = 0.62 (acceptable)  Fragment ion 160 m/z  0.5 g/kg, recovery 86.7%, RSD = 1.95%  Horwitz value = 0.46 (acceptable)  5 g/kg, recovery 96.4%, RSD = 1.80%  Horwitz value = 0.60 (acceptable)  **2,3,5-trichlorophenol**  Fragment ion 200 m/z  0.5 g/kg, recovery 84.1%, RSD = 1.65%  Horwitz value = 0.39 (acceptable)  5 g/kg, recovery 94.4%, RSD = 1.98%  Horwitz value = 0.67 (acceptable)  Fragment ion 198 m/z  0.5 g/kg, recovery 85.2%, RSD = 1.05%  Horwitz value = 0.25 (acceptable)  5 g/kg, recovery 94.2%, RSD = 1.27%  Horwitz value = 0.43 (acceptable)  Fragment ion 196 m/z  0.5 g/kg, recovery 80.2%, RSD = 1.70%  Horwitz value = 0.40 (acceptable)  5 g/kg, recovery 95.3%, RSD = 2.64%  Horwitz value = 0.89 (acceptable)  **2,4,5-trichlorophenol**  Fragment ion 200 m/z  0.5 g/kg, recovery 84.6%, RSD = 1.72%  Horwitz value = 0.41 (acceptable)  5 g/kg, recovery 94.8%, RSD = 2.14%  Horwitz value = 0.72 (acceptable)  Fragment ion 198 m/z  0.5 g/kg, recovery 87.3%, RSD = 1.35%  Horwitz value = 0.32 (acceptable)  5 g/kg, recovery 94.2%, RSD = 2.24%  Horwitz value = 0.75 (acceptable)  Fragment ion 196 m/z  0.5 g/kg, recovery 90.9%, RSD = 1.67%  Horwitz value = 0.40 (acceptable)  5 g/kg, recovery 98.5%, RSD = 0.785%  Horwitz value = 0.26 (acceptable)  **2,4,6-trichlorophenol**  Fragment ion 198 m/z  0.5 g/kg, recovery 83.2%, RSD = 3.26%  Horwitz value = 0.77 (acceptable)  5 g/kg, recovery 93.9%, RSD = 2.11%  Horwitz value = 0.71 (acceptable)  Fragment ion 196 m/z  0.5 g/kg, recovery 79.9%, RSD = 1.65%  Horwitz value = 0.39 (acceptable)  5 g/kg, recovery 85.5%, RSD = 2.30%  Horwitz value = 0.77 (acceptable)  Fragment ion 160 m/z  0.5 g/kg, recovery 83.1%, RSD = 3.50%  Horwitz value = 0.83 (acceptable)  5 g/kg, recovery 91.4%, RSD = 1.78%  Horwitz value = 0.60 (acceptable)  **3,4,5-trichlorophenol**  Fragment ion 200 m/z  0.5 g/kg, recovery 85.9%, RSD = 3.98%  Horwitz value = 0.95 (acceptable)  5 g/kg, recovery 105%, RSD = 1.96%  Horwitz value = 0.66 (acceptable)  Fragment ion 198 m/z  0.5 g/kg, recovery 85.2%, RSD = 3.26%  Horwitz value = 0.77 (acceptable)  5 g/kg, recovery 109%, RSD = 0.96%  Horwitz value = 0.32 (acceptable)  Fragment ion 133 m/z  0.5 g/kg, recovery 99.7%, RSD = 2.40%  Horwitz value = 0.57 (acceptable)  5 g/kg, recovery 112%, RSD = 1.59%  Horwitz value = 0.53 (acceptable) |
| Interference/Specificity | Retention time for chlorophenols matches between reference item and test item, confirming the identity of the analyte.  No interference was observed in solvent blank, reference item and test item at the retention time of each chlorophenol |
| Limit of quantification | LOQ for each individual chlorophenol impurity was 0.5 g/kg or 1.0 g/kg (sum of 2,4-dichlorophenol and 2,5-dichlorophenol only) |
| Comment | Acceptable against SANCO/3030/99 rev.5 criteria |

Conclusion

The analytical method for the determination of chlorophenols in ADM.09250.H.1.A is fully validated according to SANCO/3030/99 rev. 5. The results obtained show that this method is suitable for the detection and quantitation of the chlorophenols in the formulation ADM.09250.H.1.A.

**Dioxins and furans**

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

|  |  |
| --- | --- |
| Comments of zRMS: | The method is accepted for analysing 17 relevant dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) congeners in the ADM.09250.H.1.A. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.1/03 |
| Report | Bacher, R. 2023  Method validation and analysis of impurities of polychlorinated dibenzodioxins and dibenzofurans in a batch of Pielik 95 SP  Report No.: S23-100476  Sponsor reference No.: 000115192  ~~Study plan only. Report not available.~~ |
| Guideline(s): | SANCO/3030/99 Rev. 5 |
| GLP: | No |

Materials and methods

The analysis of 17 relevant 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) in ADM.09250.H.1.A was performed by gas chromatography with mass selective detection (GC-MS). The target limit of quantification was ≤ 0.01 mg/kg (WHO-TCDD-TEQ) for the 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF). Individual analytes were validated at 0.5 ng/kg.

Samples (1 g) of the test item were diluted with toluene (50 mL). Internal standard solution (50 µL) including all individual 13C labelled 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF, except OCDF) (0.5 to 1.0 ng) was added. The resulting sample solution was further purified applying silica gel, alumina oxide and florisil column clean-up steps. Thereafter, the purified sample solution was evaporated to dryness and redissolved in a recovery standard solution (13C6-labelled 1,2,3,4-TCDD (0.5 ng) and 13C12-labelled 1,2,3,4,6,8,9-HpCDF (1.0 ng. 50µL). The concentration of the respective 13C-labelled PCDD and PCDF isomers was always 100 ng/mL (exception: OCDD: 200 ng/mL). Analysis was performed using gas chromatography (GC) with mass selective detection employing an Supelco SLB-5 MS, column (30 m x 0.25 mm i.d x 0.1 µm) monitoring 2 fragment ions for each analyte using external calibration standards. Analytes and the corresponding fragment ions are shown below:

Table 5.2‑4: Analytes monitored and corresponding fragment ions

|  |  |
| --- | --- |
| Analyte monitored | Fragment ions monitored (m/z) |
| 2,3,7,8-TCDD | 319.8965 Da, 321.8937 Da |
| 1,2,3,7,8-PeCDD | 355.8547 Da, 357.8518 Da |
| 1,2,3,4,7,8-HxCDD,  1,2,3,6,7,8- HxCDD,  1,2,3,7,8,9- HxCDD | 389.8157 Da, 391.8128 Da |
| 1,2,3,4,6,7,8-HpCDD | 427.7710 Da, 425.7738 Da |
| 1,2,3,4,6,7,8,9-PCDD (OCDD) | 461.7320 Da, 463.7291 Da |
| 2,3,7,8-TCDF | 303.9014 Da, 305.8988 Da |
| 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF | 339.8598 Da, 337.8627 Da |
| 1,2,3,4,7,8-HxCDF,  1,2,3,6,7,8- HxCDF,  1,2,3,7,8,9- HxCDF,  2,3,4,6,7,8- HxCDF | 373.8208 Da, 371.8237 Da |
| 1,2,3,4,6,7,8-HpCDF,  1,2,3,4,7,8,9- HpCDF | 407.7818 Da, 409.7788 Da |
| OCDD | 461.7320 Da, 463.7291 Da |
| OCDF | 441.7428 Da, 439.7457 Da |

Validation - Results and discussions

Table 5.2‑5: Method used for the determination of PCDD/PCDF in ADM.09250.H.1.A

|  | Dioxin Congener PCDD/PCDF |
| --- | --- |
| Author(s), year | Bacher, R. 2023 |
| Principle of method | GC-MS |
| Linearity  (linear between mg/L/% range of the declared content)  (correlation coefficient, expressed as r) | In the range 0.25 ng/mL and 1000 ng/mL (for all analytes)  Equivalent to approximately 0.3 μg/kg to 200 μg/kg  r2 ≥ 0.99 for all analytes  linear 1/x weighting |
| Accuracy and Precision  n=5 for each level  (% Recovery)  (%RSD) | **13C12-2,3,7,8-TCDD**  0.5 ng/kg, recovery 77%, RSD = 16%  **13C12-1,2,3,7,8-PeCDD**  0.5 ng/kg, recovery 84%, RSD = 17%  **13C12-1,2,3,4,7,8-HxCDD**  0.5 ng/kg, recovery 73%, RSD = 14%  **13C12-1,2,3,6,7,8-HxCDD**  0.5 g/kg, recovery 71%, RSD = 14%  **13C12-1,2,3,7,8,9-HxCDD**  0.5 g/kg, recovery 78%, RSD = 19%  **13C12-1,2,3,4,6.7.8-HpCDD**  0.5 g/kg, recovery 85%, RSD = 16%  **13C12-OCDD**  0.5 g/kg, recovery 71%, RSD = 11%  **13C12-2,3,7,8-TCDF**  0.5 g/kg, recovery 79%, RSD = 19%  **13C12-1,2,3,7,8-PeCDF**  0.5 g/kg, recovery 84%, RSD = 14%  **13C12-2,3,4,7,8-PeCDF**  0.5 g/kg, recovery 83%, RSD = 17%  **13C12-1,2,3,4,7,8-HxCDF**  0.5 g/kg, recovery 74%, RSD = 15%  **13C12-1,2,3,6,7,8-HxCDF**  0.5 g/kg, recovery 71%, RSD = 13%  **13C12-1,2,3,7,8,9-HxCDF**  0.5 g/kg, recovery 75%, RSD = 15%  **13C12-2,3,4,6,7,8-HxCDF**  0.5 g/kg, recovery 78%, RSD = 17%  **13C12-1,2,3,4,6.7,8-HpCDF**  0.5 g/kg, recovery 86%, RSD = 16%  **13C12-1,2,3,4,7.8,9-HpCDF**  0.5 g/kg, recovery 85%, RSD = 15%  **OCDF**  0.5 g/kg, recovery 83%, RSD = 20%  Accuracy and precison applying addition evaluation for 2,3,7,8-TCDF  Fortification level 29.2 µg/kg, recovery 91%, RSD 5.9%  Fortification level 55.8 µg /kg recovery 101%, RSD 2.8%  Horrat ratio <1 (acceptable) |
| Interference/Specificity | Retention time for PCDD/PCDF matches between reference item and test item, confirming the identity of the analyte.  No interference was observed in solvent blank, reference item and test item at the retention time of each analyte. |
| Limit of quantification | LOQ for each individual dioxin impurity was 0.5 ng/kg.  Theoretical LOQ based on calibration level:  TCDD / TCDF 0.25 ng/kg  PeCDD / PeCDF to HpCDD / HpCDF 1.25 ng/kg  OCDD 2.5 ng/kg |
| Comment | Acceptable against SANCO/3030/99 rev.5 criteria |

Conclusion

The analytical method for the determination of 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) in ADM.09250.H.1.A is fully validated according to SANCO/3030/99 rev. 5. The results obtained show that this method is suitable for the detection and quantitation of PCDD and PCDF in the formulation ADM.09250.H.1.A.

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

ADM.09250.H.1.A does not contain any additional formulants.

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

CIPAC Method 1 is available for the determination of 2,4-D.

### 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. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.2‑6: Validated methods for the generation of pre-authorization data

| 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/EU agreed |
| Wheat  (Residues) | primary | LOQ 0.01 mg/kg | HPLC-MS/MS | | Spence, C. 2016, 698822 missing / see Appendix 2 |
| confirmatory | See primary method | | | |
| Plants, plant products,...  (Residues) | Not required | | | | |
| Animal products, food of animal origin,...  (Residues) | Not required | | | | |
| Soil, water, sediment,...  (Environmental fate) | Not required | | | | |
| Soil, water,...  (Efficacy) | Not required | | | | |
| Feed, body fluids,...  (Toxicology) | Not required | | | | |
| Body fluids, air,....  (Exposure) | Not required | | | | |
| Water  (Ecotoxicology) | Primary | 2.0 µg/mL | HPLC-UV | | Jarratt, N. 2022, FR/002603-08 000109115 missing / see Appendix 2 |
| Confirmatory | See primary method | | | |
| Water  (Ecotoxicology) | Primary | 2.0 µg/mL | HPLC-UV | | Jarratt, N. 2022, FR/002603-09 000109114 missing / see Appendix 2 |
| Confirmatory | See primary method | | | |
| Dosed feeding solutions (50% aqueous sucrose) | Primary | 130 mg/kg | HPLC-UV | | Wilkins, S. 2022, FR/002602-10 000109119 missing / see Appendix 2 |
| Confirmatory | See primary method | | | |
| Larval diet | Primary | 15 mg/kg | | HPLC-MS/MS | Wilkins, S. 2022, FR/002602-11 000109120 missing / see Appendix 2 |
| Water, buffer solutions,...  (Properties) | Not required | | | | |

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

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

Analytical methods for the determination of the active substance and relevant impurities in the plant protection product shall be submitted, unless the applicant shows that these methods already submitted in accordance with the requirements set out in point 5.2.1 can be applied.

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

#### 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 | 2,4-D (sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D) | 0.05 mg/kg | Reg. (EU) 2022/1363 |
| Plant, high acid content | 0.05 mg/kg | Reg. (EU) 2022/1363 |
| Plant, high protein/high starch content (dry commodities) | 0.05 mg/kg | Reg. (EU) 2022/1363 |
| Plant, high oil content | 0.05 mg/kg | Reg. (EU) 2022/1363 |
| Plant, difficult matrices (hops, spices, tea) | 0.1 mg/kg | Reg. (EU) 2022/1363 |
| Muscle | 2,4-D (sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D) | 0.05 mg/kg | Reg. (EU) 2022/1363 |
| Milk | 0.01 mg/kg | Reg. (EU) 2022/1363 |
| Eggs | 0.01 mg/kg | Reg. (EU) 2022/1363 |
| Fat | 0.05 mg/kg | Reg. (EU) 2022/1363 |
| Liver, kidney | 0.05 mg/kg | Reg. (EU) 2022/1363 |
| Soil  (Ecotoxicology) | 2,4-D (sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D), 2,4-DCP, 4-CP, 2,4-DCA | 0.05 mg/kg | common limit |
| Drinking water  (Human toxicology) | 2,4-D (sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D), 2,4-DCP, 4-CP, 2,4-DCA | 0.1 µg/L | general limit for drinking water |
| Surface water  (Ecotoxicology) | 2,4-D (sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D), 2,4-DCP, 4-CP, 2,4-DCA | 0.22 mg/L | lowest NOEC/EC 50 from aquatic toxicity study |
| Air | 2,4-D | LOQ 4.5 µg/m3 | AOEL inhal: 0.02 mg/kg bw/d |
| Tissue (meat or liver) | 2,4-D (sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D) | 0.05 mg/kg | LOQ of analytical method |
| Body fluids | 2,4-D | 0.05 mg/kg | EFSA Journal 2014;12(9):3812 |

#### 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. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

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 and its esters 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 | LCMS/MS | Gessell, J.T., Li, Q., 2013a/EU agreed |
| ILV | 0.01 mg/kg | LCMS/MS | Langridge, G, 2012/EU agreed |
| Confirmatory  (if required) | Refer to primary method | | |
| High acid content | Primary | 0.01 mg/kg | LCMS/MS | Gessell, J.T., Li, Q., 2013a/EU agreed |
| ILV | 0.01 mg/kg | LCMS/MS | Langridge, G, 2012/EU agreed |
| Confirmatory  (if required) | Refer to primary method | | |
| High oil content | Primary | 0.01 mg/kg | LCMS/MS | Gessell, J.T., Li, Q., 2013a/EU agreed |
| ILV | 0.01 mg/kg | LCMS/MS | Langridge, G, 2012/EU agreed |
| Confirmatory  (if required) | Refer to primary method | | |
| High protein/high starch content (dry) | Primary | 0.01 mg/kg | LCMS/MS | Gessell, J.T., Li, Q., 2013a/EU agreed |
| ILV | 0.01 mg/kg | LCMS/MS | Langridge, G, 2012/EU agreed |
| Confirmatory  (if required) | Refer to primary method | | |
| Difficult (if required, depends on intended use) | Not required | | | |

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

Table 5.3‑3: Statement on extraction efficiency

The following data and resulting conclusions have been derived in order to demonstrate the analytical methods (Gesell, J T and Li, Q; 2013, Study Number 130886 and 130887) are sufficient to satisfy the residue definition for monitoring and risk assessment consisting of the sum of 2,4-D, its salts, esters and conjugates for materials of plant and animal origin, therefore satisfying the data requirements set forth in the following guidance documents: SANCO/825/00 rev. 8.1., SANCO/3029/99 rev. 4.

The Applicant developed its analytical method for determination of 2,4-D in materials of plant origin in alignment with analytical techniques employed in historical 14C plant metabolism studies. Metabolism studies have historically observed moderate levels of free, unconjugated 2,4-D, while extracted 14C residues are then subjected to either acid or base treatment to hydrolyse conjugates. For example, in wheat forage and wheat straw, unconjugated 2,4-D consisted of 9% and 6% of the TRR, respectively, while base-lable 2,4-D conjugates accounted for 64% of the total residues, in each matrix (1). The hydrolysis of these 2,4-D conjugates by a mild base suggests esterification with indigenous substances, presumably sugars. Additionally in grain, low levels of free 2,4-D (<6% of the total grain residues) were observed in extracts, while remaining tissues was determined for non-extractable residues, which were subjected to bound residue determination such as pectin, acid-detergent fiber, lignin and cellulose isolation. Extensive metabolism of 2,4-D was observed as demonstrated by incorporation or encapsulation of radioactivity into natural plant constituents such as starch, pectin, and lignin. Alternatively, wheat forage was found to contain ring-hydroxylated 2,4-D derivatives in the form of free extractable residues, while additional polar conjugates were also identified. In summary, the radioactive residues in wheat were characterized as free 2,4-D, base-labile conjugates, and polar conjugates.

Additionally, soybean and corn nature of residue studies have been conducted with [14C]-2,4-D DMA, where in corn matrices, the procedure implemented within the nature of residue study resulted in the major component in forage and fodder as parent, 2,4-D – 67.5% and 51.3% TRR, respectively (2). In both the forage and fodder, approximately 1% was observed as free 2,4‑DCP, while 17% and 24%, respectively, were identified as base-labile 2,4-DCP conjugates. The nature of residue studies utilized a neutral solvent extraction (methanol/water), followed by a methanolic base extraction (methanol/1.0 N NaOH). Remaining tissue was determined for non-extractable residues, which were subjected to bound residue determination such as pectin, acid-detergent fiber, lignin and cellulose isolation. Approximately 30% of the TRR was associated with starch. Extensive metabolism of 2,4-D was observed as demonstrated by incorporation or encapsulation of radioactivity into natural plant constituents such as starch, pectin, and lignin. In soybean, 2,4-D comprised 85.8% and 59.4% of the TRR in forage and hay. The 2,4-DCP-acetyl-glucose conjugate comprised 12.4% and 13.4% of the TRR, respectively in forage and hay. In hay, three additional metabolites were readily converted to 2,4-DCP under acidic conditions. In summary, the majority of the radioactive residues in soybean were characterized as 2,4-D and free or conjugated 2,4-DCP in forage and hay. In seed, very little 2,4-D and 2,4-DCP were observed, while approximately 70% of the TRR remained in the extracted tissue and was thought to consist of highly polar radioactive residues from metabolism and/or the incorporation of 14CO2 from the soil into natural plant incorporation. Similarly, the majority of the radioactive residues in corn were characterized as 2,4‑D , while 2,4-DCP conjugates were also identified in forage. Approximately 30% of the TRR in grain was associated with starch, while extensive metabolism of 2,4-D was demonstrated by incorporation or encapsulation into the plant.

Furthermore, the alkaline extraction solution of methanol/1.0 N NaOH (90/10) was demonstrated within the enforcement analytical method (DAS study ID 130886) in order to validate the hydrolysis of 2,4‑dichlorophenoxyacetic acid 2-ethylhexyl ester (2‑EHE) to the free acid in agricultural commodities. Samples of agricultural commodities were fortified with 2,4-D 2-EHE and taken through the analytical methodology. Recovery of the ester as 2,4-D acid was achieved with mean recoveries in the range 81‑111% across all matrices.

Alternatively, 14C animal nature of residue studies in goats and poultry found the predominant component in goats (urine, kidneys, fat, liver and muscle) and in poultry (eggs and liver) as free residues of 2,4-D, while poultry fat resulted in a large proportion of extractable 14C residues which were released by base hydrolysis, suggesting conjugates of 2,4-D (3,4). In goats, 2,4-D acid was found to be the predominant component in urine, kidney, fat, liver and muscle. In milk, free 2,4-D was identified as the most significant residue, while certain polar conjugates were observed. Of which, these polar conjugates were hydrolysed under acidic conditions to o- and p-chlorophenoxyacetic acid (CPA) and 2,4‑dichlorophenol (2,4-DCP), both of which are non-relevant metabolites not included in the residue definition for enforcement or risk assessment.

Furthermore, the alkaline extraction solution of methanol/1.0 N NaOH (90/10) was demonstrated within the enforcement analytical method (DAS study ID 130887) in order to validate the hydrolysis of 2,4‑dichlorophenoxyacetic acid 2-ethylhexyl ester (2‑EHE) to the free acid in animal matrices. Samples of animal origin were fortified with 2,4-D 2-EHE and taken through the analytical methodology. Following the hydrolysis, recovery of the ester as 2,4-D acid was achieved with mean recoveries in the range 86–113% across all matrices.

In conclusion, the Applicant considers there to be sufficient evidence from existing studies to demonstrate the extraction efficiency and hydrolysis steps employed in the enforcement methods submitted for the Annex I Renewal are effective.

#### 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. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3‑4: Validated methods for food and feed of animal origin (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/EU agreed | |
| Milk | Primary | 0.01 mg/kg | | LCMS/MS | Gesell, J.T., Li, Q., 2013b/EU agreed | |
| ILV | 0.01 mg/kg | | LCMS/MS | Garcia-Alix, M., 2012a/EU agreed | |
| Confirmatory  (if required) | Refer to primary method | | | | |
| Eggs | Primary | 0.01 mg/kg | | LCMS/MS | Gesell, J.T., Li, Q., 2013b/EU agreed | |
| ILV | 0.01 mg/kg | | LCMS/MS | Garcia-Alix, M., 2012a/EU agreed | |
| Confirmatory  (if required) | Refer to primary method | | | | |
| Muscle | Primary | 0.01 mg/kg | | LCMS/MS | Gesell, J.T., Li, Q., 2013b/EU agreed | |
| ILV | 0.01 mg/kg | | LCMS/MS | Garcia-Alix, M., 2012a/EU agreed | |
| Confirmatory  (if required) | Refer to primary method | | | | |
| Fat | Primary | 0.01 mg/kg | | LCMS/MS | Gesell, J.T., Li, Q., 2013b/EU agreed | |
| ILV | 0.01 mg/kg | | LCMS/MS | Garcia-Alix, M., 2012a/EU agreed | |
| Confirmatory  (if required) | Refer to primary method | | | | |
| Kidney, liver | Primary | 0.01 mg/kg | | LCMS/MS | Gesell, J.T., Li, Q., 2013b/EU agreed | |
| ILV | 0.01 mg/kg | | LCMS/MS | Garcia-Alix, M., 2012a/EU agreed | |
| Confirmatory  (if required) | Refer to primary method | | | | |
| Honey | Primary | 0.01 mg/kg | LCMS/MS | | | Winter, O., 2023a/missing/see Appendix 2 |
| ILV | 0.01 mg/kg | LCMS/MS | | | Jooß, S. 2023/missing/see Appendix 2 |
| Confirmatory  (if required) | Refer to primary method | | | | |

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

The following data and resulting conclusions have been derived in order to demonstrate the analytical methods (Gesell, J T and Li, Q; 2013, Study Number 130886 and 130887) are sufficient to satisfy the residue definition for monitoring and risk assessment consisting of the sum of 2,4-D, its salts, esters and conjugates for materials of plant and animal origin, therefore satisfying the data requirements set forth in the following guidance documents: SANCO/825/00 rev. 8.1., SANCO/3029/99 rev. 4.

The Applicant developed its analytical method for determination of 2,4-D in materials of plant origin in alignment with analytical techniques employed in historical 14C plant metabolism studies. Metabolism studies have historically observed moderate levels of free, unconjugated 2,4-D, while extracted 14C residues are then subjected to either acid or base treatment to hydrolyse conjugates. For example, in wheat forage and wheat straw, unconjugated 2,4-D consisted of 9% and 6% of the TRR, respectively, while base-lable 2,4-D conjugates accounted for 64% of the total residues, in each matrix (1). The hydrolysis of these 2,4-D conjugates by a mild base suggests esterification with indigenous substances, presumably sugars. Additionally in grain, low levels of free 2,4-D (<6% of the total grain residues) were observed in extracts, while remaining tissues was determined for non-extractable residues, which were subjected to bound residue determination such as pectin, acid-detergent fiber, lignin and cellulose isolation. Extensive metabolism of 2,4-D was observed as demonstrated by incorporation or encapsulation of radioactivity into natural plant constituents such as starch, pectin, and lignin. Alternatively, wheat forage was found to contain ring-hydroxylated 2,4-D derivatives in the form of free extractable residues, while additional polar conjugates were also identified. In summary, the radioactive residues in wheat were characterized as free 2,4-D, base-labile conjugates, and polar conjugates.

Additionally, soybean and corn nature of residue studies have been conducted with [14C]-2,4-D DMA, where in corn matrices, the procedure implemented within the nature of residue study resulted in the major component in forage and fodder as parent, 2,4-D – 67.5% and 51.3% TRR, respectively (2). In both the forage and fodder, approximately 1% was observed as free 2,4‑DCP, while 17% and 24%, respectively, were identified as base-labile 2,4-DCP conjugates. The nature of residue studies utilized a neutral solvent extraction (methanol/water), followed by a methanolic base extraction (methanol/1.0 N NaOH). Remaining tissue was determined for non-extractable residues, which were subjected to bound residue determination such as pectin, acid-detergent fiber, lignin and cellulose isolation. Approximately 30% of the TRR was associated with starch. Extensive metabolism of 2,4-D was observed as demonstrated by incorporation or encapsulation of radioactivity into natural plant constituents such as starch, pectin, and lignin. In soybean, 2,4-D comprised 85.8% and 59.4% of the TRR in forage and hay. The 2,4-DCP-acetyl-glucose conjugate comprised 12.4% and 13.4% of the TRR, respectively in forage and hay. In hay, three additional metabolites were readily converted to 2,4-DCP under acidic conditions. In summary, the majority of the radioactive residues in soybean were characterized as 2,4-D and free or conjugated 2,4-DCP in forage and hay. In seed, very little 2,4-D and 2,4-DCP were observed, while approximately 70% of the TRR remained in the extracted tissue and was thought to consist of highly polar radioactive residues from metabolism and/or the incorporation of 14CO2 from the soil into natural plant incorporation. Similarly, the majority of the radioactive residues in corn were characterized as 2,4‑D , while 2,4-DCP conjugates were also identified in forage. Approximately 30% of the TRR in grain was associated with starch, while extensive metabolism of 2,4-D was demonstrated by incorporation or encapsulation into the plant.

Furthermore, the alkaline extraction solution of methanol/1.0 N NaOH (90/10) was demonstrated within the enforcement analytical method (DAS study ID 130886) in order to validate the hydrolysis of 2,4‑dichlorophenoxyacetic acid 2-ethylhexyl ester (2‑EHE) to the free acid in agricultural commodities. Samples of agricultural commodities were fortified with 2,4-D 2-EHE and taken through the analytical methodology. Recovery of the ester as 2,4-D acid was achieved with mean recoveries in the range 81‑111% across all matrices.

Alternatively, 14C animal nature of residue studies in goats and poultry found the predominant component in goats (urine, kidneys, fat, liver and muscle) and in poultry (eggs and liver) as free residues of 2,4-D, while poultry fat resulted in a large proportion of extractable 14C residues which were released by base hydrolysis, suggesting conjugates of 2,4-D (3,4). In goats, 2,4-D acid was found to be the predominant component in urine, kidney, fat, liver and muscle. In milk, free 2,4-D was identified as the most significant residue, while certain polar conjugates were observed. Of which, these polar conjugates were hydrolysed under acidic conditions to o- and p-chlorophenoxyacetic acid (CPA) and 2,4‑dichlorophenol (2,4-DCP), both of which are non-relevant metabolites not included in the residue definition for enforcement or risk assessment.

Furthermore, the alkaline extraction solution of methanol/1.0 N NaOH (90/10) was demonstrated within the enforcement analytical method (DAS study ID 130887) in order to validate the hydrolysis of 2,4‑dichlorophenoxyacetic acid 2-ethylhexyl ester (2‑EHE) to the free acid in animal matrices. Samples of animal origin were fortified with 2,4-D 2-EHE and taken through the analytical methodology. Following the hydrolysis, recovery of the ester as 2,4-D acid was achieved with mean recoveries in the range 86–113% across all matrices.

In conclusion, the Applicant considers there to be sufficient evidence from existing studies to demonstrate the extraction efficiency and hydrolysis steps employed in the enforcement methods submitted for the Annex I Renewal are effective.

#### 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. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

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/EU agreed |
| Primary | 0.05 mg/kg | LCMS/MS | Gesell, J.T., 2012/EU agreed |
| Confirmatory | Refer to primary method | | |

| Component of residue definition: 2,4-DCP | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year/missing/EU agreed |
| Primary | 0.05 mg/kg | LCMS/MS | Gesell, J.T., 2012/EU agreed |
| Confirmatory | Refer to primary method | | |

| Component of residue definition: 4-CP | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year/missing/EU agreed |
| Primary | 0.05 mg/kg | LCMS/MS | Gesell, J.T., 2012/EU agreed |
| Confirmatory | Refer to primary method | | |

| Component of residue definition: 2,4-DCA | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year/missing/EU agreed |
| Primary | 0.05 mg/kg | GC-MS | Gesell, J.T., 2012/EU agreed |
| Confirmatory | Refer to primary method | | |

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

#### 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. For the detailed valuation of new/additional studies it is referred to Appendix 2.

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/EU agreed |
| Drinking water | Primary | 0.1 μg/L | LCMS/MS | Gesell, J.T., 2012/EU agreed |
| ILV | 0.1 μg/L | LCMS/MS | Garcia-Alix, M., 2012/EU agreed |
| Confirmatory | Refer to primary method | | |
| Surface water | Primary | 0.1 μg/L | LCMS/MS | Gesell, J.T., 2012/EU agreed |
| ILV | 0.1 μg/L | LCMS/MS | Garcia-Alix, M., 2012/EU agreed |
| Confirmatory | Refer to primary method | | |

| Component of residue definition: 2,4-DCP | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year/missing/EU agreed |
| Drinking water | Primary | 0.1 μg/L | LCMS/MS | Gesell, J.T., 2012/EU agreed |
| ILV | 0.1 μg/L | LCMS/MS | Garcia-Alix, M., 2012/EU agreed |
| Confirmatory | Refer to primary method | | |
| Surface water | Primary | 0.1 μg/L | LCMS/MS | Gesell, J.T., 2012/EU agreed |
| ILV | 0.1 μg/L | LCMS/MS | Garcia-Alix, M., 2012/EU agreed |
| Confirmatory | Refer to primary method | | |

| Component of residue definition: 4-CP | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year/missing/EU agreed |
| Drinking water | Primary | 0.1 μg/L | LCMS/MS | Gesell, J.T., 2012/EU agreed |
| ILV | 0.1 μg/L | LCMS/MS | Garcia-Alix, M., 2012/EU agreed |
| Confirmatory | Refer to primary method | | |
| Surface water | Primary | 0.1 μg/L | LCMS/MS | Gesell, J.T., 2012/EU agreed |
| ILV | 0.1 μg/L | LCMS/MS | Garcia-Alix, M., 2012/EU agreed |
| Confirmatory | Refer to primary method | | |

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

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

#### 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. For the detailed evaluation of new/additional studies please refer to Appendix 2.

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/EU agreed |
| Primary | 4.5 μg/m3 | LCMS/MS | Class, T., 2011/EU agreed |
| Confirmatory | Refer to primary method | | |

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

#### 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 2,4D in body fluids and tissues is given in the following table. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

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/EU agreed |
| Blood  Primary | 0.05 mg/kg / mg/L | LCMS/MS | Senciuc, M., 2011/EU agreed |
| Confirmatory | Refer to primary method | | |
| Blood  Primary | 0.01 mg/L | LCMS/MS | Winter, O., 2023b/missing/see Appendix 2 |
| Confirmatory | Refer to primary method | | |
| Urine  Primary | 0.05 mg/kg / mg/L | LCMS/MS | Senciuc, M., 2011/EU agreed |
| Confirmatory | Refer to primary method | | |

For any special comments or remarkable points concerning the analytical methods for body fluids and tissues please refer to Appendix 2.

#### Other studies/ information

No other studies or information are required.

### Description of analytical methods for the determination of residues of active substance 2 (KCP 5.2)

Not applicable, ADM.09250.H.1.A contains one single active.

# 

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 | Comb, T | 2022 | 2,4 D 95 SP: Method validation  Report No.: ACE-21-387, Sponsor reference No.: 000109838  Agrochemex Environmental Ltd, UK  GLP, Unpublished | N | Adama |
| KCP 5.1.1/02 | Bacher, R. | 2023 | Method validation and analysis of impurities of chlorophenols in a batch of Pielik 95 SP  **~~Draft~~** Report No.: S21-07464  Reference No.: 000109288  Eurofins Agroscience Services EAG Laboratories GmbH, Germany  GLP, unpublished | N | Adama |
| KCP 5.1.1/03 | Bacher, R. | 2023 | Method validation and analysis of impurities of polychlorinated dibenzodioxins and dibenzofurans in a batch of Pielik 95 SP  **~~Study Plan~~**Report No.: S23-100476  Reference No.: 000115192  Eurofins Agroscience Services EAG Laboratories GmbH, Germany  Non- GLP, unpublished | N | Adama |
| KCA 6.3/01 | Spence, C. | 2016 | Residues of 2,4-D DMA in spring wheat following a single application of LAF-74 – Northern and Southern European zones – 2014.  Charles River, Tranent, Edinburgh, EH33 2NE, UK; Report No. 36122, DAS Study ID 140657.  XXXX Report No. 90019777 – report amendment 1  GLP, unpublished | N | EU 2,4-D Task Force |
| KCP 5.1.2/01 | Jarratt, N. | 2022 | 2,4-D 95 SP: Terrestrial plant seedling emergence test  Study number: FR/002603-08, Sponsor reference number 000109115  Fera science Ltd., UK  GLP, unpublished  → KCP 10.6.2/01 | N | Adama |
| KCP 5.1.2/02 | Jarratt, N. | 2022 | 2,4-D 95 SP: Terrestrial plant test: vegetative vigour Test  Study number: FR/002603-09, Sponsor reference number 000109114  Fera science Ltd., UK  GLP, unpublished  → KCP 10.6.2/02 | N | Adama |
| KCP 5.1.2/03 | Wilkins, S | 2022a | 2,4-D 95 SP: 10-Day chronic oral toxicity test for adult honeybees (*Apis mellifera* L.)  Study number: FR/002602-10, Sponsor reference number 000109119  Fera science Ltd., UK  GLP, unpublished  → KCP 10.3.1.2/01 | N | Adama |
| CP 5.1.2/04 | Wilkins, S | 2022b | 2,4-D 95 SP: *In vitro* 22-day toxicity test - repeated exposure to larval stage honeybees (*Apis mellifera* L.)  Study number: FR/002602-11, Sponsor reference number 000109120  Fera science Ltd., UK  GLP, unpublished  → KCP 10.3.1.3/01 | N | Adama |
| CP 5.2.1/01 | Winter, O. | 2023a | Development and Validation of an analytical method based on multi-residue method QuPPe for Determination of 2,4-Dichlorophenoxyacetic acid(2,4-D) in Honey  Study number: S23-101772, Sponsor reference number 000115746  Eurofins Agroscience Services Chem GmbH, Germany  GLP, unpublished | N | Adama |
| CP 5.2.1/02 | Jooß, S. | 2023 | Independent laboratory validation of an analytical method for the determination of 2,4-dichlorophenoxyacetic acid (2,4-D) in honey  Study number: S23-102036, Sponsor reference number 000115744  Eurofins Agroscience Services EAG Laboratories GmbH, Germany  GLP, unpublished | N | Adama |
| CP 5.2.1/03 | Winter, O. | 2023b | Validation of an analytical method for the Determination of 2,4-Dichlorophenoxyacetic acid(2,4-D)  in Body Fluid(s)  Study number: S21-101771, Sponsor reference number 000115745  Eurofins Agroscience Services Chem GmbH, Germany  GLP, unpublished | N | Adama |

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

| Data point | Author(s) | Year | Title Company Report No.  Source (where different from company) GLP or GEP status Published or not | Vertebrate study Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
| CP 5.2 | Gesell, J.T., Li, Q. | 2013a | Revision 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 spectroscopy detection Company Report No 110357 Dow AgroScieces LLC, Indianapolis, USA GLP, Unpublished | N | European Union 2,4-D Task Force 2012 |
| CP 5.2 | Langridge, G. | 2012 | Independent laboratory validation of an analytical method for the determination of (2,4-dichlorophenoxy)acetic acid in crops  Company Report No. CEMS-5229, DAS Protocol No. 110762 CEM Analytical Services, UK GLP, Unpublished | N | European Union 2,4-D Task Force 2012 |
| CP 5.2 | Gesell, J.T., Li, Q. | 2013b | Revision 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 spectroscopy detection Company Report No 110468 Dow AgroScieces LLC, Indianapolis, USA GLP, Unpublished | N | European Union 2,4-D Task Force 2012 |
| CP 5.2 | Garcia-Alix, M. | 2012a | Independent laboratory validation of an analytical method for the determination of (2,4-dichlorophenoxy)acetic acid in animal matrices Company Report No. CEMS-5230, DAS Protocol No. 110763 CEM Analytical Services, UK GLP, Unpublished | N | European Union 2,4-D Task Force 2012 |
| CP 5.2 | Gesell, J.T. | 2012a | Method validation study for the determination of residues of (2,4-dichlorophenoxy)acetic acid and its metabolites in soil Company Report No 110503 Dow AgroScieces LLC, Indianapolis, USA GLP, Unpublished | N | European Union 2,4-D Task Force 2012 |
| CP 5.2 | 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 Company Report No 110504 Dow AgroScieces LLC, Indianapolis, USA GLP, Unpublished | N | European Union 2,4-D Task Force 2012 |
| CP 5.2 | Garcia-Alix, M. | 2012b | Independent laboratory validation of an analytical method for the determination of (2,4-dichlorophenoxy)acetic acid in water Company Report No. CEMS-5324, DAS Protocol No. 110821 CEM Analytical Services, UK GLP, Unpublished | N | European Union 2,4-D Task Force 2012 |
| CP 5.2 | Class, T. | 2011 | 2,4-D: Development and validation of an analytical method for the determination of 2,4-D in air Company Report No. P 2166G, DAS Protocol No. 110026 PTRL Europe GmbH, Germany GLP, Unpublished | N | European Union 2,4-D Task Force 2012 |
| CP 5.2 | Senciuc, M. | 2011 | 2,4-D: Development and validation of an analytical method for the determination of 2,4-D in body fluid(s) Company Report No. P 2167G, DAS Protocol No. 110027 PTRL Europe GmbH, Germany GLP, Unpublished | N | European Union 2,4-D Task Force 2012 |

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 |
| --- | --- | --- | --- | --- | --- |
| CP XX | Author | YYYY | Title  Company Report No  Source  GLP/Not GLP/GEP/Not GEP, Published/Unpublished | 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 |
| --- | --- | --- | --- | --- | --- |
| CP XX | Author | YYYY | Title  Company Report No  Source  GLP/Not GLP/GEP/Not GEP, Published/Unpublished | Y/N | Owner |
|  |  |  |  |  |  |

1. Detailed evaluation of submitted analytical methods
   1. Analytical methods for 2,4-D
      1. Methods used for the generation of pre-authorization data (KCP 5.1)
         1. Description of analytical methods for the determination of residues in plant matrices (KCP 5.1)
            1. Analytical method

Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The study has been accepted (see B7). |

|  |  |
| --- | --- |
| Reference: | KCA 6.3/01 |
| Report | Residues of 2,4-D DMA in spring wheat following a single application of LAF-74 – Northern and Southern European zones – 2014. 2016, Spence, C. Report No. 36122, DAS Study ID 140657, XXXX Report No. 90019777 – report amendment 1 |
| Guideline(s): | None specified |
| Deviations: | Not specified |
| GLP: | Yes (certified laboratory) |
| Acceptability: | yes |

Materials and methods

The analytical method referenced for the determination of 2,4-D was validated in wheat (whole plant, grain and straw). The method was based on method 130886.

Samples (5 g) were extracted with methanol:1.0 N sodium hydroxide (90:10 v/v, 100 mL). The sample is homogenised, shaken and centrifuged. An aliquot (2 mL) was acidified using hydrochloric acid (0.2 M, 2 mL). An aliquot (2 mL) of the acidified aliquot was cleaned up through a Strata-X SPE cartridge. The sample was eluted twice with methanol:water (10:90 v/v, 500 µL). Stable isotope internal standard solution (13C6-2,4-D, 12.5 ng/mL in methanol water (10:90 v/v containing 0.5% acetic acid), 1.0 mL) was then added prior to analysis by LC-MS/MS.

Analysis of 2,4-D was by LC-MS/MS employing a Zorbax SB C8 HPLC column monitoring two transitions.

Quantification was performed using internal solvent calibration standards over the range 0.075 to 25 ng/mL, equivalent to 0.003 to 1.0 mg/kg in samples.

Results and discussions

The analytical method for the determination of 2,4-D in wheat (whole plant, grain and straw) was successfully validated with a limit of quantification (LOQ) of 0.01 mg/kg. No additional confirmatory method is required.

Table A 1: Recovery results from method validation of 2,4-D using the analytical method

| Matrix | Fortification level (mg/kg) (n=x) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- |
| Quantitation ion transition 219→161 *m/z* | | | | |
| Wheat whole plant | 0.01 (n=5) | 99 | 5 | Range 94-105% |
| 0.1 (n=5) | 103 | 5 | Range 96-111% |
| Overall (n=10) | 101 | 5 | Range 94-111% |
| Wheat grain | 0.01 (n=5) | 101 | 8 | Range 92-111% |
| 0.1 (n=5) | 111 | 7 | Range 99-121% |
| Overall (n=10) | 106 | 9 | Range 92-121% |
| Wheat straw | 0.01 (n=5) | 104 | 6 | Range 98-113% |
| 0.1 (n=5) | 91 | 5 | Range 85-97% |
| Overall (n=10) | 98 | 9 | Range 85-113% |
| Confirmation ion transition 221→163 *m/z* | | | | |
| Wheat whole plant | 0.01 (n=5) | 98 | 5 | Range 92-104% |
| 0.1 (n=5) | 98 | 5 | Range 92-106% |
| Overall (n=10) | 98 | 5 | Range 92-106% |
| Wheat grain | 0.01 (n=5) | 104 | 9 | Range 92-115% |
| 0.1 (n=5) | 115 | 8 | Range 101-125% |
| Overall (n=10) | 109 | 10 | Range 92-125% |
| Wheat straw | 0.01 (n=5) | 98 | 6 | Range 92-107% |
| 0.1 (n=5) | 90 | 5 | Range 84-95% |
| Overall (n=10) | 94 | 7 | Range 84-107% |

Table A 2: Characteristics for the analytical method used for validation of 2,4-D residues in wheat

|  |  |
| --- | --- |
|  | 2,4-D |
| Specificity | blank value <30% LOQ) |
| Calibration (type, number of data points) | individual calibration data presented|  calibration line equation presented  linear, 1/x weighting  9 data points |
| Calibration range | 0.075 to 25 ng/mL  Equivalent to 0.003 to 1.0 mg/kg |
| Assessment of matrix effects is presented | No, however the use of stable isotope internal standard prevented any matrix effects |
| Stability | Standard solutions stable for 41 days when stored refrigerated |
| Limit of determination/quantification | 0.01 mg/kg |

Conclusion

The analytical method for the determination of 2,4-D in wheat (whole plant, grain and straw) was successfully validated with a limit of quantification (LOQ) 2,4-D of 0.01 mg/L in all matrices The data meets the acceptance criteria of SANTE/2020/12830 rev.1.

* + - 1. Description of analytical methods for the determination of residues in animal matrices (KCP 5.1)

No new or additional studies have been submitted

* + - 1. Description of analytical methods for the determination of residues in support of environmental fate studies (KCP 5.1)

No new or additional studies have been submitted

* + - 1. Description of analytical methods for the determination of residues in support of toxicological studies (KCP 5.1)

No new or additional studies have been submitted

* + - 1. Description of analytical methods for the determination of residues in support of operator, worker, resident and bystander exposure studies (KCP 5.1)

No new or additional studies have been submitted

* + - 1. Description of analytical methods for the determination of residues in support of ecotoxicology studies (KCP 5.1)
         1. Analytical method

Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  Mean recoveries were determined within the acceptable range (70–120%), %RSD (%CV) of the recovery values, was ≤ 20%.  The LOQ was 2.0 µg/mL. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/01 |
| Title: | 2,4-D 95 SP: Terrestrial plant seedling emergence test |
| Report: | Jarratt, N., 2022, FR/002603-08, 000109115 |
| Guideline(s): | SANTE/2020/12830 rev. 1 |
| Deviations: | Not specified |
| GLP/GEP: | Yes (certified laboratory) |
| Acceptability: | yes |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/02 |
| Title: | 2,4-D 95 SP: Terrestrial plant test: Vegetative vigour test |
| Report: | Jarratt, N., 2022, FR/002603-09, 000109114 |
| Guideline(s): | SANTE/2020/12830 rev. 1 |
| Deviations: | Not specified |
| GLP/GEP: | Yes (certified laboratory) |
| Acceptability: | yes |

Materials and methods

The analytical method was developed for the determination of 2,4-D in test water from ecotoxicology studies by HPLC-UV.

To aqueous samples, an equal volume of acetonitrile was added. If considered necessary, samples were further diluted using acetonitrile:water (1:1 v/v).

All samples were analysed by HPLC-UV at 284 nm employing a Zorbax eclipse XDB C18 column.

Quantification was performed using external solvent calibration standards over the range 0.5 to 50 µg/mL equivalent to 1 to 100 µg/mL in samples, assuming no further dilution.

Results and discussions

The analytical method for the determination of 2,4-D in water was successfully validated with a limit of quantification (LOQ) of 2 µg/mL.

Table A 3: Recovery results from method validation of 2,4-D in water using the analytical method

| Matrix | Analyte | Fortification level (µg/mL) (n=x) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| Water | 2,4-D | 2 (n=5) | 112.17 | 0.18 | Range 111.91-112.38% |
| 20000 (n=5) | 109.63 | 0.24 | Range 109.42-110.02% |

Table A 4: Characteristics for the analytical method used for validation of 2,4-D residues in water

|  |  |
| --- | --- |
|  | 2,4-D |
| Specificity | Blank value < 30 % LOQ)  No residues detected in control samples. |
| Calibration (type, number of data points) | individual calibration data presented|  calibration line equation presented  r2 = 0.99995  linear, no weighting  >4 data points (in duplicate) |
| Calibration range | 0.5 to 50 µg/mL equivalent to 1 to 100 µg/mL in samples, assuming no further dilution |
| Assessment of matrix effects is presented | No, nevertheless, matrix matched calibration standards were used |
| Stability | Standard solutions stable for 28 days |
| Limit of determination/quantification | 2 µg/mL |

Conclusion

The analytical method for the determination of 2,4-D in water was successfully validated with a limit of quantification (LOQ) for 2,4-D of 2 µg/mL.

* + - * 1. Analytical method

Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  Mean recoveries were determined within the acceptable range (70–120%), %RSD (%CV) of the recovery values, was ≤ 20%.  The LOQ was 130 mg/kg. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/03 |
| Title: | 2,4-D 95 SP: 10-Day chronic oral toxicity test for adult honeybees (*Apis mellifera* L.) |
| Report: | Wilkins, S., 2022a, FR/002602-10, 000109119 |
| Guideline(s): | SANTE/2020/12830 rev. 1 |
| Deviations: | Not specified |
| GLP/GEP: | Yes (certified laboratory) |
| Acceptability: | yes |

Materials and methods

The analytical method was developed for the determination of 2,4-D in 50% (w/v) aqueous sucrose solutions from ecotoxicology studies by HPLC-UV.

To aqueous sucrose samples (1 mL), methanol (10 mL) was added. The samples are vortexed and ultrasonicated. Water (9 mL) was added prior to vortexing and ultrasonication. If considered necessary, samples were further diluted using methanol:water (1:1 v/v).

All samples were analysed by HPLC-UV at 225 nm employing a Zorbax eclipse XDB C18 column.

Quantification was performed using external solvent calibration standards over the range 2 to 12 µg/mL equivalent to 33 to 198 mg/kg in samples, assuming no further dilution.

Results and discussions

The analytical method for the determination of 2,4-D in 50% aqueous sucrose solution was successfully validated with a limit of quantification (LOQ) of 130 mg/kg.

Table A 5: Recovery results from method validation of 2,4-D in 50% aqueous sucrose solution using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n=x) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| 50% aqueous sucrose solution | 2,4-D | 130 (n=5) | 101.5 | 0.38 | Range 101.0-102.0% |
| 3000 (n=5) | 103.9 | 0.33 | Range 103.5-104.4% |

Table A 6: Characteristics for the analytical method used for validation of 2,4-D residues in 50% aqueous sucrose solution

|  |  |
| --- | --- |
|  | 2,4-D |
| Specificity | Blank value < 30 % LOQ)  No residues detected in control samples. |
| Calibration (type, number of data points) | individual calibration data presented|  calibration line equation presented  r2 = 0.99942  linear, no weighting  >4 data points (in duplicate) |
| Calibration range | 2 to 12 µg/mL equivalent to 33 to 198 mg/kg in samples, assuming no further dilution |
| Assessment of matrix effects is presented | Yes, no significant enhancement or suppression (≥20%) therefore solvent standards used |
| Stability | Standard solutions stable for 7 days. For each analysis, fresh solutions were used. |
| Limit of determination/quantification | 130 mg/kg |

Conclusion

The analytical method for the determination of 2,4-D in 50% aqueous sucrose solution was successfully validated with a limit of quantification (LOQ) for 2,4-D of 130 mg/kg.

* + - * 1. Analytical method

Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  Mean recoveries were determined within the acceptable range (70–120%), %RSD (%CV) of the recovery values, was ≤ 20%.  The LOQ was 15 mg/kg. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/04 |
| Title: | 2,4-D 95 SP: *In vitro* 22-day toxicity test - repeated exposure to larval stage honeybees (*Apis mellifera* L.) |
| Report: | Wilkins, S., 2022b, FR/002602-11, 000109120 |
| Guideline(s): | SANTE/2020/12830 rev. 1 |
| Deviations: | Not specified |
| GLP/GEP: | Yes (certified laboratory) |
| Acceptability: | yes |

Materials and methods

The analytical method was developed for the determination of 2,4-D in larval diet (50% (w/v) aqueous glucose/fructose solutions and royal jelly) from ecotoxicology studies by HPLC-MS/MS.

To larval diet samples are suspended in methanol:water (70:30 v/v) and further diluted with methanol:water (1:1 v/v) as needed. If required, samples are diluted further with methanol.

All samples were analysed by HPLC-MS/MS employing a Kinetex biphenyl column monitoring two mass transitions.

Quantification was performed using external solvent calibration standards over the range 0.0005 to 0.004 µg/mL equivalent to 4.2 to 33.6 mg/kg in samples assuming LOQ dilution factor.

Results and discussions

The analytical method for the determination of 2,4-D in larval diet was successfully validated with a limit of quantification (LOQ) of 130 mg/kg.

Table A 7: Recovery results from method validation of 2,4-D in 50% aqueous sucrose solution using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n=x) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| 50% aqueous sucrose solution | 2,4-D | 15 (n=5) | 101 | 2.21 | Range 99.7-103.7% |
| 1000 (n=5) | 103.2 | 1.37 | Range 101.0-104.8% |

Table A 8: Characteristics for the analytical method used for validation of 2,4-D residues in larval diet

|  |  |
| --- | --- |
|  | 2,4-D |
| Specificity | Blank value < 30 % LOQ)  No residues detected in control samples. |
| Calibration (type, number of data points) | individual calibration data presented|  calibration line equation presented  r2 = 0.999  linear, no weighting  >4 data points (in duplicate) |
| Calibration range | 0.0005 to 0.004 µg/mL, equivelent to 4.2 to 33.6 mg/kg in samples assuming LOQ dilution factor |
| Assessment of matrix effects is presented | No. Matrix matched standards were used |
| Stability | Stability was not assessed as all solutions and samples were analysed on the day of preparation |
| Limit of determination/quantification | 15 mg/kg |

Conclusion

The analytical method for the determination of 2,4-D in larval diet was successfully validated with a limit of quantification (LOQ) for 2,4-D of 15 mg/kg.

* + - 1. Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1)

No new or additional studies have been submitted

* + 1. Methods for post-authorization control and monitoring purposes (KCP 5.2)
       1. Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted

* + - 1. Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)
         1. Analytical method

Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The submitted study description cannot be evaluated because no submitted original study report. However, it is not needed for the registration decision and was not requested. Assessment requires a submission of the original study report. |

|  |  |
| --- | --- |
| Reference: | KCP 5.2.1/01 |
| Title: | Development and Validation of an analytical method based on multi-residue method QuPPe for Determination of 2,4-Dichlorophenoxyacetic acid(2,4-D) in Honey |
| Report: | Winter, O., Schwenk, M., 2023a S23-101772, 000115746 |
| Guideline(s): | SANTE/2020/12830 rev. 2 |
| Deviations: | None |
| GLP/GEP: | Yes (certified laboratory) |
| ~~Acceptability:~~ | ~~yes~~ |

Materials and methods

The analytical method was developed for the determination of 2,4-D in honey by HPLC-MS/MS.

Honey samples (5 g) were extracted with 0.1% formic acid in methanol (10 mL), formic acid (100 µL), water (2 mL) and EDTA solution (1 mL). The sample was shaken for 20 minutes and diluted to 20 mL with water and sonicated. The sample was centrifuged, and an aliquot (1 mL) was added to acetonitrile (1 mL).

All samples were analysed by HPLC-MS/MS employing a Helix Amaze SPF column, 3 µm monitoring two mass transitions.

Quantification was performed using external matrix matched calibration standards over the range 0.375 to 37.5 ng/mL, equivalent to 0.003 to 0.3 mg/kg in samples.

Results and discussions

The analytical method for the determination of 2,4-D in honey was successfully validated with a limit of quantification (LOQ) of 0.01 mg/kg.

Table A 9: Recovery results from method validation of 2,4-D in honey using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n=x) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| Quantification m/z 219→161 | | | | | |
| honey | 2,4-D | 0.01 (n=5) | 107 | 2.5 | Range 104-109% |
| 0.1 (n=5) | 104 | 0.5 | Range 104-105% |
| Overall (n=10) | 105 | 2.0 | Range 104-109% |
| Confirmation m/z 219→125 | | | | | |
| honey | 2,4-D | 0.01 (n=5) | 105 | 1.0 | Range 103-106% |
| 0.1 (n=5) | 104 | 1.2 | Range 102-105% |
| Overall (n=10) | 104 | 1.0 | Range 102-106% |

Table A 10: Characteristics for the analytical method used for validation of 2,4-D residues in honey

|  |  |
| --- | --- |
|  | 2,4-D |
| Specificity | Blank value < 30 % LOQ  No residues detected in control samples.  Mass spectra were provided. |
| Calibration (type, number of data points) | individual calibration data presented|  calibration line equation presented  r ≥ 0.9997  linear, 1/x weighting  8 data points (singular determinations) |
| Calibration range | 0.375 to 37.5 ng/mL, equivalent to 0.003 to 0.3 mg/kg in samples |
| Assessment of matrix effects is presented | Yes, matrix effects were determined to be insignificant (≤20%), nevertheless, matrix matched standards were used |
| Stability | Stability in standard solutions was established for 9 days when stored between 1 and 10°C in the dark  Stability of final extracts was established for 10 days when stored between 1 and 10°C in the dark |
| Limit of determination/quantification | 0.01 mg/kg |

Conclusion

The analytical method for the determination of 2,4-D in honey was successfully validated with a limit of quantification (LOQ) for 2,4-D of 0.01 mg/kg.

* + - * 1. Analytical method

Independent Laboratory Validation

|  |  |
| --- | --- |
| Comments of zRMS: | The submitted study description cannot be evaluated because no submitted original study report. However, it is not needed for the registration decision and was not requested. Assessment requires a submission of the original study report. |

|  |  |
| --- | --- |
| Reference: | KCP 5.2.1/02 |
| Title: | Independent laboratory validation of an analytical method for the determination of 2,4-dichlorophenoxyacetic acid (2,4-D) in honey |
| Report: | Jooß, S., 2023 S23-102036, 000115744 |
| Guideline(s): | SANTE/2020/12830 rev. 2 |
| Deviations: | None |
| GLP/GEP: | Yes (certified laboratory) |
| ~~Acceptability:~~ | ~~yes~~ |

Materials and methods

The analytical method was independently validated for the determination of 2,4-D in honey by HPLC-MS/MS.

Honey samples (5 g) were extracted with 0.1% formic acid in methanol (10 mL), formic acid (100 µL), water (2 mL) and EDTA solution (1 mL). The sample was shaken for 20 minutes and diluted to 20 mL with water and sonicated. The sample was centrifuged, and an aliquot (1 mL) was added to acetonitrile (1 mL).

All samples were analysed by HPLC-MS/MS employing a Helix Amaze SPF column, 3 µm monitoring two mass transitions.

Quantification was performed using external matrix matched calibration standards over the range 0.375 to 37.5 ng/mL, equivalent to 0.003 to 0.3 mg/kg in samples.

Results and discussions

The analytical method for the determination of 2,4-D in honey was successfully independently validated with a limit of quantification (LOQ) of 0.01 mg/kg.

Table A 11: Recovery results from independent laboratory validation of 2,4-D in honey using the analytical method

| Matrix | Analyte | Fortification level (mg/kg) (n=x) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| Quantification m/z 219→161 | | | | | |
| honey | 2,4-D | 0.01 (n=5) | 104 | 1.3 | Range 103-106% |
| 0.1 (n=5) | 101 | 0.7 | Range 100-102% |
| Overall (n=10) | 103 | 1.8 | Range 100-106% |
| Confirmation m/z 219→125 | | | | | |
| honey | 2,4-D | 0.01 (n=5) | 101 | 1.7 | Range 98-103% |
| 0.1 (n=5) | 102 | 0.7 | Range 101-102% |
| Overall (n=10) | 102 | 1.3 | Range 98-103% |

Table A 12: Characteristics for the analytical method used for independent laboratory validation of 2,4-D residues in honey

|  |  |
| --- | --- |
|  | 2,4-D |
| Specificity | Blank value < 30 % LOQ  No residues detected in control samples.  Mass spectra were provided. |
| Calibration (type, number of data points) | individual calibration data presented|  calibration line equation presented  r ≥ 0.9997  linear, 1/x weighting  8 data points (singular determinations) |
| Calibration range | 0.375 to 37.5 ng/mL, equivalent to 0.003 to 0.3 mg/kg in samples |
| Assessment of matrix effects is presented | Yes, matrix effects were determined to be insignificant (≤20%), nevertheless, matrix matched standards were used |
| Stability | Stability was not assessed as it was performed in the primary validation study |
| Limit of determination/quantification | 0.01 mg/kg |

Conclusion

The analytical method for the determination of 2,4-D in honey was successfully independently validated with a limit of quantification (LOQ) for 2,4-D of 0.01 mg/kg.

* + - 1. Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

* + - 1. Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted

* + - 1. Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

* + - 1. Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)
         1. Analytical method

Method validation

|  |  |
| --- | --- |
| Comments of zRMS: | The submitted study description cannot be evaluated because no submitted original study report. However, it is not needed for the registration decision and was not requested. Assessment requires a submission of the original study report. |

|  |  |
| --- | --- |
| Reference: | KCP 5.2.1/03 |
| Title: | Validation of an analytical method for the Determination of 2,4-Dichlorophenoxyacetic acid (2,4-D) in Body Fluid(s) |
| Report: | Winter, O., Schwenk, M., 2023a, S23-101771, 000115745 |
| Guideline(s): | SANTE/2020/12830 rev. 2 |
| Deviations: | None |
| GLP/GEP: | Yes (certified laboratory) |
| ~~Acceptability:~~ | ~~yes~~ |

Materials and methods

The analytical method was developed for the determination of 2,4-D in bovine blood by HPLC-MS/MS.

Blood samples (1 mL) were diluted to 20 mL with acetonitrile:water (1:1 v/v) containing 0.1% acetic acid. The sample was sonicated. An aliquot (1 mL) was added to acetonitrile (1 mL) and centrifuged. An aliquot of the supernatant solution (0.8 mL) was added to water containing 0.2% acetic acid (0.2 mL).

All samples were analysed by HPLC-MS/MS employing a Helix Amaze SPF column, 3µm monitoring two mass transitions.

Quantification was performed using external matrix matched calibration standards over the range 0.06 to 6.0 ng/mL, equivalent to 0.003 to 0.3 mg/kg in samples.

Results and discussions

The analytical method for the determination of 2,4-D in blood was successfully validated with a limit of quantification (LOQ) of 0.01 mg/L.

Table A 13: Recovery results from method validation of 2,4-D in blood using the analytical method

| Matrix | Analyte | Fortification level (mg/L) (n=x) | Mean  recovery (%) | RSD (%) | Comments |
| --- | --- | --- | --- | --- | --- |
| Quantification m/z 219→161 | | | | | |
| Bovine blood | 2,4-D | 0.01 (n=5) | 76 | 2.6 | Range 74-78% |
| 0.1 (n=5) | 80 | 3.0 | Range 77-83% |
| Overall (n=10) | 78 | 3.7 | Range 74-83% |
| Confirmation m/z 219→125 | | | | | |
| Bovine blood | 2,4-D | 0.01 (n=5) | 77 | 4.3 | Range 73-80% |
| 0.1 (n=5) | 81 | 3.2 | Range 78-84% |
| Overall (n=10) | 79 | 4.4 | Range 73-84% |

Table A 14: Characteristics for the analytical method used for validation of 2,4-D residues in blood

|  |  |
| --- | --- |
|  | 2,4-D |
| Specificity | Blank value < 30 % LOQ  No residues detected in control samples.  Mass spectra were provided. |
| Calibration (type, number of data points) | individual calibration data presented|  calibration line equation presented  r ≥ 0.9989  linear, 1/x weighting  8 data points (singular determinations) |
| Calibration range | 0.06 to 6.0 ng/mL, equivalent to 0.003 to 0.3 mg/kg in samples |
| Assessment of matrix effects is presented | Yes, matrix effects were determined to be insignificant (<20%), nevertheless, matrix matched standards were used |
| Stability | Stability in standard solutions was established for 7 days when stored between 1 and 10°C in the dark  Stability of final extracts was established for 10 days when stored between 1 and 10°C in the dark |
| Limit of determination/quantification | 0.01 mg/L |

Conclusion

The analytical method for the determination of 2,4-D in blood was successfully validated with a limit of quantification (LOQ) for 2,4-D of 0.01 mg/L.

* + - 1. Other Studies/Information

No new or additional studies have been submitted