REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: AG-F8-250 CS

Chemical active substance:

Flurochloridone, 250 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT/

(authorization)

Sponsor: ADAMA Agan Ltd

Applicant: Country organisation/representative of ADAMA Agan Ltd. as reported in Part A

Submission date: January 2020

MS Finalisation date: October 2020 (initial Core Assessment)

March 2021 (final Core Assessment)

Version history

When	What
January 2020	dRR submitted by the Applicant
October 2020	Initial zRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through and shaded for transparency~~.
March 2021	Final report (Core Assessment updated following the commenting period) Additional information/assessments included by the zRMS in the report in response to comments recieved from the cMS and the Applicant are highlighted in yellow. No comments after the commenting period.

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5 Analytical methods

This document summarises the analytical methods on the plant protection product AG-F8-250 CS, a capsule suspension containing 250 g/L flurochloridone for use in potato in Central Zone according to article 33 of the Regulation 1107/2009.
This application follows the data requirements for the active substance laid down in Regulation (EC) No. 544/2011 and the data requirements for the plant protection product laid down in Regulation (EC) No. 284/2013.

The active substance flurochloridone was included into Annex I of Directive 91/414 (2011/34/EU) with entry into force by 01 June 2011. This inclusion was reported in the EC regulation 540/2011 further the application of the regulation 1107/2009. ADAMA Agan Ltd. was the sole notifier.

5.1 Conclusion and summary of assessment

zRMS conclusions:
In according to the EFSA Journal 2010;8(12):1869 - Conclusion on the peer review of the pesticide risk assessment of the active substance:
Residues of FLC in plants can be analysed with GC-MS methods. A method of analysis for products of animal origin is not required as no MRL's are proposed. For soil, water and air GC-MS methods are available. The active substance is classified as toxic (T) and therefore a data gap is identified for a method of analysis for body fluids and tissues.

Analytical methods for residues (Annex IIA, point 4.1)
Residue definitions for monitoring purposes

Food of plant origin		FLC sum of isomers
Food of animal origin		No required for the supported use
Soil		FLC sum of isomers
Water	surface	FLC sum of isomers
	drinking/ground	FLC sum of isomers
Air		FLC sum of isomers

Monitoring/Enforcement methods

Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)	For sunflower seeds: DFG method S19 (GC/MS), LOQ = 0.01 mg/kg For potatoes tubers: modified version of a published multiresidue method, Fillion et al., 2000 (GC/MS), LOQ = 0.01 mg/kg
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)	Not required.
Soil (analytical technique and LOQ)	FLC: GC/MSD, 0.01 mg/kg
Water (analytical technique and LOQ)	FLC: Ground, GC/MS, LOQ = 0.05 µg/L Surface, GC/MS, LOQ = 0.05 µg/L Drinking, GC/MS, LOQ = 0.05 µg/L
Air (analytical technique and LOQ)	FLC: GC/MS LOQ = 0.75 µg/m^3
Body fluids and tissues (analytical technique and LOQ)	The active substance is classified as T and a data gap is identified for a method of analysis.

Summary:
Soil
The analytical method of Wolf, S. (2006, A47272) using GC-MS for the determination of flurochloridone residue in soil was provided by the Applicant and validated at EU level (DAR of flurochloridone) with a LOQ=0.01 mg/kg.
Water
The analytical method of Wolf S. (2005, 85708) using GC-MS for the determination of flurochloridone residue in drinking, ground and surface water was provided by the Applicant and validated at EU level (DAR of

flurochloridone) with a LOQ = 0.05 µg/L.

Air

The analytical method of Wolf, S.(2006, 90009363) using GC-MS for the determination of flurochloridone residue in air was provided by the Applicant and validated at EU level (DAR of flurochloridone) with a LOQ=0.75 µg/m^3.

Body fluids and tissues

The active substance is classified as toxic (T) and therefore a data gap is identified for a method of analysis for body fluids and tissues. according to the EFSA Journal 2010;8(12):1869.

As above mentioned methods are not considered highly specific (one ion monitoring), a confirmatory method should be required for the determination of flurochloridone residue in soil, water and air according to SANCO 825/00 rev. 8.1 at the renewal of the active substance.

Therefore the Applicant has already submitted a number of methods for analysis of residues of flurochloridone for the generation of pre-authorization data and methods for post-authorization control and monitoring purposes.

The details of the evaluation of new and additional studies are referred in Appendix 2 and the conslusion is presented below:

- Different analytical methods to determine concentrations of flurochloridone in ecotoxicology media for risk assessment have been developed and validated in accordance with SANCO/3029/99 rev. 4.
- The analytical methods were successfully validated for the determination of flurochloridone in the 4 matrix groups of **plant matrices** (dry high starch (dried peas), high water (apples), high acid (grapes) and high oil (sunflower seeds) content) and in **animal matrices** (milk, fat, eggs, muscle and kidney) with a limit of quantification (LOQ) of 0.01 mg/kg, in accordance to SANCO/825/00 rev. 8.1 requirements.
- The analytical method for the determination of flurochloridone (2 isomers: FND-311-Trans and FND-311-Cis) and metabolite R406639in three different **soils** was fully validated with a limit of quantification (LOQ) of 0.01 mg/kg for flurochloridone and 0.001 mg/kg for metabolite R406639, in accordance to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 requirements.
- The analytical methods for the determination of flurochloridone and metabolites R42819 and R406639 in **drinking water and surface water** were fully validated with a limit of quantification (LOQ) of 0.05 µg/L in accordance to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 requirements.
- The analytical method for the determination of flurochloridone in **body fluids** (human urine and whole blood) was fully validated with a limit of quantification (LOQ) of 0.01 mg/L in accordance to SANCO/825/00 rev. 8.1 requirements.
- No new method validations will be submitted for the analysis of flurochloridone in **air**.

Studies have been accepted.

Additionally excerpt from EFSA Journal 2018;16(1):5144- Review of the existing MRLs for flurochloridone:

Methods of analysis in plants

In the framework of this review, a gas chromatography (GC) method with tandem mass spectrometry (MS/MS) detection (GC–MS/MS) was considered suitable for monitoring flurochloridone in high water and high acid commodities, with a limit of quantification (LOQ) of 0.01 mg/kg. The method was validated in potatoes; however, an independent laboratory validation (ILV) was not available (Spain, 2006). A confirmatory GC–MS method with a LOQ of 0.01 mg/kg in high water and high oil content commodities (potatoes and sunflower seeds) was provided during this review (France, 2017).

A GC-MS method with a LOQ of 0.01 mg/kg flurochloridone, validated in sunflower seeds, supported by an ILV and confirmatory method, is available for monitoring in high oil commodities (Spain, 2006; France, 2017).

According to the information provided by the EURLs during the completeness check, a LOQ of 0.01 mg/kg for flurochloridone (validated in high water, high acid, high oil content and dry commodities) is achievable for routine analyses by using a validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) method (EURL, 2017).

Conclusions

Fully validated analytical methods are available for the enforcement of the proposed residue definition in high water, high acid, high oil content and dry commodities with a LOQ of 0.01 mg/kg, respectively.

Residues in livestock

The residue definition in these commodities can be defined as flurochloridone (cis and trans isomers) by default for both enforcement and risk assessment. A method for enforcement of the proposed residue definition in ruminants and swine tissues and in milk (goat and sheep) is not available. Therefore, the current LOQ of 0.05 mg/kg as reported in the EU legislation and considered in this review should be confirmed by the submission of a fully validated analytical method for enforcement. MRLs for cattle milk, poultry tissues and eggs are not required.

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

- None.

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

- None.

Commodity/crop	**Supported/ Not supported**
Potato	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

An overview on the acceptable methods and possible data gaps for analysis of the active substances in the plant protection product AG-F8-250 CS (RACER 25 CS; CS formulation containing 250 g/L flurochloridone) is provided as follows.

A GC-FID method for the determination of flurochloridone in AG-F8-250 CS was fully validated by the EU agreed method presented in the DAR from March 2006 performed by Gorban I. (2005). This validated method also used for sample measurement during storage stability testing of AG-F8-250 CS by Gorban I. (2006), as presented below.

Comments of zRMS:	The description of the analytical method is considered sufficient.

Reference:	**5.2.1.1/01 (KCP 5.1.1/01)**
Report	Gorban, I. (2006) RACER 25 CS (Flurochloridone 250 g/L CS) Determination of Storage Stability and Shelf Life Specification Data of RACER 25 CS Stored at 54°C for 14 Days Report No. F06-07, Sponsor Reference No. 90009450
Guideline(s):	SANCO/3030/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analysis of the active ingredient flurochloridone in AG-F8-250 CS was done by gas chromatograph (GC) with FID detection using internal standard technique.
The study objective was to develop and validate an analytical method for the determination of flurochloridone (cis and trans isomers) in AG-F8-250 CS. The test item AG-F8-250 CS was dissolved in acetone/water and the content of active ingredients was determined by gas chromatograph (GC) with FID detection using internal standard technique.

Results and discussions

Table 5.2-1: Methods suitable for the determination of active substance flurochloridone in plant protection product AG-F8-250 CS

	Flurochloridone
Author(s), year	Gorban, I. (2006)
Principle of method	GC with FID detection using internal standard technique
Linearity (linear between mg/L / % range of the declared content)	A series of four standard stock solutions were prepared in acetone, with internal standard. The linearity was determined with four calibration standards injected in triplicate in the range of interest (1mg/mL to 2.75 mg/mL for cis-flurochloridone;

<table>
<tr><th></th><th>Flurochloridone</th></tr>
<tr><td>(correlation coefficient, expressed as r)</td><td>2.75 mg/mL to 8.5 mg/mL for trans-flurochloridone). The calibrations were linear in the following ranges with the following correlation coefficients r:
Trans-flurochloridone: Y = $6.56311e^{-1}$ x – $2.50828e^{-3}$, r = 0.99998
Cis-flurochloridone: Y = $6.49267e^{-1}$ x + $1.03345e^{-3}$, r = 0.99996</td></tr>
<tr><td>Precision – Repeatability Mean
from Report No. 05-11/6
n = 30
(%RSD)</td><td>The relative standard deviation (RSD) for the whole procedure of sample preparation and measurement of 10 independent samples of the test item, each injected in triplicate, was found to be:
<table>
<tr><td>Active ingredient</td><td>Mean concentration of analyte in the test item (%, (w/w))</td><td>RSD (%)</td><td>Proposed acceptable RSD for the concentration of analyte (Horwitz) (%) (1)</td></tr>
<tr><td>Trans-flurochloridone</td><td>17.38</td><td>0.54</td><td>1.74</td></tr>
<tr><td>Cis-flurochloridone</td><td>5.01</td><td>1.66</td><td>2.10</td></tr>
</table></td></tr>
<tr><td>Accuracy
from Report No. 05-11/6
n = 4
(% Recovery)</td><td>Accuracy was determined by a recovery fortification of known amounts of the analytes to four sample solutions of the formulation. Four recovery determinations were performed:
<table>
<tr><td>Recovery Level (% (w/w))</td><td>Cis-flurochloridone Recovery (%)</td></tr>
<tr><td>0.130</td><td>100.1</td></tr>
<tr><td>0.453</td><td>97.9</td></tr>
<tr><td>0.797</td><td>97.0</td></tr>
<tr><td>1.066</td><td>98.1</td></tr>
</table>
<table>
<tr><td>Recovery Level (% (w/w))</td><td>Trans-flurochloridone (%)</td></tr>
<tr><td>1.209</td><td>99.96</td></tr>
<tr><td>2.165</td><td>101.9</td></tr>
<tr><td>3.085</td><td>101.4</td></tr>
<tr><td>3.975</td><td>101.6</td></tr>
</table></td></tr>
<tr><td>Interference/ Specificity</td><td>The blank samples showed no relevant interferences with the signals of the active ingredient, hence the specificity of the method is confirmed.
Confirmation of analyte identification was done by GC-MS. The similarity of the mass spectra from standard and sample solutions confirms the identity of the active ingredient peak.</td></tr>
<tr><td>Comment</td><td>Acceptable</td></tr>
</table>

Conclusion

The analytical method for the active ingredient flurochloridone determination in AG-F8-250 CS was fully validated according to SANCO/3030/99 rev. 4, 11 July 2000.
The results obtained prove that this method is suitable for the detection and quantitation of the active substance flurochloridone in the formulation AG-F8-250 CS.

A GC-FID method for the determination of total flurochloridone and free flurochloridone in AG-F8-250 CS was fully validated by Ricau, H. (2018) and is presented below.

Comments of zRMS:	Study accepted. The analytical method for total flurochloridone and free flurochloridone determination in AG-F8-250 CS is fully validated according to SANCO/3030/99 rev. 4, 11 July 2000.

Reference: **5.2.1.1/02 (KCP 5.1.1/02)**

Report: Ricau, H. (2018)
Validation of the analytical methods for the determination of total flurochloridone and free flurochloridone in AG-F8-250 CS
Report No. 17-901066-005, Sponsor Reference No. 90021272

Guideline(s): SANCO/3030/99 rev. 4

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

The analysis of total flurochloridone and free flurochloridone in AG-F8-250 CS was done by gas chromatograph (GC) with FID detection using internal standard technique.
The analysis of the active ingredient flurochloridone in AG-F8-250 CS was done by gas chromatograph (GC) with FID detection using internal standard technique.
The study objective was to develop and validate an analytical method for the determination of total flurochloridone and free flurochloridone in AG-F8-250 CS. The test item AG-F8-250 CS was dissolved in acetone/water 90/10 v/v (total flurochloridone) or acetone (free flurochloridone) and the content of active ingredients was determined by gas chromatograph (GC) with FID detection using internal standard technique.

Results and discussions

Table 5.2-2: Methods suitable for the determination of total flurochloridone and free flurochloridone in plant protection product AG-F8-250 CS

<table>
<tr><th></th><th>Total Flurochloridone</th><th>Free Flurochloridone</th></tr>
<tr><td>Author(s), year</td><td colspan="2">Ricau, H. (2018)</td></tr>
<tr><td>Principle of method</td><td colspan="2">GC with FID detection using internal standard technique</td></tr>
<tr><td>Linearity
(linear between mg/L / % range of the declared content)
(correlation coefficient, expressed as r)</td><td>A series of five calibration standard solutions were prepared in water/acetone (10/90 v/v), with internal standard. The linearity was determined with five calibration standards injected in duplicate ranging from 221.27 mg/L to 726.78 mg/L (between 50% and 150% of the reference items). The calibrations were linear with correlation coefficients r >0.99:
$Y = 6.68E^{-03} x – 2.13E^{-01}$, r = 0.9997</td><td>A series of five calibration standard solutions were prepared in hexane/acetone (50/50 v/v), with internal standard. The linearity was determined with five calibration standards injected in duplicate ranging from 21.79 mg/L to 73.60 mg/L (between 50% and 150% of the reference items). The calibrations were linear with correlation coefficients r >0.99:
$Y = 2.02E^{-02} x – 1.40E^{-01}$, r = 0.9991</td></tr>
<tr><td>Precision – Repeatability
Mean
n = 10
(%RSD)</td><td>The relative standard deviation (RSD) for the whole procedure of sample preparation and measurement of 5 independent samples of the test item, each injected in duplicate, was found to be:
<table>
<tr><th>Mean concentration of analyte in the test item (g/L)</th><th>RSD (%)</th><th>Proposed acceptable RSD (Horwitz) (%) (1)</th></tr>
<tr><td>240*</td><td>0.45</td><td>1.69</td></tr>
</table>
* equivalent to 21.8 % (w/w)</td><td>The relative standard deviation (RSD) for the whole procedure of sample preparation and measurement of 5 independent samples of the test item, each injected in duplicate, was found to be:
<table>
<tr><th>Mean concentration of analyte in the test item (g/L)</th><th>RSD (%)</th><th>Proposed acceptable RSD (Horwitz) (%) (1)</th></tr>
<tr><td>10.69</td><td>2.55</td><td>2.69</td></tr>
</table>
* equivalent to 0.971 % (w/w)</td></tr>
<tr><td>Accuracy
n = 4
(% Recovery)</td><td>Accuracy was determined by a recovery fortification of known amounts of the analytes to two formulation blank solutions, each injected in duplicate.
<table>
<tr><th>Recovery Level (mg/L)*</th><th>Recovery (%)</th><th>Mean recovery (%)</th></tr>
</table></td><td>The accuracy of the free flurochloridone was not performed because this parameter cannot be adequately measured with the free flurochloridone method.
No free flurochloridone was detected after the addition of a known quantity of reference item of flurochloridone on the test item AG-F8-250 CS</td></tr>
</table>

<table>
<tr><th></th><th colspan="2">Total Flurochloridone</th><th>Free Flurochloridone</th></tr>
<tr><td></td><td colspan="2"><table>
<tr><td>455.30</td><td>100.2
99.7</td><td>100.0</td></tr>
<tr><td>468.79</td><td>99.2
99.1</td><td>99.1</td></tr>
</table>
* equivalent to 29.4 % (w/w) (nominal)

Recovery results are in the range 98% - 102%.</td><td>and after extraction according to the free flurochloridone method. It is assumed that the reference item is incorporated into the capsules during the preparation and is therefore not measured with the free flurochloridone method which intend to measure only releasing active ingredient.
Consequently, the recovery of the free flurochloridone is impossible to calculate.</td></tr>
<tr><td>Interference/ Specificity</td><td colspan="2">Retention times for each analyte match between reference item and test item, confirming the identity of the analyte.
No interference was observed in solvent blank, formulation blank and test item at the retention times of each analyte.
Therefore, the analytical method showed a good specificity for analysis of total flurochloridone and free flurochloridone in the product of AG-F8-250 CS.</td></tr>
<tr><td>Comment</td><td colspan="2">Acceptable</td><td>Acceptable</td></tr>
</table>

Conclusion

The analytical method for total flurochloridone and free flurochloridone determination in AG-F8-250 CS was fully validated according to SANCO/3030/99 rev. 4, 11 July 2000.
The results obtained prove that this method is suitable for the detection and quantitation of total flurochloridone and free flurochloridone in the formulation AG-F8-250 CS.

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

An overview on the acceptable methods and possible data gaps for analysis of relevant impurities in the plant protection product AG-F8-250 CS (RACER 25 CS; CS formulation containing 250 g/L flurochloridone) is provided as follows.

Toluene is present as a residual solvent used in the manufacturing process of technical Fluorochloridone, however, the level of this substance will not increase during storage of the final CS-formulation, since there are no chemical or physical processes, through toluene can be formed from the molecule Fluorochloridone. As toluene is considered to be of toxicological concern a method for its determination was provided during the EU review and considered acceptable (see point IIIA 5.2.4 below).

Comments of zRMS:	The following study has already been evaluated as part of the EU review.

The following analytical method for the determination of the relevant impurity toluene was assessed in the EU review. The report is enclosed with this submission but since the study was deemed to be acceptable during the EU review a full summary of the study has not been provided.

Report:	**KIIIA 5.2.4/01, Guzikevich, G., 2003**
Title:	Flurochloridone technical five lots analysis and method validation
Document No:	Report no. 03-03, Agan report no. 90006021, October 08, 2003
Guidelines:	EC Commission Directive 94/37/EC SANCO/3030/99 rev. 4 EPA Guidelines OPPTS 830 Series Deviations (to SANCO/3030/99 rev. 4): None
GLP	Yes

The study was performed to characterise the chemical composition of flurochloridone technical and its components occurring at level ≥0.1% (w/w).The quantification was done by capillary gas chromatography with a flame ionisation detector (FID).
The analytical method proved to be precise, repeatable, linear, accurate and specific for the determination of flurochloridone and for the relevant impurity toluene in technical material.

Conclusion: the method is acceptable.

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

This is not an EC data requirement / not required by Regulation EC 1107/2009.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

AG-F8-250 CS contains one active substance (flurochloridone).

CIPAC method 430 is available for the analysis of flurochloridone but no publication is available.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Flurochloridone / Pre-registration methods

Additionally to the unprotected data available on EU level, the applicant has developed a set of own methods to support ecotoxicology studies.

An overview on the acceptable methods and possible data gaps for analysis of residues of flurochloridone and its relevant metabolites for the generation of pre-authorisation data is given in the following table. For detailed evaluation of new/additional studies it is referred to Appendix 2.

Residue definition according to EFSA conclusion, 2010 (EFSA Journal 2010;8(12):1869):
Plant residue definition for risk assessment:
Flurochloridone FLC (sum cis + trans isomers)
Animal residue definition for risk assessment:
Not relevant

Residue definition according to EFSA conclusion, 2018 (EFSA Journal 2018;16(1):5144):
Plant residue definition for risk assessment:
Flurochloridone (sum of cis and trans isomers) by default
Animal residue definition for risk assessment:
Flurochloridone (sum of cis and trans isomers) by default

With regard to the performed standard ecotoxicologial studies (aquatic, terrestrial) performed with the product, the following residue definition was published by EFSA Journal 2010;8(12):1869:

Soil	Flurochloridone FLC
Surface water	Flurochloridone FLC, R42819
Sediment	Flurochloridone FLC, R406639, R42819
Ground water	Flurochloridone FLC, R406639, R42819
Air	Flurochloridone FLC

Table 5.2-3: Validated methods for the generation of pre-authorisation data – Flurochloridone

Residue definition: Flurochloridone (sum cis + trans isomers)

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)	No additional data.			
	Confirmatory (if required)	-	-	-
Animal products, food of animal origin,... (Residues)	No additional data.			
	Confirmatory (if required)	-	-	-
Soil, water,... (Efficacy)	No additional data.			
	Confirmatory (if required)	-	-	-
Feed, body fluids,... (Toxicology)	No additional data.			
	Confirmatory (if required)	-	-	-
Body fluids, air,.... (Exposure)	No additional data.			
	Confirmatory (if required)	-	-	-

Residue definition: Flurochloridone, R42819, and R406639 (sediment and ground water only)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Daphnia toxicity study OECD 211 (Ecotoxicology)	Primary	LOQ = 1.02 mg/L of test item (flurochloridone technical)	HPLC-MS/MS m/z 312 → 292	KCP 5.1.2/01, Weber, B. (2012) Report No. D45354 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Algae toxicity study OECD 201 (Ecotoxicology)	Primary	LOQ = 2.41 µg/L of test item (0.595 µg/L of flurochloridone)	HPLC-MS/MS m/z 312 → 292	KCP 5.1.2/02, Liedtke, A. (2012a) Report No. D54231 See Appendix 2
	Confirmatory (if required)		Not required, highly specific detection system was used (MS/MS)	
Algae toxicity study OECD 201 (Ecotoxicology)	Primary	LOQ = 13.5 µg/L of test item (flurochloridone technical)	HPLC-MS/MS m/z 312 → 292	KCP 5.1.2/03, Liedtke, A. (2013a) Report No. D65727 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Algae toxicity study OECD 201 (Ecotoxicology)	Primary	LOQ = 1.96 µg/L of test item (flurochloridone technical)	HPLC-MS/MS m/z 312 → 292	KCP 5.1.2/04, Liedtke, A. (2013b) Report No. D65738 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Algae toxicity study OECD 201 (Ecotoxicology)	Primary	LOQ = 1.36 µg/L of test item (flurochloridone technical)	HPLC-MS/MS m/z 312 → 292	KCP 5.1.2/05, Liedtke, A. (2013c) Report No. D65740 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Algae toxicity study OECD 201	Primary	LOQ = 3.09 µg/L of test item (trans-flurochloridone)	HPLC-MS/MS m/z 312 → 292	KCP 5.1.2/06, Liedtke, A. (2013d) Report No. D59890

Residue definition: Flurochloridone, R42819, and R406639 (sediment and ground water only)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
(Ecotoxicology)				See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Algae toxicity study OECD 201 (Ecotoxicology)	Primary	LOQ = 0.451 µg/L of test item (trans-flurochloridone)	HPLC-MS/MS m/z 312 → 292	KCP 5.1.2/07, Liedtke, A. (2013e) Report No. D65547 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Algae toxicity study OECD 201 (Ecotoxicology)	Primary	LOQ = 0.01 µg/L of test item (flurochloridone technical)	HPLC-MS/MS m/z 312 → 292	KCP 5.1.2/08, Scheerbaum, D. (2013a) Report No. SPO15371 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Algae toxicity study OECD 201 (Ecotoxicology)	Primary	LOQ = 0.01 µg/L of test item (flurochloridone technical)	HPLC-MS/MS m/z 312 → 292	KCP 5.1.2/09, Scheerbaum, D. (2013b) Report No. SNC15371 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Algae toxicity study OECD 201 (Ecotoxicology)	Primary	LOQ = 0.01 µg/L of test item (flurochloridone technical)	HPLC-MS/MS m/z 312 → 292	KCP 5.1.2/10, Scheerbaum, D. (2013c) Report No. SSL15371 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Algae toxicity study OECD 201 (Ecotoxicology)	Primary	LOQ = 0.01 µg/L of test item (flurochloridone technical)	HPLC-MS/MS m/z 312 → 292	KCP 5.1.2/11, Scheerbaum, D. (2013d) Report No. SCN15371 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Algae toxicity study OECD 201 (Ecotoxicology)	Primary	LOQ = 0.01 µg/L of test item (flurochloridone technical)	HPLC-MS/MS m/z 312 → 292	KCP 5.1.2/12, Scheerbaum, D. (2013e) Report No. SAF15371 See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS/MS)	
Algae toxicity study OECD 201 (Ecotoxicology)	Primary	LOQ = 4.0 µg/L of flurochloridone (sum of isomers)	GC-MS m/z 311 m/z 187	KCP 5.1.2/13, Wenzel, A. (2015a) Report No. ADM-003/4-10/B See Appendix 2
	Confirmatory (if required)	-	Not required, highly specific detection system was used (MS)	
Lemna toxicity study OECD 221 (Ecotoxicology)	Primary	LOQ = 11.4 µg/L of test item (2.81 µg/L of flurochloridone)	HPLC-MS/MS m/z 312 → 292	KCP 5.1.2/14, Liedtke, A. (2012b) Report No. D54308 See Appendix 2
	Confirmatory (if required)		Not required, highly specific detection system was used (MS/MS)	
Lemna toxicity study	Primary	LOQ = 5.0 µg/L of	GC-MS	KCP 5.1.2/15,

Residue definition: Flurochloridone, R42819, and R406639 (sediment and ground water only)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
OECD 221 (Ecotoxicology)		flurochloridone trans isomer	m/z 311 m/z 187	Wenzel, A. (2015b) Report No. ADM-005/4-11/I See Appendix 2
	Confirmatory (if required)		Not required, highly specific detection system was used (MS/MS)	
Myriophyllum toxicity study OECD 238 (Ecotoxicology)	Primary	LOQ = 5.0 µg/L of flurochloridone trans isomer	GC-MS m/z 311 m/z 187	KCP 5.1.2/16, Wenzel, A. (2015c) Report No. ADM-005/4-13/K, See Appendix 2
	Confirmatory (if required)		Not required, highly specific detection system was used (MS/MS)	
Honey Bee toxicity study OECD guideline proposal (2016) (Ecotoxicology)	Primary	LOQ = 39.0 mg/kg of test item (9.52 mg/kg of flurochloridone)	HPLC-MS/MS m/z 312 → 292	KCP 5.1.2/17, Molitor, A.M. (2017) Report No. S17-00282 See Appendix 2
	Confirmatory (if required)		Not required, highly specific detection system was used (MS/MS)	
Honey Bee Larval toxicity study OECD 239 (2016) (Ecotoxicology)	Primary	LOQ = 50.0 mg/kg of test item (12.2 mg/kg of Flurochloridone)	HPLC-MS/MS m/z 312 → 292	KCP 5.1.2/18, Molitor, A.M. (2018) Report No. S17-00318 See Appendix 2
	Confirmatory (if required)		Not required, highly specific detection system was used (MS/MS)	

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

Flurochloridone

With regard to monitoring enforcement methods (post-registration methods) for flurochloridone reference is made to the analytical methods available on EU level.

For summary, a reference is made to the data compiled in the Draft Renewal Assessment Report of flurochloridone (February 2006) and the EFSA Journal 2010;8(12):1869, Peer review of the pesticide risk assessment of the active substance flurochloridone).

These data are considered to provide the relevant dossier information on the active substance. Besides, own ADAMA studies on the active substance are available where necessary. An overview on the acceptable methods and possible data gaps for analysis of residues of flurochloridone for the generation of post-authorisation data is given in the following table. For detailed evaluation of new/additional studies, it is referred to Appendix 2.

5.3.1 Analysis of the plant protection product (KCP 5.2)

Please refer to the analytical methods for the determination of the active substance and relevant impurities in the plant protection product as provided in chapter 5.2.1.

5.3.2 Description of analytical methods for the determination of residues of flurochloridone (KCP 5.2)

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

Matrix	Residue Definition	Reference
Plant commodities	Flurochloridone (FLC) sum of isomers	EFSA Journal 2010;8(12):1869, Peer review of the pesticide risk assessment of the active substance flurochloridone
	~~Flurochloridone (FLC) sum of isomers~~ Flurochloridone (sum of cis- and trans- isomers)	~~Regulation (EU) 149/2008~~ Regulation (EU) 2019/973
Animal origin	Not required for the supported use	EFSA Journal 2010;8(12):1869
	~~Flurochloridone (FLC) sum of isomers~~ Flurochloridone (sum of cis- and trans- isomers)	~~Regulation (EU) 149/2008~~ Regulation (EU) 2019/973
Soil	Flurochloridone (FLC) sum of isomers	EFSA Journal 2010;8(12):1869
Ground water	Flurochloridone (FLC) sum of isomers	EFSA Journal 2010;8(12):1869
Surface water	Flurochloridone (FLC) sum of isomers	EFSA Journal 2010;8(12):1869
Air	Flurochloridone (FLC) sum of isomers	EFSA Journal 2010;8(12):1869

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
Potato	Flurochloridone (sum of isomers)	0.01 mg/kg*	EFSA Journal 2010;8(12):1869
Sunflower		0.01 mg/kg*	EFSA Journal 2010;8(12):1869
Plant, high water content	Flurochloridone (sum of isomers)	~~0.1 mg/kg~~ 0.01** mg/kg	~~Com. Reg. (EU) 149/2008~~ Com. Reg. 2019/973
Plant, high acid content		~~0.1 mg/kg~~ 0.01** mg/kg	~~Com. Reg. (EU) 149/2008~~ Com. Reg. 2019/973
Plant, high protein/high starch content (dry commodities)		~~0.1 mg/kg~~ 0.01** mg/kg	~~Com. Reg. (EU) 149/2008~~ Com. Reg. 2019/973
Plant, high oil content		~~0.1 mg/kg~~ 0.01** mg/kg	~~Com. Reg. (EU) 149/2008~~ Com. Reg. 2019/973

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, difficult matrices (hops, spices, tea)		~~0.1 mg/kg~~ 0.01** mg/kg	~~Com. Reg. (EU) 149/2008~~ Com. Reg. 2019/973
Muscle	Flurochloridone (sum of isomers)	0.05 mg/kg	~~Com. Reg. (EU) 149/2008~~ Com. Reg. 2019/973
Milk		0.05 mg/kg	~~Com. Reg. (EU) 149/2008~~ Com. Reg. 2019/973
Eggs		0.05 mg/kg	~~Com. Reg. (EU) 149/2008~~ Com. Reg. 2019/973
Fat		0.05 mg/kg	~~Com. Reg. (EU) 149/2008~~ Com. Reg. 2019/973
Liver, kidney		0.05 mg/kg	~~Com. Reg. (EU) 149/2008~~ Com. Reg. 2019/973
Soil (Ecotoxicology)	Flurochloridone (sum of isomers)	0.05 mg/kg	SANCO/825/00 rev. 8.1
Drinking water (Human toxicology)	Flurochloridone (sum of isomers)	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Flurochloridone (sum of isomers)	Algae *S. Subspicatus* 72h (static) EbC_{50} = 2.1 µg a.s./L	EFSA Journal 2010;8(12):1869
Air	Flurochloridone (sum of isomers)	LOQ = 12 µg/m^3 for flurochloridone, based on AOEL = 0.04 mg/kg bw/day	EFSA Journal 2010;8(12):1869
Tissue (meat or liver)	Flurochloridone (sum of isomers)	0.1 mg/kg	SANCO/825/00 rev. 8.1 Classified as toxic (T)
Body fluids (blood, serum, plasma or urine)	Flurochloridone (sum of isomers)	0.05 mg/L	SANCO/825/00 rev. 8.1 Classified as toxic (T)

* According to EFSA Journal 2010;8(12):1869, no residues were detected in the supervised residues trials conducted on sunflower and potato and the MRLs were proposed at the LOQ of 0.01 mg/kg.
** Indicates lower limit of analytical determination

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

An overview on the acceptable methods and possible data gaps for analysis of flurochloridone in plant matrices is given in the following table. ADAMA performed new method validation studies to fulfil the data requirement within this dossier. For the detailed evaluation of new/additional studies, please refer 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: Flurochloridone sum of isomers				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Sunflower seeds	Primary	LOQ = 0.01 mg/kg of flurochloridone	DFG S19 GC-MS	Anspach, T. 2002 DAR, March 2006
ILV - Sunflower seeds:	Primary	LOQ = 0.01 mg/kg of flurochloridone	DFG S19 GC-MS	Wolf, S. 2003 DAR, March 2006
Potato tubers	Primary	LOQ = 0.01 mg/kg of flurochloridone	modified version of a published multi residue method (Fillion et al.,	Balluff, M. 2004 DAR, March 2006

Component of residue definition: Flurochloridone sum of isomers				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
			2000) GC-MS	
High water (apple) High acid (grape) High oil (sunflower seed) High protein /high starch, dry (dry pea)	Primary and confirmatory	LOQ = 0.01 mg/kg of flurochloridone	QuEChERS GC-MS/MS m/z 311 → 174 m/z 145 → 95	KCP 5.2/01 Garrigue, P. (2017a) Report No. BPL17-0001 See Appendix 2
ILV - apple and dry pea	ILV	LOQ = 0.01 mg/kg of flurochloridone	QuEChERS GC-MS/MS m/z 311 → 174 m/z 145 → 95	KCP 5.2/02 Hauler, C. (2018a) Report No. S17-08035 See Appendix 2

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Not required	No residue ≥ LOQ are expected in plant matrices

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

An overview on the acceptable methods and possible data gaps for analysis of flurochloridone in animal matrices is given in the following table. Additional methods are presented in Appendix 2.

Table 5.3-4: Validated methods for food of animal origin

Component of residue definition: Flurochloridone				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Animal matrices	-	-	-	No method required (EFSA Journal 2010;8(12):1869)
Animal matrices: (muscle, kidney, fat, milk, egg)	Primary and confirmatory	LOQ = 0.01 mg/kg of flurochloridone	QuEChERS GC-MS/MS m/z 311 → 174 m/z 145 → 95	KCP 5.2/03, Garrigue, P. (2017b) Report No. BPL17-0002 See Appendix 2
ILV - Fat, liver	ILV	LOQ = 0.01 mg/kg of flurochloridone	QuEChERS GC-MS/MS m/z 311 → 174 m/z 145 → 95	KCP 5.2/04, Hauler, C. (2018b) Report No. S17-08036 See Appendix 2

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Not required	No residue ≥ LOQ are expected in animal matrices

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

An overview on the acceptable methods and possible data gaps for analysis of flurochloridone in soil is given in the following table. Additional methods are presented in Appendix 2.

Table 5.3-6: Validated methods for soil matrix

Component of residue definition: Flurochloridone sum of isomers				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Soil	Primary	LOQ = 0.01 mg/kg of flurochloridone	GC-MS	Wolf, S. 2006a Addendum 1 to the DAR, November 2007
Soil	Primary and Confirmatory	LOQ = 0.01 mg/kg of flurochloridone (cis and trans isomers) 0.001 mg/kg for metabolite R406639	HPLC-MS/MS Flurochloridone m/z 312 → 292 m/z 312 → 53 m/z 312 → 89 R406639 m/z 292 → 79 m/z 292 → 64	KCP 5.2/05 Brown, D. (2018) Report No. 39322 See Appendix 2

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

An overview on the acceptable methods and possible data gaps for analysis of flurochloridone in water is given in the following table. Additional methods are presented in Appendix 2.

Table 5.3-7: Validated methods for water matrix

Component of residue definition: Flurochloridone sum of isomers				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Ground, surface and drinking water	Primary	LOQ = 0.05 µg/L of flurochloridone	GC-MS	Wolf, S. 2005 DAR, March 2006
Surface and Drinking Water	Primary and Confirmatory	LOQ = 0.05 µg/L of flurochloridone	GC-MS/MS m/z 311 → 174 m/z 145 → 95	KCP 5.2/06 Garrigue, P. (2017c) Report No.: BPL17-0004 See Appendix 2
ILV - Drinking water	ILV	LOQ = 0.05 µg/L of flurochloridone	GC-MS/MS m/z 311 → 174 m/z 145 → 95	KCP 5.2/07 Hauler, C. (2018c) Report No.: S17-08037 See Appendix 2
Surface and Drinking Water	Primary and Confirmatory	LOQ = 0.05 µg/L of metabolites R42819 and R406639	LC-MS/MS R42819 m/z 278 → 258 m/z 278 → 127 R406639 m/z 294 → 97 m/z 294 → 69	KCP 5.2/08 Garrigue, P. (2017d) Report No.: BPL17-0005 See Appendix 2

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

An overview on the acceptable methods and possible data gaps for analysis of flurochloridone in water is given in the following table. No additional / new methods are presented in this dossier.

Table 5.3-8: Validated methods for air matrix

Component of residue definition: Flurochloridone sum of isomers				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Air	Primary	LOQ = 0.75 µg/m^3 of flurochloridone	GC-MS	Wolf, S. 2006c Addendum 1 to the DAR, November 2007

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

Flurochloridone is classified as toxic (T). According to Regulation 283/2013 and according to SANCO 825/00 rev 8.1 it is necessary to supply a method for determining the residues in body fluids and tissues. Consequently, an analytical method validation for flurochloridone in body tissues was validated by Garrigue, P. (2017b). Furthermore a method validation for the determination of flurochloridone in body fluids was performed by Garrigue, P. (2017e). For details, see Appendix 2.

Table 5.3-9: Validated methods for flurochloridone in animal tissues and body fluids

Component of residue definition: Flurochloridone				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Body fluids	-	-	-	Data Gap in DAR (EFSA Journal 2010;8(12):1869)
Body tissues (muscle and kidney)	Primary and confirmatory	LOQ = 0.01 mg/kg of flurochloridone	QuEChERS GC-MS/MS m/z 311 → 174 m/z 145 → 95	KCP 5.2/03, Garrigue, P. (2017b) Report No. BPL17-0002 See Appendix 2
Body fluids (blood and urine)	Primary and confirmatory	LOQ = 0.01 mg/kg of flurochloridone	QuEChERS GC-MS/MS m/z 311 → 174 m/z 145 → 95	KCP 5.2/09, Garrigue, P. (2017e) Report No. BPL17-0003 See Appendix 2

5.3.2.8 Other studies/ information

5.3.3 No additional / other information.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner*
KCP 5.1.1/01	Gorban, I.	2006	RACER 25 CS (Flurochloridone 250 g/L CS) Determination of Storage Stability and Shelf Life Specification Data of RACER 25 CS Stored at 54°C for 14 Days Report No. F06-07, Sponsor Reference No. 90009450 ADAMA Agan, Ashdod, Israel GLP Unpublished	N	ADM
KCP 5.1.1/02	Ricau, H.	2018	Validation of the analytical methods for the determination of total flurochloridone and free flurochloridone in AG-F8-250 CS Report No. 17-901066-005, Sponsor Reference No. 90021272 ANADIAG Group DEFITRACES, Brindas, France GLP Unpublished	N	ADM
KCP 5.1.2/01	Weber, B.	2012	TEST ITEM: Flurochloridone technical Effect on Survival and Reproduction of *Daphnia magna* in a Semi-Static Test over Three Weeks Report No. D45354, Sponsor Reference No. 90015011 Harlan Laboratories Ltd., Switzerland GLP Unpublished	N	ADM
KCP 5.1.2/02	Liedtke, A.	2012a	TEST ITEM: AG-F8-250 EC (Flurochloridone) Toxicity to *Pseudokirchneriella subcapitata* in a 72 -Hour Algal Growth Inhibition Test Report No. D54231, Sponsor Reference No. 90015423 Harlan Laboratories Ltd., Switzerland GLP Unpublished	N	ADM
KCP 5.1.2/03	Liedtke, A.	2013a	Flurochloridone technical: Toxicity to *Chlamydomonas reinhardtii* in a 72-Hour Algal Growth Inhibition Test Supplemented with Testing for Recovery of Growth Report No. D65727, Sponsor Reference No. 90015442 Harlan Laboratories Ltd., Switzerland GLP Unpublished	N	ADM

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner*
KCP 5.1.2/04	Liedtke, A.	2013b	Flurochloridone technical: Toxicity to *Chlorella vulgaris* in a 72-Hour Algal Growth Inhibition Test Report No. D65738, Sponsor Reference No. 90015443 Harlan Laboratories Ltd., Switzerland GLP Unpublished	N	ADM
KCP 5.1.2/05	Liedtke, A.	2013c	Flurochloridone technical: Toxicity to *Navicula pelliculosa* in a 72-Hour Algal Growth Inhibition Test Supplemented with Testing for Recovery of Growth Report No. D65740, Sponsor Reference No. 90015444 Harlan Laboratories Ltd., Switzerland GLP Unpublished	N	ADM
KCP 5.1.2/06	Liedtke, A.	2013d	Flurochloridone (Trans isomer): Toxicity to *Desmodesmus subspicatus* in a Pulse Exposure Growth Inhibition Test Supplemented with Testing for Recovery of Growth Report No. D59890, Sponsor Reference No. 90015421 Harlan Laboratories Ltd., Switzerland GLP Unpublished	N	ADM
KCP 5.1.2/07	Liedtke, A.	2013e	Flurochloridone (Trans isomer): Toxicity to *Desmodesmus subspicatus* in a Pulse Exposure Growth Inhibition Test Supplemented with Testing for Recovery of Growth Report No. D65547, Sponsor Reference No. 90015432 Harlan Laboratories Ltd., Switzerland GLP Unpublished	N	ADM
KCP 5.1.2/08	Scheerbaum, D.	2013a	Flurochloridone technical: Alga, Growth Inhibition Test with *Pseudokirchneriella subcapitata*, 72 hours Report No. SPO15371, Sponsor Reference No. 90015448 Dr.U.Noack-Laboratorien, Germany GLP Unpublished	N	ADM
KCP 5.1.2/09	Scheerbaum, D.	2013b	Flurochloridone technical: Alga, Growth Inhibition Test with *Nitzschia communis*, 72 hours Report No. SNC15371, Sponsor Reference No. 90015449 Dr.U.Noack-Laboratorien, Germany GLP Unpublished	N	ADM
KCP 5.1.2/10	Scheerbaum, D.	2013c	Flurochloridone technical: Alga, Growth Inhibition Test with *Synechococcus leopoliensis*, 72 hours Report No. SSL15371, Sponsor Reference No. 90015450	N	ADM

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*
			Dr.U.Noack-Laboratorien, Germany GLP Unpublished		
KCP 5.1.2/11	Scheerbaum, D.	2013d	Flurochloridone technical: Alga, Growth Inhibition Test with *Chromulina nebulosa*, 72 hours Report No. SCN15371, Sponsor Reference No. 90016462 Dr.U.Noack-Laboratorien, Germany GLP Unpublished	N	ADM
KCP 5.1.2/12	Scheerbaum, D.	2013e	Flurochloridone technical: Alga, Growth Inhibition Test with *Ankistrodesmus falcatus*, 72 hours Report No. SAF15371, Sponsor Reference No. 90016463 Dr.U.Noack-Laboratorien, Germany GLP Unpublished	N	ADM
KCP 5.1.2/13	Wenzel, A.	2015a	Freshwater Alga, Growth Inhibition Test Flurochloridone (technical): *Desmodesmus subspicatus* Toxicity Test - Testing for Recovery of Growth Report No. ADM-003/4-10/B, Sponsor Reference No. 90016481 Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Germany GLP Unpublished	N	ADM
KCP 5.1.2/14	Liedtke, A.	2012b	TEST ITEM: AG-F8-250 EC (Flurochloridone) Toxicity to the Aquatic Higher Plant *Lemna gibba* in a 7-Day Growth Inhibition Test Report No. D54308, Sponsor Reference No. 90015431 Harlan Laboratories Ltd., Switzerland GLP Unpublished	N	ADM
KCP 5.1.2/15	Wenzel, A.	2015b	Macrophyte Pulse Exposure Growth Inhibition Test Flurochloridone (trans-isomer): Sediment-free *Lemna minor* Toxicity Test - testing for recovery of growth Report No. ADM-005/4-11/I, Sponsor Reference No. 90016482 Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Germany GLP Unpublished	N	ADM
KCP 5.1.2/16	Wenzel, A.	2015c	Macrophyte Pulse Exposure Growth Inhibition Test Flurochloridone (trans-isomer): Sediment-free *Myriophyllum spicatum* Toxicity Test - testing for recovery of growth Report No. ADM-005/4-13/K, Sponsor Reference No. 90016483 Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Germany	N	ADM

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner*
			GLP Unpublished		
KCP 5.1.2/17	Molitor, A.M.	2017	AG-F8-250 EC (Flurochloridone 250 EC) - Assessment of Effects on the Adult Honey Bee, *Apis mellifera* L., in a 10 Day Chronic Feeding Test under Laboratory Conditions Report No. S17-00282, Sponsor Reference No. 90020495 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH GLP Unpublished	N	ADM
KCP 5.1.2/18	Molitor, A.M.	2018	AG-F8-250 EC (Flurochloridone 250 EC) - Honey Bee (*Apis mellifera* L.) 22 Day Larval Toxicity Test (Repeated Exposure) Report No. S17-00318, Sponsor Reference No. 90020496 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH GLP Unpublished	N	ADM
KCP 5.2/01	Garrigue, P.	2017a	Validation of Residue Analytical Method for Determination of Flurochloridone in Plant Matrices Report No.: BPL17-0001, Sponsor Reference No. 90020483 SGS Multilab, Laboratory of Rouen , France GLP Unpublished	N	ADM
KCP 5.2/02	Hauler, C.	2018a	Independent Laboratory Validation of an Analytical Method for the Determination of Flurochloridone in Different Matrices of Plant Origin Report No.: S17-08035; Sponsor Reference No. 90021292 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Germany GLP Unpublished	N	ADM
KCP 5.2/03	Garrigue, P.	2017b	Validation of Residue Analytical Method for Determination of Flurochloridone in Animal Matrices Report No.: BPL17-0002, Sponsor Reference No. 90020484 SGS Multilab, Laboratory of Rouen , France GLP Unpublished	N	ADM
KCP 5.2/04	Hauler, C.	2018b	Independent Laboratory Validation of an Analytical Method for the Determination of Flurochloridone in Different Matrices of Animal Origin Report No.: S17-08036 and amendment 1; Sponsor Reference No. 90021293 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Germany GLP	N	ADM

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner***
			Unpublished		
KCP 5.2/05	Brown, D.	2018	Flurochloridone (2 isomers) and Metabolite, R406639: Non-radiolabelled Environmental Fate Method Validation in Three Soils Report No.: 39322 and amendment 1, Sponsor Reference No. 90020530 Charles River Laboratories Edinburgh Ltd, UK GLP Unpublished	N	ADM
KCP 5.2/06	Garrigue, P.	2017c	Validation of Residue Analytical Method for Determination of Flurochloridone in Water Report No.: BPL17-0004 and amendment 1, Sponsor Reference No. 90020486 SGS Multilab, Laboratory of Rouen , France GLP Unpublished	N	ADM
KCP 5.2/07	Hauler, C.	2018c	Independent Laboratory Validation of an Analytical Method for the Determination of Flurochloridone in Water Report No.: S17-08037; Sponsor Reference No. 90021294 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Germany GLP Unpublished	N	ADM
KCP 5.2/08	Garrigue, P.	2017d	Validation of Residue Analytical Method for Determination of Flurochloridone Metabolites R42819 and R406639 in Water Report No.: BPL17-0005 and amendment 1, Sponsor Reference No. 90020487 SGS Multilab, Laboratory of Rouen , France GLP Unpublished	N	ADM
KCP 5.2/09	Garrigue, P.	2017e	Validation of Residue Analytical Method for Determination of Flurochloridone in Body Fluid Matrices Report No.: BPL17-0003, Sponsor Reference No. 90020485 SGS Multilab, Laboratory of Rouen , France GLP Unpublished	N	ADM

*The sponsor company (ADM, ADAMA Agan Ltd.) is a member of ADAMA Agricultural Solutions.

List of data referred to flurochloridone 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**
IIIA 5.2.4/01	Guzikevich, G	2003	Flurochloridone technical five lots analysis and method validation Report no. 03-03, Agan report no. 90006021, October 08, 2003 GLP Unpublished	N	ADM
IIA 4.2.1/01	Gorban, I.	2005	RACER 25 C- Analysis of Cis/Trans Isomers on Active Ingredient and Method Validation Report No. F05-11/6, Sponsor Reference No. 90008149 ADAMA Agan, Ashdod, Israel GLP Unpublished	N	ADM
IIA 4.2.1/01	Anspach, T.	2002	Flurochloridone: Validation of the DFG method S19 (Extended revision) for the determination of residues of flurochloridone in/on sunflower seeds Report No.: SYN-0207V, Sponsor Reference No. 90005208 Dr. Specht & Partner GLP Unpublished	N	ADM
IIA 4.2.1/03	Wolf, S.	2003	Independent laboratory validation (ILV) of a residue analytical method for flurochloridone in sunflower seeds Report No.: 848299, Sponsor Reference No. 90006028 RCC Ltd. GLP Unpublished	N	ADM
IIA 4.2.2/01	Wolf, S.	2006a	Development and validation of a residue analytical method for Flurochloridone in soil. Report No.: A47272, Sponsor Reference No. 90009077 RCC Ltd. GLP Unpublished	N	ADM
IIA 4.2.3/02	Wolf, S.	2005	Development and validation of a residue analytical method for Flurochloridone in drinking, ground and surface water Report No.: 857308, Sponsor Reference No. 90007711 RCC Ltd. GLP Unpublished	N	ADM
IIA 4.2.4/01	Wolf, S.	2006c	Development and validation of a residue analytical method for flurochloridone in air Report No.: A81630, Sponsor Reference No. 90009363 RCC Ltd.	N	ADM

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

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for flurochloridone

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

A 2.1.1.1 Description of analytical methods for the determination of residues in water, buffer, field dilution of the preparation

A 2.1.1.1.1 Analytical method 1

Method validation for the determination of flurochloridone in plant protection product AG-F8-250 CS for content determination during storage stability testing of AG-F8-250 CS.

A 2.1.1.1.1.1 Method validation

Comments of zRMS:	Please refer to the point 5.2.1.1/01 (KCP 5.1.1/01).

Reference:	**2.1.1.1.1/01 (KCP 5.1.1/01)**
Report	Gorban, I. (2006) RACER 25 CS (Flurochloridone 250 g/L CS) Determination of Storage Stability and Shelf Life Specification Data of RACER 25 CS Stored at 54°C for 14 Days Report No. F06-07, Sponsor Reference No. 90009450
Guideline(s):	SANCO/3030/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analysis of the active ingredient flurochloridone in AG-F8-250 CS was done by gas chromatograph (GC) with FID detection using internal standard technique. The analytical method was fully validated for both isomers (cis and trans) of flurochloridone in study 05-11/6, for linearity, precision, accuracy, non-analyte interference, and specificity. Linearity and non-analyte interference tests were carried for the current study again.

Specimen preparation
Duplicate quantitative solutions were prepared. About 600 mg of Flurochloridone 25 SC and about 100 mg of DMBP standard were placed into a small Erlenmeyer flask, then 3mL water, 20mL acetone, and 2g of sodium sulfate anhydrous were added. The solutions were sonicated for about 10 minutes.

Equipment for flurochloridone determination

GC-FID system	Hewlett Packard GC system equipped with an FID detector, automatic injector and HP GC Chemstation Software
Column:	50% phenyl, 50% methyl silicone, capillary column, 30m, film thickness 0.25µm
Carrier gas:	Helium, 1 mL/min
Injector temperature:	260 °C
Detector temperature:	280 °C
Oven temperature:	260 °C
Run time:	15 min

Injection Volume:	1 µL
Split ratio:	1:50
Retention time(s):	Approx. 4.2 min (trans-flurochloridone), 4.3 min (cis-flurochloridone), 7.7 min (dimethoxy benzophenone)

Validation - Results and discussions

Table A 1: Methods suitable for the determination of active substance flurochloridone in plant protection product AG-F8-250 CS

<table>
<tr><th></th><th>Flurochloridone</th></tr>
<tr><td>Principle of method</td><td>GC with FID detection using internal standard technique</td></tr>
<tr><td>Linearity
(linear between mg/L / % range of the declared content)
(correlation coefficient, expressed as r)</td><td>A series of four standard stock solutions were prepared in acetone, with internal standard. The linearity was determined with four calibration standards injected in triplicate in the range of interest (1mg/mL to 2.75 mg/mL for cis-flurochloridone; 2.75 mg/mL to 8.5 mg/mL for trans-flurochloridone). The calibrations were linear in the following ranges with the following correlation coefficients r:
Trans-flurochloridone: Y = $6.56311e^{-1}$ x – $2.50828e^{-3}$, r = 0.99998
Cis-flurochloridone: Y = $6.49267e^{-1}$ x + $1.03345e^{-3}$, r = 0.99996</td></tr>
<tr><td>Precision – Repeatability Mean
from Report No. 05-11/6
n = 30
(%RSD)</td><td>The relative standard deviation (RSD) for the whole procedure of sample preparation and measurement of 10 independent samples of the test item, each injected in triplicate, was found to be:
<table>
<tr><td>Active ingredient</td><td>Mean concentration of analyte in the test item (%, (w/w))</td><td>RSD (%)</td><td>Proposed acceptable RSD for the concentration of analyte (Horwitz) (%) (1)</td></tr>
<tr><td>Trans-flurochloridone</td><td>17.38</td><td>0.54</td><td>1.74</td></tr>
<tr><td>Cis-flurochloridone</td><td>5.01</td><td>1.66</td><td>2.10</td></tr>
</table></td></tr>
<tr><td>Accuracy
from Report No. 05-11/6
n = 4
(% Recovery)</td><td>Accuracy was determined by a recovery fortification of known amounts of the analytes to four sample solutions of the formulation. Four recovery determinations were performed:
<table>
<tr><td>Recovery Level (% (w/w))</td><td>Cis-flurochloridone Recovery (%)</td></tr>
<tr><td>0.130</td><td>100.1</td></tr>
<tr><td>0.453</td><td>97.9</td></tr>
<tr><td>0.797</td><td>97.0</td></tr>
<tr><td>1.066</td><td>98.1</td></tr>
</table>
<table>
<tr><td>Recovery Level (% (w/w))</td><td>Trans-flurochloridone (%)</td></tr>
<tr><td>1.209</td><td>99.96</td></tr>
<tr><td>2.165</td><td>101.9</td></tr>
<tr><td>3.085</td><td>101.4</td></tr>
<tr><td>3.975</td><td>101.6</td></tr>
</table></td></tr>
<tr><td>Interference/ Specificity</td><td>The blank samples showed no relevant interferences with the signals of the active ingredient, hence the specificity of the method is confirmed.
Confirmation of analyte identification was done by GC-MS. The similarity of the mass spectra from standard and sample solutions confirms the identity of the active ingredient peak.</td></tr>
<tr><td>Comment</td><td>Acceptable</td></tr>
</table>

Conclusion

The analytical method for the active ingredient flurochloridone determination in AG-F8-250 CS was fully validated according to SANCO/3030/99 rev. 4, 11 July 2000.
The results obtained prove that this method is suitable for the detection and quantitation of the active substance flurochloridone in the formulation AG-F8-250 CS.

Gorban, I. (2006)

A 2.1.1.1.2 Analytical method 2

Method validation for the determination of total flurochloridone and free flurochloridone in plant protection product AG-F8-250 CS.

A 2.1.1.1.2.1 Method validation

Comments of zRMS:	Please refer to the point 5.2.1.1/02 (KCP 5.1.1/02).

Reference:	**2.1.1.1.2.1/01 (KCP 5.1.1/02)**
Report	Ricau, H. (2018) Validation of the analytical methods for the determination of total flurochloridone and free flurochloridone in AG-F8-250 CS Report No. 17-901066-005, Sponsor Reference No. 90021272
Guideline(s):	SANCO/3030/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analysis of total flurochloridone and free flurochloridone in AG-F8-250 CS was done by gas chromatograph (GC) with FID detection using internal standard technique. The analytical method was fully validated for total flurochloridone for specificity, linearity, accuracy, precision and reproducibility of the method. The analytical method was fully validated for free flurochloridone for specificity, linearity, precision and reproducibility of the method.

Specimen preparation for total flurochloridone
A quantity of 0.22 g of the test item was weighed into a 100 mL volumetric flask and a volume of 10 mL of water was added. The solution was manually homogenised until the complete disappearance of deposit. A volume of 2 mL of the Internal Standard solution (8 mg/mL of 4'-dimetoxybenzophenone in acetone) was added and the volume was made up with acetone. The solution was treated with ultrasounds during 20 minutes.
Preparation of the formulation blank solution for total flurochloridone
A quantity of 0.17 g of the formulation blank was weighed into a 100-mL volumetric flask and volumes of 10 mL of water and 2 mL of the Internal Standard solution were added. The volume was made up with acetone. The solution was treated with ultrasounds during 20 minutes.
Preparation of the solutions for the accuracy for total flurochloridone
Quantities of 10 mg of the reference item of flurochloridone and 34 mg of the formulation blank were weighed into a 20-mL volumetric flask. Volumes of 2 mL of water and 0.4 mL of the Internal Standard solution were added. The volume was made up with acetone. The solution was treated with ultrasounds during 20 minutes. An identical accuracy solution was prepared.

Specimen preparation for free flurochloridone
A quantity of 0.5 g of the test item was weighed into a 60 mL glass flask then a volume of 3 mL of water and 50 mL of hexane were added. The solution was put under orbital magnetic stirring during 5 minutes at a rotation of 60 rpm. A volume of 10 mL of the previous solution was taken into a 20 mL volumetric flask, a volume of 0.1 mL of the Internal Standard solution (8 mg/mL of 4'-dimetoxybenzophenone in acetone) was added and the volume was made up with acetone. The solution was homogenised.
Preparation of the formulation blank solution for free flurochloridone
A quantity of 0.39 g of the formulation blank was weighed into a 60-mL glass flask and the experimental procedure was followed like for the preparation of the test item.

Equipment for total flurochloridone and free flurochloridone determination

GC-FID system	Bruker 436 GC with Bruker CP 8400 autosampler equipped with an FID detector (total

	flurochloridone) or Thermo Scientific TRACE 1310 GC with Thermo Scientific SSL autosampler equipped with an FID detector (free flurochloridone)
Column:	Varian 5 MS, 30m x 0.25 mm, film thickness 0.25µm
Carrier gas:	Helium, 1.2 mL/min
Injector temperature:	260 °C
Detector temperature:	280 °C
Oven temperature:	Initial 80 °C, hold for 1.5 min Heat rate 20 °C/min to 250 °C, hold for 4 min Heat rate 25 °C/min to 300 °C, hold for 4 min
Run time:	20 min
Injection Volume:	1 µL
Retention time(s):	Approx. 11.1 min (flurochloridone I), 11.2 min (flurochloridone I), 13.2 min (Internal standard)

Validation - Results and discussions

Table A 2: Methods suitable for the determination of total flurochloridone and free flurochloridone in plant protection product AG-F8-250 CS

<table>
<tr><th></th><th>Total Flurochloridone</th><th>Free Flurochloridone</th></tr>
<tr><td>Principle of method</td><td colspan="2">GC with FID detection using internal standard technique</td></tr>
<tr><td>Linearity
(linear between mg/L / % range of the declared content)
(correlation coefficient, expressed as r)</td><td>A series of five calibration standard solutions were prepared in water/acetone (10/90 v/v), with internal standard. The linearity was determined with five calibration standards injected in duplicate ranging from 221.27 mg/L to 726.78 mg/L (between 50% and 150% of the reference items). The calibrations were linear with correlation coefficients r >0.99:
Y = 6.68E^{-03} x – 2.13E^{-01}, r = 0.9997</td><td>A series of five calibration standard solutions were prepared in hexane/acetone (50/50 v/v), with internal standard. The linearity was determined with five calibration standards injected in duplicate ranging from 21.79 mg/L to 73.60 mg/L (between 50% and 150% of the reference items). The calibrations were linear with correlation coefficients r >0.99:
Y = 2.02E^{-02} x – 1.40E^{-01}, r = 0.9991</td></tr>
<tr><td>Precision – Repeatability
Mean
n = 10
(%RSD)</td><td>The relative standard deviation (RSD) for the whole procedure of sample preparation and measurement of 5 independent samples of the test item, each injected in duplicate, was found to be:
<table><tr><th>Mean concentration of analyte in the test item (g/L)</th><th>RSD (%)</th><th>Proposed acceptable RSD (Horwitz) (%) (1)</th></tr><tr><td>240*</td><td>0.45</td><td>1.69</td></tr></table>* equivalent to 21.8 % (w/w)</td><td>The relative standard deviation (RSD) for the whole procedure of sample preparation and measurement of 5 independent samples of the test item, each injected in duplicate, was found to be:
<table><tr><th>Mean concentration of analyte in the test item (g/L)</th><th>RSD (%)</th><th>Proposed acceptable RSD (Horwitz) (%) (1)</th></tr><tr><td>10.69</td><td>2.55</td><td>2.69</td></tr></table>* equivalent to 0.971 % (w/w)</td></tr>
<tr><td>Accuracy
n = 4
(% Recovery)</td><td>Accuracy was determined by a recovery fortification of known amounts of the analytes to two formulation blank solutions, each injected in duplicate.
<table><tr><th>Recovery Level (mg/L)*</th><th>Recovery (%)</th><th>Mean recovery (%)</th></tr><tr><td rowspan="2">455.30</td><td>100.2</td><td rowspan="2">100.0</td></tr><tr><td>99.7</td></tr><tr><td rowspan="2">468.79</td><td>99.2</td><td rowspan="2">99.1</td></tr><tr><td>99.1</td></tr></table>* equivalent to 29.4 % (w/w) (nominal)</td><td>The accuracy of the free flurochloridone was not performed because this parameter cannot be adequately measured with the free flurochloridone method.
No free flurochloridone was detected after the addition of a known quantity of reference item of flurochloridone on the test item AG-F8-250 CS and after extraction according to the free flurochloridone method. It is assumed that the reference item is incorporated into the capsules during the preparation and is therefore not measured with the free flurochloridone method which intend to measure only releasing active ingredient.</td></tr>
</table>

	Total Flurochloridone	Free Flurochloridone
	Recovery results are in the range 98% - 102%.	Consequently, the recovery of the free flurochloridone is impossible to calculate.
Interference/ Specificity	Retention times for each analyte match between reference item and test item, confirming the identity of the analyte. No interference was observed in solvent blank, formulation blank and test item at the retention times of each analyte. Therefore, the analytical method showed a good specificity for analysis of total flurochloridone and free flurochloridone in the product of AG-F8-250 CS.	
Comment	Acceptable	Acceptable

Conclusion

The analytical method for total flurochloridone and free flurochloridone determination in AG-F8-250 CS was fully validated according to SANCO/3030/99 rev. 4, 11 July 2000.
The results obtained prove that this method is suitable for the detection and quantitation of total flurochloridone and free flurochloridone in the formulation AG-F8-250 CS.

Ricau, H. (2018)

A 2.1.1.2 Description of analytical methods for the determination of impurities in the preparation

A 2.1.1.2.1 Analytical method 1

No additional / new methods are presented in this dossier for determination of flurochloridone impurities in the preparation.

A 2.1.1.3 Description of analytical methods for the determination of flurochloridone residues in crops (Residues) (KCP 5.1)

A 2.1.1.3.1 Analytical method

No additional / new risk assessment methods are presented in this dossier for determination of flurochloridone residues in crops.

A 2.1.1.4 Description of analytical methods for the determination of flurochloridone residues in aquatic media (Ecotoxicology) (KCP 5.1)

A 2.1.1.4.1 Analytical methods

Different analytical methods to determine concentrations of flurochloridone in ecotoxicology media for risk assessment have been developed and validated in the following study reports by

- Weber, B. (2012), Report No. D45354, Daphnia toxicity study OECD 211 (Flurochloridone technical), see KCP 5.1.2/01
- Liedtke, A. (2012a), Report No. D54231, Algae toxicity study OECD 201 (AG-F8-250 EC), see KCP 5.1.2/02
- Liedtke, A. (2013a), Report No. D65727, Algae toxicity study OECD 201 (Flurochloridone technical), see KCP 5.1.2/03
- Liedtke, A. (2013b), Report No. D65738, Algae toxicity study OECD 201 (Flurochloridone technical), see KCP 5.1.2/04
- Liedtke, A. (2013c), Report No. D65740, Algae toxicity study OECD 201 (Flurochloridone technical), see KCP 5.1.2/05
- Liedtke, A. (2013d), Report No. D59890, Algae toxicity study OECD 201 (Flurochloridone trans isomer), see KCP 5.1.2/06
- Liedtke, A. (2013e), Report No. D65547, Algae toxicity study OECD 201 (Flurochloridone trans isomer), see KCP 5.1.2/07
- Scheerbaum, D. (2013a), Report No. SPO15371, Algae toxicity study OECD 201 (Flurochloridone technical), see KCP 5.1.2/08
- Scheerbaum, D. (2013b), Report No. SNC15371, Algae toxicity study OECD 201 (Flurochloridone technical), see KCP 5.1.2/09
- Scheerbaum, D. (2013c), Report No. SSL15371, Algae toxicity study OECD 201 (Flurochloridone technical), see KCP 5.1.2/10
- Scheerbaum, D. (2013d), Report No. SCN15371, Algae toxicity study OECD 201 (Flurochloridone technical), see KCP 5.1.2/11
- Scheerbaum, D. (2013e), Report No. SAF15371, Algae toxicity study OECD 201 (Flurochloridone technical), see KCP 5.1.2/12
- Wenzel, A. (2015a), Report No. ADM-003/4-10/B, Algae toxicity study OECD 201 (Flurochloridone technical), see KCP 5.1.2/13
- Liedtke, A. (2012b), Report No. D54308, Lemna toxicity study OECD 221 (AG-F8-250 EC), see KCP 5.1.2/14
- Wenzel, A. (2015b), Report No. ADM-005/4-11/I, Lemna toxicity study OECD 221 (Flurochloridone trans isomer), see KCP 5.1.2/15
- Wenzel, A. (2015c), Report No. ADM-005/4-13/K, Myriophyllum toxicity study OECD 238 (Flurochloridone trans isomer), see KCP 5.1.2/16
- Molitor, A.M. (2017), Report No. S17-00282, Bee toxicity Study OECD guideline proposal (2016) (AG-F8-250 EC), see KCP 5.1.2/17

- Molitor, A.M. (2018), Report No. S17-00318, Larval toxicity Study OECD 239 (2016) (AG-F8-250 EC), see KCP 5.1.2/18

A 2.1.1.4.2 Analytical method - Flurochloridone residues in OECD 211 test medium

A 2.1.1.4.2.1 Method validation

Comments of zRMS:	The method is satisfactorily validated in accordance with SANCO/3029/99 rev. 4. for the determination of the concentration of flurochloridone in test medium with a limit of quantification (LOQ) of 1.02 mg/L.

Reference: **2.1.1.4.2.1/01 (KCP 5.1.2/01)**

Report: Weber, B. (2012)
TEST ITEM: Flurochloridone technical.
Effect on Survival and Reproduction of *Daphnia magna* in a Semi-Static Test over Three Weeks
Report No. D45354, Sponsor Reference No. 90015011

Guideline(s): Not mentioned

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

An analytical method for flurochloridone in OECD 211 test medium was validated. The method involves dilution with methanol/test water (v/v; 1/1) if necessary, prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (LC-MS/MS) using external calibration. The limit of quantification (LOQ) of 1.02 mg/L of test item was validated.

Specimen preparation
Test samples and control samples were thawed at room temperature for two hours and shaken manually to obtain homogeneous sample solutions. If necessary, they were further diluted into the calibration range with methanol/test water (v/v; 1/1) prior to analysis by LC-MS/MS.

Equipment for flurochloridone determination

LC-MS/MS system	High pressure gradient HPLC system, consisting of two Shimadzu LC-10AD pumps, CTC PAL autosampler, Sciex API 5000 triple quadrupole mass spectrometer
Column:	Inertsil ODS-3; 2.1 mm x 33 mm; particle size 3 µm
Column temperature:	Not stated
Injection Volume:	5 µL
Mobile phases:	Eluent A: water/methanol/formic acid (95:5:0.1 v/v/v) + 5mM ammonium formate Eluent B: water/methanol/formic acid (5:95:0.1 v/v/v) + 5mM ammonium formate
Gradient:	

Time [min]	Eluent A	Eluent B	Flow rate [mL/min]
0.0	60	40	0.4
0.5	60	40	0.4
2.0	10	90	0.4
3.0	10	90	0.4
3.1	60	40	0.4
4.0	60	40	0.4

Retention time(s)	Approx. 2 min (flurochloridone)
Ionisation type:	TurboIonSpray (ESI), positive mode
Ion mass transition monitored (m/z) for flurochloridone :	312 → 292, collision energy = 32 eV (Quantifier)
	No qualifier ion monitored

Results and discussions

Table A 3: Recovery results of flurochloridone in OECD 211 test medium

Matrix	Fortification level of test item (mg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery* (%)	Overall RSD* (%)
OECD 211 test medium	1.02	98, 101, 99, 96, 98	98	2	5	101	4
	10.2	106, 103, 101, 102, 110	104	4	5		

* Calculated from reported individual recovery values

RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 4: Characteristics for the analytical method used for validation of flurochloridone in OECD 211 test medium

	Flurochloridone
Specificity	A highly specific detection system was used (HPLC-MS/MS). The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No interferences at the retention time of the analyte were detected in any untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of calibration standard solutions were prepared in methanol/water (1/1, v/v). The linearity was determined with eight solvent standards ranging from 0.0162 mg/L to 1.08 mg/L of test item. A power calibration function was obtained with coefficients of determination $R^2 \geq 0.990$: $Y = 2947030x^{0.9235}$, $R^2 = 0.9976$
Assessment of matrix effects is presented	Not assessed
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level* and was successfully established at 1.02 mg/L of test item.

* Note that the LOQ defined in the study report was derived from the lowest calibration solution as 0.0324 mg test item /L taking into account the sample preparation factor.

Conclusion

The method was found to be valid for the determination of flurochloridone in OECD 211 test medium used in ecotoxicology studies at fortification levels of 1.02 mg/L of test item and 10.2 mg/L of test item, with a limit of quantification (LOQ) of 1.02 mg/L, in accordance to SANCO/3029/99 rev. 4 requirements.

Weber, B. (2012)

A 2.1.1.4.3 Analytical method - Flurochloridone residues in OECD 201 test medium

A 2.1.1.4.3.1 Method validation 1

Comments of zRMS:	The method is validated in accordance with SANCO/3029/99 rev. 4. for the determination of the concentration of flurochloridone in test medium with a limit of quantification (LOQ) of 2.41 µg/L. In the report of Liedtke, A. (Harlan Study Number: D54231) it is stated that *"On the basis of a method provided by the sponsor, an analytical method was adapted and implemented by Harlan Laboratories Ltd.* *These experiments were not performed according to the regulations of GLP."*

Reference: **2.1.1.4.3.1/01 (KCP 5.1.2/02)**

Report: Liedtke, A. (2012a)
TEST ITEM: AG-F8-250 EC (Flurochloridone)
Toxicity to *Pseudokirchneriella subcapitata* in a 72 -Hour Algal Growth

Inhibition Test
Report No. D54231, Sponsor Reference No. 90015423

Guideline(s): Not mentioned

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

An analytical method for flurochloridone in OECD 201 test medium was validated. The method involves dilution with methanol prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (LC-MS/MS) using external calibration. The limit of quantification (LOQ) of 2.41 µg/L of test item was validated, corresponding to 0.595 µg/L of flurochloridone.

Specimen preparation
Treatment samples and control samples were thawed at room temperature for 1.5 hours and shaken manually to obtain homogeneous sample solutions. All samples were centrifuged if necessary (3500 rpm, 5 min), and diluted by a factor of two with methanol prior to analysis by LC-MS/MS.

Equipment for flurochloridone determination

LC-MS/MS system	High pressure gradient HPLC system, consisting of two Shimadzu LC-10AD pumps, CTC PAL autosampler, Sciex API 4000 triple quadrupole mass spectrometer
Column:	Inertsil ODS-3; 2.1 mm x 33 mm; particle size 3 µm
Column temperature:	Not stated
Injection Volume:	50 µL
Mobile phases:	Eluent A: water/methanol/formic acid (95:5:0.1 v/v/v) + 5mM ammonium formate Eluent B: water/methanol/formic acid (5:95:0.1 v/v/v) + 5mM ammonium formate
Gradient:	

Time [min]	Eluent A	Eluent B	Flow rate [mL/min]
0.0	60	40	0.4
0.5	60	40	0.4
2.0	10	90	0.4
2.5	10	90	0.4
2.6	100	0	0.4
3.0	100	0	0.4
3.1	60	40	0.4
4.0	60	40	0.4

Retention time(s)	Approx. 2.3 min (flurochloridone)
Ionisation type:	TurboIonSpray (ESI), positive mode
Ion mass transition monitored (m/z) for flurochloridone :	312 → 292, collision energy = 32 eV (Quantifier)
	No qualifier ion monitored

Results and discussions

Table A 5: Recovery results of flurochloridone in OECD 201 test medium (non-centrifuged)

Matrix	Fortification level of test item (µg/L)	Fortification level of active ingredient (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery* (%)	Overall RSD* (%)
OECD 201 test medium	2.41	0.595	114, 115, 106, 116, 120	114	4	5	113	4
	20.5	5.06	111, 108, 114, 109, 115	111	3	5		

* Calculated from reported individual recovery values
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 115%, which is only just outside SANCO/3029/99 rev. 4 requirements (70-110%), and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 6: Recovery results of flurochloridone in OECD 201 test medium (centrifuged)

Matrix	Fortification level of test item (µg/L)	Fortification level of active ingredient (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD* (%)
OECD 201 test medium	2.41	0.595	107, 107	107	-	2	105	3
	20.5	5.06	100, 105	103	-	2		

* Calculated from reported individual recovery values
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 7: Characteristics for the analytical method used for validation of flurochloridone in OECD 201 test medium

	Flurochloridone
Specificity	A highly specific detection system was used (HPLC-MS/MS). The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No interferences at the retention time of the analyte were detected in any untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of calibration standard solutions were prepared in methanol/water (1/1, v/v). The linearity was determined with eight solvent standards ranging from 0.121 µg/L to 39.3 µg/L. The calibrations were found linear with coefficients of determination $R^2 \geq 0.990$ (1/Y weighing): $Y = 10584x - 29.826$, $R^2 = 0.9993$
Assessment of matrix effects is presented	Not assessed
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level* and was successfully established at 2.41 µg/L of test item (0.595 µg/L of flurochloridone).

* Note that the LOQ defined in the study report was derived from the lowest calibration solution as 0.242 µg a.i. /L taking into account the sample preparation factor

Conclusion

The method was found to be valid for the determination of flurochloridone in OECD 201 test medium used in ecotoxicology studies at fortification levels of 2.41 µg/L of test item and 20.05 µg/L of test item, with a limit of quantification (LOQ) of 2.41 µg/L of test item (0.595 µg/L of flurochloridone), in accordance to SANCO/3029/99 rev. 4 requirements.

Liedtke, A. (2012a)

A 2.1.1.4.3.2 Method validation 2

Comments of zRMS:	The method is validated in accordance with SANCO/3029/99 rev. 4. for the determination of the concentration of flurochloridone in test medium with a limit of quantification (LOQ) of 13.5 µg/L. In the report of Liedtke, A. (Harlan Study Number: D65727) it is stated that *"On the basis of a method provided by the sponsor, an analytical method was adapted and implemented by Harlan Laboratories Ltd.* *These experiments were not performed according to the regulations of GLP."*

Reference: **2.1.1.4.3.2/01 (KCP 5.1.2/03)**

Report: Liedtke, A. (2013a)
Flurochloridone technical: Toxicity to *Chlamydomonas reinhardtii* in a 72-Hour Algal Growth Inhibition Test Supplemented with Testing for

Recovery of Growth
Report No. D65727, Sponsor Reference No. 90015442

Guideline(s): Not mentioned

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

An analytical method for flurochloridone in OECD 201 test medium was validated. The method involves dilution with test water/methanol (v/v; 1/1), prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (LC-MS/MS) using external calibration. The limit of quantification (LOQ) of 13.5 µg/L of test item was validated.

Specimen preparation

Test samples and control samples were thawed at room temperature for 30 minutes and shaken manually to obtain homogeneous sample solutions. If necessary, samples were centrifuged (3500 rpm, 5 min) before being further diluted into the calibration range with test water/methanol (v/v; 1/1), prior to analysis by LC-MS/MS.

Equipment for flurochloridone determination

LC-MS/MS system	High pressure gradient HPLC system, consisting of two Shimadzu LC-10AD pumps, CTC PAL autosampler, Sciex API 4000 triple quadrupole mass spectrometer
Column:	Inertsil ODS-3; 2.1 mm x 33 mm; particle size 3 µm
Column temperature:	Not stated
Injection Volume:	20 µL
Mobile phases:	Eluent A: water/methanol/formic acid (95:5:0.1 v/v/v) + 5mM ammonium formate Eluent B: water/methanol/formic acid (5:95:0.1 v/v/v) + 5mM ammonium formate
Gradient:	

Time [min]	Eluent A	Eluent B	Flow rate [mL/min]
0.0	60	40	0.4
0.5	60	40	0.4
2.0	10	90	0.4
2.5	10	90	0.4
2.6	100	0	0.4
3.0	100	0	0.4
3.1	60	40	0.4
4.0	60	40	0.4

Retention time(s)	Approx. 2 min (flurochloridone)
Ionisation type:	TurboIonSpray (ESI), positive mode
Ion mass transition monitored (m/z) for flurochloridone :	312 → 292, collision energy = 32 eV (Quantifier)
	No qualifier ion monitored

Results and discussions

Table A 8: Recovery results of flurochloridone in OECD 201 test medium (non-centrifuged)

Matrix	Fortification level of test item (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery* (%)	Overall RSD* (%)
OECD 201 test medium	13.5	100, 114, 108, 114, 115	110	6	5	110	6
	206	97, 114, 110, 112, 113	109	6	5		

* Calculated from reported individual recovery values
Recoveries fortified at 13.5 µg/L and 206 µg/L were diluted by 2 and 51 respectively for analysis
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 9: Recovery results of flurochloridone in OECD 201 test medium (centrifuged)

Matrix	Fortification level of test item (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD* (%)
OECD 201 test medium	13.5	94, 112	103	-	2	104	10
	206	95, 113	104	-	2		

* Calculated from reported individual recovery values
Recoveries fortified at 13.5 µg/L and 206 µg/L were diluted by 2 and 51 respectively for analysis
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 10: Characteristics for the analytical method used for validation of flurochloridone in OECD 201 test medium

	Flurochloridone
Specificity	A highly specific detection system was used (HPLC-MS/MS). The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No significant interferences at the retention time of the analyte were detected in any untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of calibration standard solutions were prepared in methanol/test water (1/1, v/v). The linearity was determined with six solvent standards ranging from 0.0927 µg/L to 9.37 µg/L of test item. The calibrations were found linear with coefficients of determination $R^2 \geq 0.990$ ($1/Y^2$ weighing): $y = 23324x - 165.68$, $R^2 = 0.9978$
Assessment of matrix effects is presented	Not assessed
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level* and was successfully established at 13.5 µg/L of test item.

* Note that the LOQ defined in the study report was derived from the lowest calibration solution as 0.185 µg test item /L taking into account the sample preparation factor

Conclusion

The method was found to be valid for the determination of flurochloridone in OECD 201 test medium used in ecotoxicology studies at fortification levels of 13.5 µg/L of test item and 206 µg/L of test item, with a limit of quantification (LOQ) of 13.5 µg/L of test item, in accordance to SANCO/3029/99 rev. 4 requirements.

Liedtke, A. (2013a)

A 2.1.1.4.3.3 Method validation 3

Comments of zRMS:	The method is validated in accordance with SANCO/3029/99 rev. 4. for the determination of the concentration of flurochloridone in test medium with a limit of quantification (LOQ) of 1.96 µg/L. In the report of Liedtke, A. (Harlan Study Number: D65738) it is stated that *The method was developed by Harlan Laboratories Ltd. These experiments were not performed according to the regulations of GLP.*

Reference: **2.1.1.4.3.3/01 (KCP 5.1.2/04)**

Report: Liedtke, A. (2013b)
Flurochloridone technical: Toxicity to *Chlorella vulgaris* in a 72-Hour Algal Growth Inhibition Test
Report No. D65738, Sponsor Reference No. 90015443

Guideline(s): Not mentioned

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

An analytical method for flurochloridone in OECD 201 test medium was validated. The method involves dilution with methanol or test water/methanol (v/v; 1/1) prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (LC-MS/MS) using external calibration. The limit of quantification (LOQ) of 1.96 µg/L of test item was validated.

Specimen preparation

Test samples and control samples were thawed at room temperature between 30 min and 3 hours and shaken manually to obtain homogeneous sample solutions. If necessary, samples were centrifuged (3500 rpm, 5 min) and if necessary, they were further diluted into the calibration range by a factor of 2 with methanol or by a factor of 21 with test water/methanol (v/v; 1/1)), prior to analysis by LC-MS/MS.

Equipment for flurochloridone determination

LC-MS/MS system	High pressure gradient HPLC system, consisting of two Shimadzu LC-10AD pumps, CTC PAL autosampler, Sciex API 5000 triple quadrupole mass spectrometer
Column:	Inertsil ODS-3; 2.0 mm x 33 mm; particle size 3 µm
Column temperature:	Not stated
Injection Volume:	20 µL
Mobile phases:	Eluent A: water/methanol/formic acid (95:5:0.1 v/v/v) + 5mM ammonium formate Eluent B: water/methanol/formic acid (5:95:0.1 v/v/v) + 5mM ammonium formate
Gradient:	

Time [min]	Eluent A	Eluent B	Flow rate [mL/min]
0.0	80	20	0.4
2.0	60	40	0.4
2.5	10	90	0.4
2.7	10	90	0.4
2.8	80	20	0.4
5.0	80	20	0.4

Retention time(s)	Approx. 2.2 min (flurochloridone)
Ionisation type:	TurboIonSpray (ESI), positive mode
Ion mass transition monitored (m/z) for flurochloridone :	312 → 292, collision energy = 32 eV (Quantifier)
	No qualifier ion monitored

Results and discussions

Table A 11: Recovery results of flurochloridone in OECD 201 test medium (non-centrifuged)

Matrix	Fortification level of test item (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery* (%)	Overall RSD* (%)
OECD 201 test medium	1.96	86, 97, 93, 95, 95	93	5	5	94	3
	294	92, 94, 97, 93, 96	94	2	5		

* Calculated from reported individual recovery values
Recoveries fortified at 1.96 µg/L and 294 µg/L were diluted by 2 and 201 respectively for analysis
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 12: Recovery results of flurochloridone in OECD 201 test medium (centrifuged)

Matrix	Fortification level of test item (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD* (%)
OECD 201 test medium	2.23	98, 106	102	-	2	104	4
	300	107, 107	107	-	2		

* Calculated from reported individual recovery values
Recoveries fortified at 2.23 µg/L and 300 µg/L were diluted by 2 and 202 respectively for analysis
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 13: Characteristics for the analytical method used for validation of flurochloridone in OECD 201 test medium

	Flurochloridone
Specificity	A highly specific detection system was used (HPLC-MS/MS). The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No significant interferences at the retention time of the analyte were detected in any untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of calibration standard solutions were prepared in methanol/test water (1/1, v/v). The linearity was determined with seven solvent standards ranging from 0.0889 µg/L to 9.54 µg/L of test item. The calibrations were found linear with coefficients of determination $R^2 \geq 0.990$ (1/Y weighing): $y = 26857x + 722.63$, $R^2 = 0.9991$
Assessment of matrix effects is presented	Not assessed
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level* and was successfully established at 1.96 µg/L of test item.

* Note that the LOQ defined in the study report was derived from the lowest calibration solution as 0.18 µg test item /L taking into account the sample preparation factor

Conclusion

The method was found to be valid for the determination of flurochloridone in OECD 201 test medium used in ecotoxicology studies at fortification levels between 1.96 µg/L of test item and 300 µg/L of test item, with a limit of quantification (LOQ) of 1.96 µg/L of test item, in accordance to SANCO/3029/99 rev. 4 requirements.

Liedtke, A. (2013b)

A 2.1.1.4.3.4 Method validation 4

Comments of zRMS:	The method is validated in accordance with SANCO/3029/99 rev. 4. for the determination of the concentration of flurochloridone in test medium with a limit of quantification (LOQ) of 1.36 µg/L. In the report of Liedtke, A. (Harlan Study Number: D65740) it is stated that *The method was developed by Harlan Laboratories Ltd. These experiments were not performed according to the regulations of GLP.*

Reference: **2.1.1.4.3.4/01 (KCP 5.1.2/05)**

Report: Liedtke, A. (2013c)
Flurochloridone technical: Toxicity to *Navicula pelliculosa* in a 72-Hour Algal Growth Inhibition Test Supplemented with Testing for Recovery of Growth
Report No. D65740, Sponsor Reference No. 90015444

Guideline(s): Not mentioned

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

An analytical method for flurochloridone in OECD 201 test medium was validated. The method involves dilution with methanol and if necessary with test water/methanol (v/v; 1/1), prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (LC-MS/MS) using external calibration. The limit of quantification (LOQ) of 1.36 µg/L of test item was validated.

Specimen preparation

Test samples and control samples were thawed at room temperature at 1.5 hours and shaken manually to obtain homogeneous sample solutions. If necessary, samples were centrifuged (3500 rpm, 5 min) before being diluted with methanol by a factor of two. If necessary, they were further diluted into the calibration range with test methanol/test water (v/v; 1/1), prior to analysis by LC-MS/MS.

Equipment for flurochloridone determination

LC-MS/MS system	High pressure gradient HPLC system, consisting of two Shimadzu LC-10AD pumps, CTC PAL autosampler, Sciex API 4000 triple quadrupole mass spectrometer
Column:	Inertsil ODS-3; 2.1 mm x 33 mm; particle size 3 µm
Column temperature:	Not stated
Injection Volume:	20 or 50 µL
Mobile phases:	Eluent A: water/methanol/formic acid (95:5:0.1 v/v/v) + 5mM ammonium formate Eluent B: water/methanol/formic acid (5:95:0.1 v/v/v) + 5mM ammonium formate
Gradient:	
Retention time(s)	Approx. 2 min (flurochloridone)
Ionisation type:	TurboIonSpray (ESI), positive mode
Ion mass transition monitored (m/z) for flurochloridone :	312 → 292, collision energy = 32 eV (Quantifier) No qualifier ion monitored

Time [min]	Eluent A	Eluent B	Flow rate [mL/min]
0.0	60	40	0.4
0.5	60	40	0.4
2.0	10	90	0.4
2.5	10	90	0.4
2.6	100	0	0.4
3.0	100	0	0.4
3.1	60	40	0.4
4.0	60	40	0.4

Results and discussions

Table A 14: Recovery results of flurochloridone in OECD 201 test medium (non-centrifuged)

Matrix	Fortification level of test item (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery* (%)	Overall RSD* (%)
OECD 201 test medium	1.36	115, 110, 113, 113, 112	113	2	5	106	7
	1053	97, 99, 96, 99, 102	99	2	5		

* Calculated from reported individual recovery values
Recoveries fortified at 1.36 µg/L and 1053 µg/L were diluted by 2 and 200 respectively for analysis
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 115%, which is only just outside SANCO/3029/99 rev. 4 requirements (70-110%), and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 15: Recovery results of flurochloridone in OECD 201 test medium (centrifuged)

Matrix	Fortification level of test item (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD* (%)
OECD 201 test medium	1.36	111, 107	109	-	2	104	7
	1053	93, 106	99	-	2		

* Calculated from reported individual recovery values
Recoveries fortified at 1.36 µg/L and 1053 µg/L were diluted by 2 and 200 respectively for analysis
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 16: Characteristics for the analytical method used for validation of flurochloridone in OECD 201 test medium

	Flurochloridone
Specificity	A highly specific detection system was used (HPLC-MS/MS). The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No significant interferences at the retention time of the analyte were detected in any untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of calibration standard solutions were prepared in methanol/test water (1/1, v/v). The linearity was determined with seven solvent standards ranging from 0.0966 µg/L to 10.5 µg/L of test item. The calibrations were found linear with coefficients of determination $R^2 \geq 0.990$ (1/Y weighing): $y = 22472x + 1692.3$, $R^2 = 0.9986$
Assessment of matrix effects is presented	Not assessed
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level* and was successfully established at 1.36 µg/L of test item.

* Note that the LOQ defined in the study report was derived from the lowest calibration solution as 0.2 µg test item /L taking into account the sample preparation factor

Conclusion

The method was found to be valid for the determination of flurochloridone in OECD 201 test medium used in ecotoxicology studies at fortification levels of 1.36 µg/L of test item and 1053 µg/L of test item, with a limit of quantification (LOQ) of 1.36 µg/L of test item, in accordance to SANCO/3029/99 rev. 4 requirements.

Liedtke, A. (2013c)

A 2.1.1.4.3.5 Method validation 5

Comments of zRMS:	The method is validated in accordance with SANCO/3029/99 rev. 4. for the determination of the concentration of flurochloridone in test medium with a limit of quantification (LOQ) of 3.09 µg/L. In the report of Liedtke, A. (Harlan Study Number: D59890) it is stated that *The method was developed by Harlan Laboratories Ltd. These experiments were not performed according to the regulations of GLP.*

Reference: **2.1.1.4.3.5/01 (KCP 5.1.2/06)**

Report: Liedtke, A. (2013d)
Flurochloridone (Trans isomer): Toxicity to *Desmodesmus subspicatus* in a Pulse Exposure Growth Inhibition Test Supplemented with Testing for Recovery of Growth
Report No. D59890, Sponsor Reference No. 90015421

Guideline(s): Not mentioned

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

An analytical method for flurochloridone in OECD 201 test medium was validated. The method involves dilution with methanol and if necessary further dilution with test water/methanol (v/v; 1/1), prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (LC-MS/MS) using external calibration. The limit of quantification (LOQ) of 3.09 µg/L of test item was validated.

Specimen preparation

Test samples, solvent control samples and the application solution were thawed at room temperature for 4 hours and shaken manually to obtain homogeneous sample solutions. The old test samples were centrifuged (3500 rpm, 5 min) due to the presence of algae, before dilution of a supernatant aliquot with methanol by a factor of two. The application solution was diluted with methanol and methanol/test water (v/v; 1/1) giving a sample preparation factor of 40401, prior to analysis by LC-MS/MS.

Equipment for flurochloridone determination

LC-MS/MS system	High pressure gradient HPLC system, consisting of two Shimadzu LC-10AD pumps, CTC PAL autosampler, Sciex API 5000 triple quadrupole mass spectrometer
Column:	Inertsil ODS-3; 2.1 mm x 33 mm; particle size 3 µm
Column temperature:	Not stated
Injection Volume:	5 µL
Mobile phases:	Eluent A: water/methanol/formic acid (95:5:0.1 v/v/v) + 5mM ammonium formate Eluent B: water/methanol/formic acid (5:95:0.1 v/v/v) + 5mM ammonium formate
Gradient:	

Time [min]	Eluent A	Eluent B	Flow rate [mL/min]
0.0	60	40	0.4
0.5	60	40	0.4
2.0	10	90	0.4
3.0	10	90	0.4
3.1	60	40	0.4
4.0	60	40	0.4

Retention time(s)	Approx. 2.17 min (flurochloridone)
Ionisation type:	TurboIonSpray (ESI), positive mode
Ion mass transition monitored (m/z) for flurochloridone :	312 → 292, collision energy = 32 eV (Quantifier)
	No qualifier ion monitored

Results and discussions

Table A 17: Recovery results of flurochloridone in OECD 201 test medium

Matrix	Fortification level of test item (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery* (%)	Overall RSD* (%)
OECD 201 test medium	3.09	97, 98, 97, 101, 93	97	3	5	100	3
	35.2	101, 101, 103, 102, 103	102	1	5		

* Calculated from reported individual recovery values
All recoveries were diluted by 2 for analysis
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 18: Characteristics for the analytical method used for validation of flurochloridone in OECD 201 test medium

	Flurochloridone

	Flurochloridone
Specificity	A highly specific detection system was used (HPLC-MS/MS). The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No significant interferences at the retention time of the analyte were detected in any untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of calibration standard solutions were prepared in methanol/test water (1/1, v/v). The linearity was determined with five solvent standards ranging from 1.02 µg/L to 19.2 µg/L of test item. The calibrations were found linear with coefficients of determination $R^2 \geq 0.990$: $y = 4315x + 747.05$, $R^2 = 0.9990$
Assessment of matrix effects is presented	Not assessed
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level* and was successfully established at 3.09 µg/L of test item.

* Note that the LOQ defined in the study report was derived from the lowest calibration solution as 2.04 µg test item /L taking into account the sample preparation factor

Conclusion

The method was found to be valid for the determination of flurochloridone in OECD 201 test medium used in ecotoxicology studies at fortification levels of 3.09 µg/L of test item and 35.2 µg/L of test item, with a limit of quantification (LOQ) of 3.09 µg/L of test item, in accordance to SANCO/3029/99 rev. 4 requirements.

Liedtke, A. (2013d)

A 2.1.1.4.3.6 Method validation 6

Comments of zRMS:	The method is validated in accordance with SANCO/3029/99 rev. 4. for the determination of the concentration of flurochloridone in test medium with a limit of quantification (LOQ) of 0.451 µg/L. In the report of Liedtke, A. (Harlan Study Number: D65547) it is stated that *On the basis of a method provided by the sponsor, an analytical method was adapted and implemented by Harlan Laboratories Ltd. These experiments were not performed according to the regulations of GLP*.

Reference:	**2.1.1.4.3.6/01 (KCP 5.1.2/07)**
Report:	Liedtke, A. (2013e) Flurochloridone (Trans isomer): Toxicity to *Desmodesmus subspicatus* in a Pulse Exposure Growth Inhibition Test Supplemented with Testing for Recovery of Growth Report No. D65547, Sponsor Reference No. 90015432
Guideline(s):	Not mentioned
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

An analytical method for flurochloridone in OECD 201 test medium was validated. The method involves dilution with dimethylformamide and water if necessary, prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (LC-MS/MS) using external calibration. The limit of quantification (LOQ) of 0.451 µg/L of test item was validated.

Specimen preparation

Test samples, control samples and the application solution were thawed at room temperature for 1.5 hours and shaken manually to obtain homogeneous sample solutions. The old test and control samples were

centrifuged (3500 rpm, 5 min) due to the presence of algae before aliquots of all samples were analyzed. The application solutions were diluted into the calibration range in a first step with dimethylformamide and in the following two steps with water, prior to analysis by LC-MS/MS.

Equipment for flurochloridone determination

LC-MS/MS system	High pressure gradient HPLC system, consisting of two Shimadzu LC-10AD pumps, CTC PAL autosampler, Sciex API 4000 triple quadrupole mass spectrometer
Column:	Inertsil ODS-3; 2.1 mm x 33 mm; particle size 3 µm
Column temperature:	Not stated
Injection Volume:	50 µL
Mobile phases:	Eluent A: water/methanol/formic acid (95:5:0.1 v/v/v) + 5mM ammonium formate Eluent B: water/methanol/formic acid (5:95:0.1 v/v/v) + 5mM ammonium formate
Gradient:	

Time [min]	Eluent A	Eluent B	Flow rate [mL/min]
0.0	60	40	0.4
0.5	60	40	0.4
2.0	10	90	0.4
2.5	10	90	0.4
2.6	100	0	0.4
3.0	100	0	0.4
3.1	60	40	0.4
4.0	60	40	0.4

Retention time(s)	Approx. 2 min (flurochloridone)
Ionisation type:	TurboIonSpray (ESI), positive mode
Ion mass transition monitored (m/z) for flurochloridone :	312 → 292, collision energy = 32 eV (Quantifier)
	No qualifier ion monitored

Results and discussions

Table A 19: Recovery results of flurochloridone in OECD 201 test medium

Matrix	Fortification level of test item (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery* (%)	Overall RSD* (%)
OECD 201 test medium	0.451	80, 78, 81, 81, 80	80	1	5	84	5
	5.47	86, 88, 89, 85, 87	87	2	5		

* Calculated from reported individual recovery values
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 20: Characteristics for the analytical method used for validation of flurochloridone in OECD 201 test medium

	Flurochloridone
Specificity	A highly specific detection system was used (HPLC-MS/MS). The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No significant interferences at the retention time of the analyte were detected in any untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of calibration standard solutions were prepared in methanol/water (1/1, v/v). The linearity was determined with eight solvent standards ranging from 0.110 µg/L to 8.42 µg/L of test item. The calibrations were found linear with coefficients of determination $R^2 \geq 0.990$ (1/y weighting): $y = 56147x + 1781.4$, $R^2 = 0.9997$
Assessment of matrix effects is presented	Not assessed
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level* and was successfully established at 0.451 µg/L of test item.

* Note that the LOQ defined in the study report was derived from the lowest calibration solution as 0.110 µg test item /L

Conclusion

The method was found to be valid for the determination of flurochloridone in OECD 201 test medium used in ecotoxicology studies at fortification levels of 0.451 µg/L of test item and 5.47 µg/L of test item, with a limit of quantification (LOQ) of 0.451 µg/L of test item, in accordance to SANCO/3029/99 rev. 4 requirements.

Liedtke, A. (2013e)

A 2.1.1.4.4 Analytical method - Flurochloridone residues in OECD 201 test medium

A 2.1.1.4.4.1 Method validation 1

Comments of zRMS:	The methods (KCP 5.1.2/08 - KCP 5.1.2/12) are satisfactorily validated in accordance with SANCO/3029/99 rev. 4. for the determination of the concentration of flurochloridone in test medium with a limit of quantification (LOQ) of 0.01 µg/L.

Reference: **2.1.1.4.4.1/01 (KCP 5.1.2/08)**

Report: Scheerbaum, D. (2013a)
Flurochloridone technical: Alga, Growth Inhibition Test with *Pseudokirchneriella subcapitata*, 72 hours
Report No. SPO15371, Sponsor Reference No. 90015448

And

Reference: **2.1.1.4.4.1/02 (KCP 5.1.2/09)**

Scheerbaum, D. (2013b)
Flurochloridone technical: Alga, Growth Inhibition Test with *Nitzschia communis*, 72 hours
Report No. SNC15371, Sponsor Reference No. 90015449

And

Reference: **2.1.1.4.4.1/03 (KCP 5.1.2/10)**

Scheerbaum, D. (2013c)
Flurochloridone technical: Alga, Growth Inhibition Test with *Synechococcus leopoliensis*, 72 hours
Report No. SSL15371, Sponsor Reference No. 90015450

And

Reference: **2.1.1.4.4.1/04 (KCP 5.1.2/11)**

Scheerbaum, D. (2013d)
Flurochloridone technical: Alga, Growth Inhibition Test with *Chromulina nebulosa*, 72 hours
Report No. SCN15371, Sponsor Reference No. 90016462

And

Reference: **2.1.1.4.4.1/05 (KCP 5.1.2/12)**

Scheerbaum, D. (2013e)
Flurochloridone technical: Alga, Growth Inhibition Test with *Ankistrodesmus falcatus*, 72 hours
Report No. SAF15371, Sponsor Reference No. 90016463

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

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

An analytical method for flurochloridone in OECD 201 test medium was validated. The method involves clean-up with Strata X cartridges and concentration into acetonitrile, prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (LC-MS/MS) using external calibration. The limit of quantification (LOQ) of 0.01 µg/L of test item was validated.

Specimen preparation

Test samples and control samples were enriched with Strata X cartridges on top with glass wool (preconditioned with 5 mL acetonitrile and 5 mL HPLC water) and eluted with 10 mL acetonitrile. The solutions were evaporated to dryness at 40 °C, filled up to 1 mL with acetonitrile and sonicated for 60 sec and if necessary centrifuged for 3 min at 10000 rpm (20 °C). If necessary, samples were diluted by a factor of 10 to 100 in order to compensate matrix effect, prior to analysis by LC-MS/MS.

Equipment for flurochloridone determination

LC-MS/MS system	Waters Acquity UPLC pump and autosampler, Waters Xevo Acquity UPLC triple quadrupole mass spectrometer
Column:	Waters Acquity UPLC BEH C18; 2.1 mm x 50 mm; particle size 1.7 µm
Column temperature:	30 °C
Injection Volume:	5 µL
Mobile phases:	Eluent A: HPLC water + 1 % formic acid Eluent B: acetonitrile + 1 % formic acid
Gradient:	

Time [min]	Eluent A	Eluent B	Flow rate [mL/min]
0.0	90	10	0.5
0.2	90	10	0.5
1.0	10	90	0.5
2.0	10	90	0.5
2.1	90	10	0.5
3.0	90	10	0.5

Retention time(s)	Approx. 1..3 min (flurochloridone) as displayed on chromatograms
Ionisation type:	TurboIonSpray (ESI), positive mode
Ion mass transition monitored (m/z) for flurochloridone :	312 → 292, collision energy = 20 eV (Quantifier) 312 → 145, collision energy = 48 eV (Qualifier)

Results and discussions

Table A 21: Recovery results of flurochloridone in OECD 201 test medium

Matrix	Fortification level of test item (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery* (%)	Overall RSD* (%)
OECD 201 test medium	0.01	97, 98, 96, 90, 91	94	3.2	5	100	7.0
	1.0	108, 105, 107, 110, 101	106	2.8	5		

* Calculated from reported individual recovery values
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

The recovery results of the method validation presented above are applicable to all study reports by Scheerbaum, D. (2013a, 2013b, 2013c, 2013d, 2013e), as they were conducting within one month (February 11 to March 14, 2013):
Report No. SPO15371: February 11 to 14
Report No. SNC15371: March 11 to 14

Report No. SSL15371: February 18 to 21
Report No. SCN15371: February 11 to 14
Report No. SAF15371: February 25 to 28

In addition the following procedural recoveries were analysed:

Table A 22: Recovery results of flurochloridone in test mediums

Matrix	Fortification level of test item (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
WARIS-H test medium (Report No. SNC15371)	0.01	108, 104	106	-	2	-	-
AAP test medium (Report No. SSL15371)	0.01	74, 92	83	-	2	-	-
UTEX DYIII test medium (Report No. SCN15371)	1.0	81, 80	81	-	2	-	-

RSD = Relative Standard Deviation

Mean recovery for each test medium in each study was between 70 and 110% in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 23: Characteristics for the analytical method used for validation of flurochloridone in OECD 201 test medium

	Flurochloridone
Specificity	A highly specific detection system was used (HPLC-MS/MS). The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No significant interferences at the retention time of the analyte were detected in any untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of calibration standard solutions were prepared in acetonitrile. The linearity was determined with seven solvent standards ranging from 3.0 µg/L to 50.0 µg/L of test item. The calibrations were found linear with coefficients of determination $R^2 \geq 0.990$ (no weighing): Report No. SPO15371: y = 57.1985x – 10.0859, R^2= 0.999507 Report No. SNC15371: y = 38.9721x – 9.0642, R^2= 0.998911 Report No. SSL15371: y = 55.8072x + 11.0512, R^2= 0.999488 Report No. SCN15371: y = 54.0444x + 5.16162, R^2= 0.998141 Report No. SAF15371: y = 9.99268x + 2.05243, R^2= 0.997889
Assessment of matrix effects is presented	Not assessed
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.01 µg/L of test item.

Conclusion

The method was found to be valid for the determination of flurochloridone in OECD 201 test medium used in ecotoxicology studies at fortification levels of 0.01 µg/L of test item and 1.0 µg/L of test item, with a limit of quantification (LOQ) of 0.01 µg/L of test item, in accordance to SANCO/3029/99 rev. 4 requirements.

Scheerbaum, D. (2013a, 2013b, 2013c, 2013d, 2013e)

A 2.1.1.4.5 Analytical method - Flurochloridone residues in OECD 201 test medium

A 2.1.1.4.5.1 Method validation

Comments of zRMS:	The validation of the analytical method was performed in compliance with GLP and according to the guideline SANCO/3029/99 rev.4 (11/07/00). The method is applicable for flurochloridone in algae medium in concentrations above the validated limit of quantification (LOQ) of 4.0 µg/L.

Reference: **2.1.1.4.5.1/01 (KCP 5.1.2/13)**

Report: Wenzel, A. (2015a)
Freshwater Alga, Growth Inhibition Test Flurochloridone (technical): *Desmodesmus subspicatus* Toxicity Test - Testing for Recovery of Growth
Report No. ADM-003/4-10/B, Sponsor Reference No. 90016481

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

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

An analytical method for flurochloridone in OECD 201 test medium was validated. The method involves extraction with n-hexane and concentration into n-hexane, prior to analysis by gas chromatography coupled with mass spectrometric detection (GC-MS) using internal calibration. The limit of quantification (LOQ) of 4.0 µg/L of flurochloridone (sum of isomers) was validated.

Specimen preparation
The sample preparation was made by spiking 50 µL of internal standard 1,1-Dichloro-2,2-bis(4-chlorophenyl)ethene (4,4-DDE) solution to a volume of aqueous sample (25, 50 or 2x 50 mL). Recoveries were spiked on 25 mL of aqueous sample.
After extraction with 5 mL of n-hexane or with 2 x 5 mL of n-hexane for 2x 50mL initial sample volume by shaking for 45 min and short centrifugation, the organic phase was dried with Na_2SO_4 and concentrated to 1 mL, prior to analysis by GC-MS.

Equipment for flurochloridone determination

GC-MS system	GC 6890N with MSD 5973 inert (Agilent), with MPS 2 autosampler with 10µL-liquid injection unit (Gerstel)
Column:	DB-5MS UI; 30 m, 0.25 mm ID, 0.25 µm film (Agilent)
Oven:	2.2 min 80°C, then 25°C/min to 275°C for 4.0 min
Carrier gas:	Helium, constant flow, 0.8 mL/min
Inlet:	Splitless Inlet at 280°C
MS source temperature:	250 °C
MS quadrupole temperature:	200 °C
Solvent delay:	9 min
Run time:	14 min
Injection Volume:	Not stated
Retention time(s):	Approx. 10.06 min (flurochloridone) and 10.81 min (4,4-DDE (IS))
Acquisition mode:	SIM-Mode
Ion masses monitored (m/z) for flurochloridone :	311 (Quantifier)
	187 (Qualifier)
Ion masses monitored (m/z) for 4,4-DDE (internal Standard):	246 (Quantifier)
	317.9 (Qualifier)

Results and discussions

Table A 24: Recovery results of flurochloridone in OECD 201 test medium

Matrix	Fortification level of active ingredient (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
OECD 201 test medium	4.0	92.75, 89.25, 96.00, 96.50, 94.25	93.75	3.12	5	94.37	2.69
	40.0	97.83, 96.33, 92.85, 92.63, 95.33	94.99	2.36	5		

RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 110% and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 25: Characteristics for the analytical method used for validation of flurochloridone in OECD 201 test medium

	Flurochloridone
Specificity	A highly specific detection system was used (GC-MS). The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No interferences at the retention time of the analyte were detected in any untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of calibration standard solutions were prepared in acetone the range from 1.00 to 25.00 mg/L with addition of internal standard and 50 µL of each calibration solution were spiked into 25 mL of algae matrix and extracted with 5 mL n-hexane to produce final matrix matched standards used for quantitation. The linearity was determined with seven matrix standards ranging from 2.0 µg/L to 50.0 µg/L. The calibration was obtained by exponential regression analysis with coefficients of determination $R^2 \geq 0.990$: $Y = 0.0004x^2 + 0.1730x – 0.0442$, $R^2 = 0.9990$ As the calculated correlation coefficient R^2 for flurochloridone was very close to 1, the applicability of the exponential calibration function was accepted.
Assessment of matrix effects is presented	Not assessed, however matrix matched standards were used for quantitation
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 4.0 µg/L of flurochloridone.

Conclusion

The method was found to be valid for the determination of flurochloridone (sum of isomers) in OECD 201 test medium used in ecotoxicology studies at fortification levels of 4.0 µg/L and 40.0 µg/L of flurochloridone, with a limit of quantification (LOQ) of 4.0 µg/L of flurochloridone, in accordance to SANCO/3029/99 rev. 4 requirements.

Wenzel, A. (2015a)

A 2.1.1.4.6 Analytical method - Flurochloridone residues in OECD 221 test medium

A 2.1.1.4.6.1 Method validation

Comments of zRMS:	The method is validated in accordance with SANCO/3029/99 rev. 4. for the determination of the concentration of flurochloridone in test medium with a limit of quantification (LOQ) of 11.4 µg/L. In the report of Liedtke, A. (Harlan Study Number: D54308) it is stated that *On the basis of a method provided by the sponsor, an analytical method was adapted and implemented by Harlan Laboratories Ltd. These experiments were not performed according to the regulations of GLP.*

Reference: **2.1.1.4.6.1/01 (KCP 5.1.2/14)**

Report: Liedtke, A. (2012b)
TEST ITEM: AG-F8-250 EC (Flurochloridone)
Toxicity to the Aquatic Higher Plant *Lemna gibba* in a 7-Day Growth Inhibition Test
Report No. D54308, Sponsor Reference No. 90015431

Guideline(s): Not mentioned

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

An analytical method for flurochloridone in OECD 221 test medium was validated. The method involves dilution with methanol prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (LC-MS/MS) using external calibration. The limit of quantification (LOQ) of 11.4 µg/L of test item was validated, corresponding to 2.81 µg/L of flurochloridone.

Specimen preparation
Treatment samples and control samples were thawed at room temperature for 1.5 hours and shaken manually to obtain homogeneous sample solutions. All samples were diluted by a factor of two with methanol prior to analysis by LC-MS/MS.

Equipment for flurochloridone determination

LC-MS/MS system	High pressure gradient HPLC system, consisting of two Shimadzu LC-10AD pumps, CTC PAL autosampler, Sciex API 4000 triple quadrupole mass spectrometer
Column:	Inertsil ODS-3; 2.1 mm x 33 mm; particle size 3 µm
Column temperature:	Not stated
Injection Volume:	50 µL
Mobile phases:	Eluent A: water/methanol/formic acid (95:5:0.1 v/v/v) + 5mM ammonium formate Eluent B: water/methanol/formic acid (5:95:0.1 v/v/v) + 5mM ammonium formate
Gradient:	

Time [min]	Eluent A	Eluent B	Flow rate [mL/min]
0.0	60	40	0.4
0.5	60	40	0.4
2.0	10	90	0.4
2.5	10	90	0.4
2.6	100	0	0.4
3.0	100	0	0.4
3.1	60	40	0.4
4.0	60	40	0.4

Retention time(s)	Approx. 2.3 min (flurochloridone)
Ionisation type:	TurboIonSpray (ESI), positive mode

Ion mass transition monitored (m/z) for flurochloridone :	312 → 292, collision energy = 32 eV (Quantifier)
	No qualifier ion monitored

Results and discussions

Table A 26: Recovery results of flurochloridone in OECD 221 test medium

Matrix	Fortification level of test item (µg/L)	Fortification level of active ingredient (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery* (%)	Overall RSD* (%)
OECD 221 test medium	11.4	2.81	114, 111, 112, 112, 111	112	1	5	111	3
	241	59.5	108, 106, 116, 110, 107	109	3	5		

* Calculated from reported individual recovery values
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 115%, which is only just outside SANCO/3029/99 rev. 4 requirements (70-110%), and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 27: Characteristics for the analytical method used for validation of flurochloridone in OECD 221 test medium

	Flurochloridone
Specificity	A highly specific detection system was used (HPLC-MS/MS). The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No interferences at the retention time of the analyte were detected in any untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of calibration standard solutions were prepared in methanol/water (1/1, v/v). The linearity was determined with eight solvent standards ranging from 0.121 µg/L to 39.3 µg/L. The calibrations were found linear with coefficients of determination $R^2 \geq 0.990$ (1/Y weighing): $Y = 10261x + 28.945$, $R^2 = 0.9998$
Assessment of matrix effects is presented	Not assessed
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 11.4 µg/L of test item (2.81 µg/L of flurochloridone).

Conclusion

The method was found to be valid for the determination of flurochloridone in OECD 221 test medium used in ecotoxicology studies at fortification levels of 11.4 µg/L of test item and 241 µg/L of test item, with a limit of quantification (LOQ) of 11.4 µg/L of test item (2.81 µg/L of flurochloridone), in accordance to SANCO/3029/99 rev. 4 requirements.

Liedtke, A. (2012b)

A 2.1.1.4.7 Analytical method - Flurochloridone residues in OECD 221 test medium

A 2.1.1.4.7.1 Method validation

Comments of zRMS:	The analytical method for the analyte trans-flurochloridone in aqueous samples has been successfully validated with LOQ of 5.0 µg/L in accordance with the EU guidance document SANCO/3029/99 rev. 4 (11/07/00).

Reference: **2.1.1.4.7.1/01 (KCP 5.1.2/15)**

Report: Wenzel, A. (2015b)
Macrophyte Pulse Exposure Growth Inhibition Test.
Flurochloridone (trans-isomer): Sediment-free *Lemna minor* Toxicity Test -

	testing for recovery of growth Report No. ADM-005/4-11/I, Sponsor Reference No. 90016482
And	
Reference:	**2.1.1.4.8.1/01 (KCP 5.1.2/16)**
Report:	Wenzel, A. (2015c) Macrophyte Pulse Exposure Growth Inhibition Test. Flurochloridone (trans-isomer): Sediment-free *Myriophyllum spicatum* Toxicity Test - testing for recovery of growth Report No. ADM-005/4-13/K, Sponsor Reference No. 90016483
Guideline(s):	SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

An analytical method for flurochloridone trans isomer in OECD 221 and 238 test mediums was validated. The method involves extraction with n-hexane, prior to analysis by gas chromatography coupled with mass spectrometric detection (GC-MS) using internal calibration. The limit of quantification (LOQ) of 5.0 µg/L of flurochloridone trans isomer was validated.

The analytical procedure was conducted on the same days of analysis for both studies therefore the method validation is applicable to both studies and was reported in both study reports.

Specimen preparation
The sample preparation was made by spiking 50 µL of internal standard 1,1-Dichloro-2,2-bis(4-chlorophenyl)ethene (4,4-DDE) solution to a volume of aqueous sample (25 mL). Recoveries were spiked on 25 mL of aqueous sample.
After extraction with 5 mL of n-hexane by shaking for 45 min and short centrifugation, 1 mL of the organic phase was transferred for analysis by GC-MS.

Equipment for flurochloridone trans isomer determination

GC-MS system	GC 6890N with MSD 5973 inert (Agilent), with MPS 2 autosampler with 10µL-liquid injection unit (Gerstel)
Column:	DB-5MS UI; 30 m, 0.25 mm ID, 0.25 µm film (Agilent)
Oven:	2.2 min 80°C, then 25°C/min to 275°C for 4.0 min
Carrier gas:	Helium, constant flow, 0.8 mL/min
Inlet:	Splitless Inlet at 280°C
MS source temperature:	250 °C
MS quadrupole temperature:	200 °C
Solvent delay:	9 min
Run time:	14 min
Injection Volume:	Not stated
Retention time(s):	Approx. 10.0 min (flurochloridone trans isomer) and 10.8 min (4,4-DDE (IS))
Acquisition mode:	SIM-Mode
Ion masses monitored (m/z) for flurochloridone trans isomer:	311 (Quantifier) 187 (Qualifier)
Ion masses monitored (m/z) for 4,4-DDE (internal Standard):	246 (Quantifier) 317.9 (Qualifier)

Results and discussions

Table A 28: Recovery results of flurochloridone trans isomer in OECD 221 test medium

Matrix	Fortification level of active ingredient (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)

Matrix	Fortification level of active ingredient (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
OECD 221 test medium	5.0	116.90, 111.60, 113.30, 110.80, 111.90	112.90	2.14	5	105.5	7.64
	50.0	99.34, 99.53, 99.37, 96.52, 95.68	98.09	1.88	5		

RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 113%, which is only just outside SANCO/3029/99 rev. 4 requirements (70-110%), and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 29: Characteristics for the analytical method used for validation of flurochloridone trans isomer in OECD 221 test medium

	Flurochloridone trans isomer
Specificity	A highly specific detection system was used (GC-MS). The retention time of flurochloridone trans isomer in the samples matches the retention time in the standard solutions. No interferences at the retention time of the analyte were detected in any untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of calibration standard solutions were prepared in acetone in the range from 2.00 to 35.00 mg/L with addition of internal standard and 50 µL of each calibration solution were spiked into 25 mL of test medium and extracted with 5 mL n-hexane to produce final matrix matched standards used for quantitation. The linearity was determined with eight matrix standards ranging from 4.0 µg/L to 70.0 µg/L. The calibration was obtained by exponential regression analysis with coefficients of determination $R^2 \geq 0.990$: $Y = 0.0004x^2 + 0.1846x$, $R^2 = 0.9993$ As the calculated correlation coefficient R^2 for flurochloridone trans isomer was very close to 1, the applicability of the exponential calibration function was accepted.
Assessment of matrix effects is presented	Not assessed, however matrix matched standards were used for quantitation
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 5.0 µg/L of flurochloridone trans isomer.

Conclusion

The method was found to be valid for the determination of flurochloridone trans isomer in OECD 221 test medium used in ecotoxicology studies at fortification levels of 5.0 µg/L and 50.0 µg/L of flurochloridone trans isomer, with a limit of quantification (LOQ) of 5.0 µg/L of flurochloridone trans isomer, in accordance to SANCO/3029/99 rev. 4 requirements.

Wenzel, A. (2015b, 2015c)

A 2.1.1.4.8 Analytical method - Flurochloridone residues in honey bee feeding solutions

A 2.1.1.4.8.1 Method validation

Comments of zRMS:	An analytical method for the determination of flurochloridone in honey bee feeding solutions 50% (w/v) aqueous sucrose solution was validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000. The limit of quantification (LOQ) of the analytical method was 39.0 mg/kg of test item (9.52 mg/kg of flurochloridone).

Reference: **2.1.1.4.9.1/01 (KCP 5.1.2/17)**

Report: Molitor, A.M. (2017)
AG-F8-250 EC (Flurochloridone 250 EC) -
Assessment of Effects on the Adult Honey Bee, *Apis mellifera* L., in a 10 Day Chronic Feeding Test under Laboratory Conditions
Report No. S17-00282, Sponsor Reference No. 90020495

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

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

An analytical method for flurochloridone in honey bee feeding solutions (50 % (w/v) aqueous sucrose solutions) was validated. The method involves dilution with acetonitrile/water (1:1, v/v) prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (LC-MS/MS) using external calibration. The limit of quantification (LOQ) of 39.0 mg/kg of test item was validated, corresponding to 9.52 mg/kg of flurochloridone.

Specimen preparation

Samples were stored deep-frozen (≤ - 18 °C) after sampling. At the day of analysis, the samples were thawed to ambient temperature and shaken well using a Vortex-Mixer for 30 sec and ultrasonicated for 15 min. The samples were diluted by a factor of 1000 with the dilution solvent acetonitrile/water (1:1, v/v): an aliquot of 500 µL was diluted in a 50 mL volumetric flask and 100 µL of this diluted sample were further diluted with 900 µL dilution solvent. If necessary, further dilution was performed with matrix blank extract prior analysis by HPLC/MS-MS.

Equipment for flurochloridone determination

LC-MS/MS system	Agilent 1290 infinity HPLC system, Sciex API 5500 triple quadrupole mass spectrometer
Column:	Phenomenex Kinetex C18 (100A), 50 mm x 2.1 mm i.d., 2.6 µm mean particle size (No.00B-4462-AN) with 2.1 mm guard column
Column temperature:	20 °C
Injection Volume:	5 µL
Mobile phases:	Eluent A: Water + 0.1 % (v/v) formic acid Eluent B: Methanol + 0.1 % (v/v) formic acid
Gradient:	

Time [min]	Eluent A	Eluent B	Flow rate [mL/min]
0.0	95	5	0.35
0.1	95	5	0.35
2.0	5	95	0.35

	3.2	5	95	0.35
	3.3	95	5	0.35
	5.5	95	5	0.35
Retention time(s)	Approx. 2.8 min (flurochloridone)			
Ionisation type:	TurboIonSpray (ESI), positive mode			
Ion mass transition monitored (m/z) for flurochloridone:	312 → 292, collision energy = 31 eV (Quantifier) 312 → 145, collision energy = 73 eV (Qualifier)			

Results and discussions

Table A 30: Recovery results of flurochloridone in honey bee feeding solutions

Matrix	Fortification level of test item (mg/kg)	Fortification level of active ingredient (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery* (%)	Overall RSD* (%)
Honey bee feeding solutions	39.0	9.52	73, 74, 79, 78, 86	78	7	5	88	12
	7800	1900	97, 96, 99, 95, 98	97	2	5		

* Calculated from reported individual recovery values
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 110%, and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 31: Characteristics for the analytical method used for validation of flurochloridone in honey bee feeding solutions

	Flurochloridone
Specificity	A highly specific detection system was used (HPLC-MS/MS). The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No significant interference (< 30 % of LOQ) at the retention time of the analyte were detected in any untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of calibration standard solutions were prepared in matrix blank extract. The linearity was determined with seven solvent standards ranging from 1 ng/mL to 20 ng/mL, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a diluted sample. The calibrations were found linear with correlation coefficients $r \geq 0.995$ (1/x weighing): $y = 1.72e^{+004} x + 3.55e^{+003}$, r = 0.9981
Assessment of matrix effects is presented	Not assessed, however matrix matched standards were used for quantitation.
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 39.0 mg/kg of test item (9.52 mg/kg of flurochloridone). The limit of detection (LOD) was defined as 30 % of the limit of quantification (2.86 mg/kg of flurochloridone).
Specimen storage stability	Flurochloridone was found to be stable in 50 % (w/v) aqueous sucrose solution samples when stored under deep-frozen conditions (≤ - 18°C) for 52 days. This time period covers the longest storage period of samples of 52 days for flurochloridone in test medium.

Conclusion

The method was found to be valid for the determination of flurochloridone in honey bee feeding solutions (50 % (w/v) aqueous sucrose solutions) used in ecotoxicology studies at fortification levels of 39.0 mg/kg of test item and 7800 mg/kg of the test item, with a limit of quantification (LOQ) of 39.0 mg/kg of test item (9.52 mg/kg of flurochloridone), in accordance to SANCO/3029/99 rev. 4 requirements .

Molitor, A.M. (2017)

A 2.1.1.4.9 Analytical method - Flurochloridone residues in larval diet solutions

A 2.1.1.4.9.1 Method validation

Comments of zRMS:	An analytical method for the determination of flurochloridone in larval diet was validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000. The limit of quantification (LOQ) of the analytical method was 50.0 mg/kg of test item (12.2 mg/kg of flurochloridone).

Reference: **2.1.1.4.10.1/01 (KCP 5.1.2/18)**

Report: Molitor, A.M. (2018)
AG-F8-250 EC (Flurochloridone 250 EC) -
Honey Bee (*Apis mellifera* L.) 22 Day Larval Toxicity Test (Repeated Exposure)
Report No. S17-00318, Sponsor Reference No. 90020496

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

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

An analytical method for flurochloridone in larval diet (50 % weight of fresh royal jelly + 50 % weight of an aqueous solution containing yeast extract, glucose and fructose) was validated. The method involves extraction acetonitrile/water (1:1, v/v) and dilution with acetonitrile/water (1:1, v/v), prior to analysis by liquid chromatography coupled with a tandem mass spectrometric detection (LC-MS/MS) using external calibration. The limit of quantification (LOQ) of 50.0 mg/kg of test item was validated, corresponding to 12.2 mg/kg of flurochloridone.

Specimen preparation
Samples were stored deep-frozen (≤ - 18 °C) after sampling. At the day of analysis, the samples were thawed to ambient temperature and shaken well using a Vortex-Mixer for 30 sec and ultrasonicated for 30 sec. An aliquot of about 500 mg larval diet sample were weighed into a 15 mL plastic tube. About 10 mL extraction solvent (acetonitrile/water (1:1, v/v)) were added and the samples were homogenized using a Vortex-Mixer (a fixed ratio of 500 mg diet / 10 mL extraction solvent was used). The samples were homogenized on a flatbed shaker (5 min) and subsequently centrifuged (4000 rpm, 5 min). After phase separation an aliquot of the supernatant was diluted with acetonitrile/water (1:1, v/v). If necessary further dilution steps were performed with matrix blank extract prior to analysis by HPLC-MS/MS.

Equipment for flurochloridone determination

LC-MS/MS system	Agilent 1290 infinity HPLC system, Sciex API 5500 triple quadrupole mass spectrometer
Column:	Phenomenex Kinetex C18 (100A), 50 mm x 2.1 mm i.d., 2.6 µm mean particle size (No.00B-4462-AN) with 4 mm guard column
Column temperature:	20 °C
Injection Volume:	20 µL
Mobile phases:	Eluent A: Water + 0.1 % (v/v) formic acid Eluent B: Methanol + 0.1 % (v/v) formic acid
Gradient:	

Time [min]	Eluent A	Eluent B	Flow rate [mL/min]
0.0	95	5	0.35
0.1	95	5	0.35
2.0	5	95	0.35
3.2	5	95	0.35
3.3	95	5	0.35
5.5	95	5	0.35

Retention time(s)	Approx. 2.8 min (flurochloridone)
Ionisation type:	TurboIonSpray (ESI), positive mode
Ion mass transition monitored (m/z) for flurochloridone:	312 → 292, collision energy = 31 eV (Quantifier)
	312 → 145, collision energy = 73 eV (Qualifier)
	312 → 198, collision energy = 57 eV (Qualifier)

Results and discussions

Table A 32: Recovery results of flurochloridone in larval diet

Matrix	Fortification level of test item (mg/kg)	Fortification level of active ingredient (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery* (%)	Overall RSD* (%)
Larval diet	50	12.2	119, 116, 113, 102, 102	110	7	5	97	16
	10600	2590	86, 81, 82, 85, 82	83	3	5		

* Calculated from reported individual recovery values
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 110%, and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

In addition the following procedural recoveries were analysed:

Table A 33: Recovery results of flurochloridone in larval diet

Matrix	Fortification level of test item (mg/kg)	Fortification level of active ingredient (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Larval diet	10600	2590	86, 89, 97	91	6	3	-	-

RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 110%, and the overall RSD was below 20%, in compliance with SANCO/3029/99 rev. 4 requirements.

Table A 34: Characteristics for the analytical method used for validation of flurochloridone in larval diet

	Flurochloridone
Specificity	A highly specific detection system was used (HPLC-MS/MS). The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No significant interference (< 30 % of LOQ) at the retention time of the analyte were detected in any untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of calibration standard solutions were prepared in matrix blank extract. The linearity was determined with seven solvent standards ranging from 1.0 ng/mL to 40 ng/mL, covering the working range of no more than 30 % of the LOQ to at least + 20 % of the highest analyte concentration level in a diluted sample. The calibrations were found linear with correlation coefficients $r \geq 0.995$ (1/x weighing): $y = 4.91e^{+004} x - 1.4e^{+003}$, r = 0.9990
Assessment of matrix effects is presented	Not assessed, however matrix matched standards were used for quantitation.
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 50.0 mg/kg of test item (12.2 mg/kg of Flurochloridone). The limit of detection (LOD) was defined as 30 % of the limit of quantification (3.66 mg/kg of Flurochloridone).
Specimen storage stability	Flurochloridone was found to be stable in larval diet samples when stored under deep-frozen conditions (≤ - 18°C) for 232 days. This time period covers the longest storage period of samples of 216 days for flurochloridone in test medium.

Conclusion

The method was found to be valid for the determination of flurochloridone in larval diet solutions used in

ecotoxicology studies at fortification levels of 10600 mg/kg of test item and 50 mg/kg of the test item, with a limit of quantification (LOQ) of 50.0 mg/kg of test item (12.2 mg/kg of Flurochloridone), in accordance to SANCO/3029/99 rev. 4 requirements .

Molitor, A.M. (2018)

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

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

A 2.1.2.1.1 Analytical method 1

An analytical method for the determination of the active substance flurochloridone in different plant commodities has been validated by Garrigue, P. (2017a) (see KCP 5.2/01). This method was independently validated by Hauler, C. (2018a) (see KCP 5.2/02).

A 2.1.2.1.1.1 Method validation

Comments of zRMS:	It was demonstrated (in compliance with the principles of Good Laboratory Practices (GLP)), that the validation method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries of SANCO/825/00 rev. 8.1 and is therefore applicable to correctly determine residues of flurochloridone in the 4 matrix groups of plant matrices: dry high starch (dried peas), high water (apples), high acid (grapes) and high oil (sunflower seeds) content with a limit of quantification (LOQ) of 0.01 mg/kg. The study is acceptable.

Reference:	**2.1.2.1.1.1/01 (KCP 5.2/01)**
Report	Garrigue, P. (2017a) Validation of Residue Analytical Method for Determination of Flurochloridone in Plant Matrices Report No. BPL17-0001, Sponsor Reference No. 90020483
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No.
GLP	Yes
Acceptability:	Yes

Materials and Methods

A residue analytical method for flurochloridone in plant commodities (dried peas (dry high starch), apples (high water), grapes (high acid) and sunflower seeds (high oil)) in analogy to the multi-residue method QuEChERS (EN15662) was validated. The method involves extraction of flurochloridone with acetonitrile / water, followed by liquid-liquid partition using QuEChERS EN15662 salt-mixture, then C18/PSA clean-up, freezing step, and concentration into hexane / acetone prior to analysis by gas chromatography coupled with tandem mass spectrometric detection (GC-MS/MS) using external calibration. The limit of quantification (LOQ) was set to 0.01 mg/kg, expressed as sum of isomers, for all matrices.

Specimen preparation
5 g of homogenized specimen were weighed into a 50 mL plastic centrifuge tube. 5 mL (or 10 mL for dry matrices) of water was added. 10 mL acetonitrile were added and the material was shaken vigorously for 1 min using a vortex. Thereafter, QuEChERS EN15662 salt-mixture (1 g sodium citrate, 0.5 g sodium hydrogencitrate sequihydrate, 4 g magnesium sulphate, 1 g sodium chloride) was added and shaken vertically for an additional minute. The samples were centrifuged at > 3000 rpm for 5 minutes at room temperature.

An aliquot of the supernatant (6 mL) was transferred into a centrifuge tube containing the QuEChERs dispersive kit (900 mg of magnesium sulphate, 150 mg of C18 and 150 mg of PSA) and shaken vertically for 1 min. The samples were centrifuged at > 3000 rpm for 5 min at room temperature. An aliquot of the supernatant (4 mL) was transferred into a centrifuge tube and stored 1 hour in a freezer. It was then transferred immediately into another centrifuge tube.
An aliquot (2 mL) was transferred into another centrifuge tube to which 20 µL of Protectant Solution (10g olive oil dissolved in 50 mL of hexane) was added. The extract was evaporated to dryness under nitrogen flow at room temperature. 200 µL of lindane solution (200 ng/mL) was added and the extract was brought to a volume of 2 mL with Hexane/Acetone (70/30; v/v) prior to analysis by GC-MS/MS.
Note: Lindane was used as injection qualifier and not as internal standard for quantification

Equipment for flurochloridone determination

GC-MS/MS system	Agilent 7890A GC system with 7693 Autosampler and 7000B MS/MS and Mass Hunter software
Column:	Restek Rxi 17 Sil, 30 m x 0.250 mm x 0.25 µm
Injection:	1 µL, pulse splitless (25 psi for 0.5 min)
Injection Liner:	Agilent 2mm dimpled
Injector:	Initial 50 °C (hold for 0.05 min) Heat rate 720 °C/min to 300 °C, hold for 14 min
Oven:	Initial 50 °C (hold for 1 min) Heat rate 40 °C/min to 150 °C, hold for 0 min Heat rate 20 °C/min to 340 °C, hold for 1 min
Transfer line temperature:	340 °C
Source temperature	280 °C
Quadrupoles temperature:	150 °C
Carrier gas	Helium, 1.7 mL/min
Retention time(s)	Approx. 9.2 min (flurochloridone), approx. 8.1 min (lindane)
Ionisation type:	Electron ionization
Ion mass transition monitored (m/z) for flurochloridone :	311 → 174, collision energy = 15 eV (Quantifier) 145 → 95, collision energy = 20 eV (Qualifier)
Ion mass transition monitored (m/z) for lindane :	225 → 189, collision energy = 5 eV

Results and discussions

Table A 35: Recovery results from method validation of flurochloridone in matrices of plant origin

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 311 → 174 m/z (Quantification)							
Dried peas	0.01	121, 108, 123, 111, 112	115	5.8	5	117	4.9
	0.10	115, 116, 122, 122, 124	120	3.4	5		
Apples	0.01	94, 107, 101, 95, 99	99	5.2	5	92	9.1
	0.10	88, 88, 84, 85, 82	85	3.4	5		
Grapes	0.01	113, 115, 108, 115, 102	111	5.0	5	107	6.2
	0.10	105, 96, 101, 110, 100	102	5.1	5		
Sunflower seed	0.01	81, 85, 90, 99, 105	92	10.9	5	98	13.1
	0.10	126, 94, 94, 108, 97	104	13.0	5		
Ion Mass Transition 145 → 95 m/z (Confirmation)							
Dried peas	0.01	121, 108, 120, 109, 110	114	5.6	5	116	4.8
	0.10	114, 113, 120, 121, 122	118	3.7	5		
Apples	0.01	88, 111, 104, 102, 109	103	8.6	5	97	9.1
	0.10	92, 94, 89, 92, 87	91	3.1	5		
Grapes	0.01	117, 111, 111, 112, 102	111	4.7	5	106	6.1

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
	0.10	103, 97, 102, 109, 99	102	4.5	5		
Sunflower seed	0.01	73, 74, 81, 104, 108	88	18.8	5	90	17.9
	0.10	122, 82, 81, 91, 84	92	18.9	5		

Calculation was performed on unrounded values. No correction by blank value was performed.
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 120% and the RSD was below 20% for flurochloridone for both transitions in all matrices, in compliance with SANCO/825/00 rev. 8.1 requirements.

Table A 36: Characteristics for the analytical method used for residue determination of flurochloridone in matrices of plant origin

	Flurochloridone
Specificity	A highly specific detection system was used (GC-MS/MS) and two mass transitions were monitored. The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No interferences above 30 % of the LOQ at the retention time of the analyte were detected in the untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of seven calibration standard solutions were prepared in a lindane/olive oil solution diluted in hexane/acetone (70/30, v/v). The linearity was determined with seven matrix or solvent standards ranging from 1.5 ng/mL to 200 ng/mL (corresponding to 0.003 mg/kg to 0.4 mg/kg in matrix). The calibrations were found linear with coefficients of determination $R^2 \geq 0.990$ (1/X weighting): Flurochloridone in solvent for apple and sunflower seeds: 311 → 174 m/z: Y = 485.980632 x - 147.940431, R^2= 0.9999 145 → 95 m/z: Y = 408.549474 x - 194.380708, R^2 = 0.9999 Flurochloridone in dried peas: 311 → 174 m/z: Y = 310.083979 x - 3.98150, R^2= 0.9997 145 → 95 m/z: Y = 218.836768 x + 23.096113, R^2 = 0.9997 Flurochloridone in grapes: 311 → 174 m/z: Y = 402.490908 x + 13.279511, R^2= 0.9993 145 → 95 m/z: Y = 370.692322 x + 25.332309, R^2 = 0.9992
Assessment of matrix effects is presented	In dried peas, significant matrix effect (≥ ±20%) was found on signal. Therefore quantification using matrix matched standards was performed. Due to non-significant matrix effect (< ±20%) on signal, quantification using standard solutions prepared in solvent was performed for apples and sunflower seeds. Although non-significant matrix effect (< ±20%) on signal was found in grapes, quantification using matrix matched standards was performed.
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.01 mg/kg. The limit of detection (LOD) was set at 0.003 mg/kg, which is 30% of the LOQ.
Extract Stability	Flurochloridone was found to be stable in final extracts of each matrix for at least 10 days when stored at < -18°C.

Conclusion

The method was found to be valid for the determination of flurochloridone in plant commodities (dried peas (dry high starch), apples (high water), grapes (high acid) and sunflower seeds (high oil)), with a limit of quantification (LOQ) of 0.01 mg/kg, in accordance to SANCO/825/00 rev. 8.1 requirements.

Garrigue, P. (2017a)

A 2.1.2.1.1.2 Independent laboratory validation

Comments of zRMS:	The method was independently and successfully validated for the determination of flurochloridone in apples and dry peas according to the guidance document SANCO/825/00, rev. 8.1, with a limit of quantification (LOQ) of 0.01 mg/kg. All mean recovery values at the fortification levels of 0.01 mg/kg and 0.1 mg/kg for both mass transitions comply with the standard acceptance criteria of SANCO/825/00, rev. 8.1 since mean recoveries were in the range of 70 - 120% with relative standard deviations of ≤20% for all matrices at each level. The study is acceptable.

Reference:	**2.1.2.1.1.2/01 (KCP 5.2/02)**
Report	Hauler, C. (2018a) Independent Laboratory Validation of an Analytical Method for the Determination of Flurochloridone in Different Matrices of Plant Origin Report No. S17-08035; Sponsor Reference No. 90021292
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No.
GLP	Yes
Acceptability:	Yes

Materials and Methods

The residue analytical method for flurochloridone in plant commodities validated by Garrigue, P. (2017a), report No. BPL17-0001, was independently validated by Hauler, C. (2018a) in apples and dried peas. The method involves extraction of flurochloridone with acetonitrile/water, followed by liquid-liquid partition using QuEChERS EN15662 salt-mixture, then C18/PSA clean-up, freezing step, and concentration into hexane / acetone, prior to analysis by gas chromatography coupled with tandem mass spectrometric detection (GC-MS/MS) using external calibration. The limit of quantification (LOQ) was set to 0.01 mg/kg, expressed as sum of isomers, for all matrices.

Primary validation and independent laboratory validation were carried out at different locations, by different study personnel, and using different instrumentation and stocks of chemicals. No addition or modification to the original method other than optimization of instrumental parameters was done. No communication with the method developers or others familiar with the method was necessary to carry out the analysis.

Specimen preparation

5 g of homogenized specimen were weighed into a 50 mL plastic centrifuge tube. 5 mL (or 10 mL for dry matrices) of water was added. 10 mL acetonitrile were added and the material was shaken vigorously for 1 min using a vortex. Thereafter, QuEChERS EN15662 salt-mixture (1 g sodium citrate, 0.5 g sodium hydrogencitrate sequihydrate, 4 g magnesium sulphate, 1 g sodium chloride) was added and shaken vertically for an additional minute. The samples were centrifuged at 4000 rpm for 5 min at room temperature.

An aliquot of the supernatant (6 mL) was transferred into a centrifuge tube containing the QuEChERs dispersive kit (900 mg of magnesium sulphate, 150 mg of C18 and 150 mg of PSA) and shaken vertically for 1 min. The samples were centrifuged at 4000 rpm for 5 min at room temperature. An aliquot of the supernatant (4 mL) was transferred into a centrifuge tube and stored 1 hour in a freezer. It was then transferred immediately into another centrifuge tube.

An aliquot (2 mL) was transferred into another centrifuge tube to which 20 µL of Protectant Solution (10g olive oil dissolved in 50 mL of hexane) was added. The extract was evaporated to dryness under nitrogen flow at room temperature. 200 µL of lindane solution (200 ng/mL) was added and the extract was brought to a volume of 2 mL with Hexane/Acetone (70/30; v/v) prior to analysis by GC-MS/MS.

Note: Lindane was used as injection qualifier and not as internal standard for quantification

Equipment for flurochloridone determination

GC-MS/MS system	Thermo TSQ 8000 Evo GC-MS/MS system with TriPlus RSH Autosampler

Column:	VF-17-MS, 30 m x 0.250 mm x 0.25 µm
Injection:	1 µL, splitless
Injection port:	Programmable Temperature Vaporizing (PTV)
Injection temperature program:	50 °C (hold for 1.0 min), 12 °C/sec to 300 °C (hold for 14.0 min)
Oven:	Initial 50 °C (hold for 1 min) Heat rate 40 °C/min to 150 °C, hold for 0 min Heat rate 20 °C/min to 340 °C, hold for 1 min
Transfer line temperature:	280 °C
Source temperature	280 °C
Purge flow:	5 mL/min
Carrier gas	Helium, 1.7 mL/min
Retention time(s)	Approx. 9.0 min (flurochloridone), approx. 7.8 min (lindane)
Ionisation type:	Electron ionization
Ion mass transition monitored (m/z) for flurochloridone :	311 → 174, collision energy = 15 eV (Quantifier)
	145 → 95, collision energy = 20 eV (Qualifier)
Ion mass transition monitored (m/z) for lindane :	219 → 183, collision energy = 5 eV

Results and discussions

Table A 37: Recovery results from independent laboratory validation of flurochloridone in matrices of plant origin

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 311 → 174 m/z (Quantification)							
Apples	0.01	87, 68, 83, 76, 92	81	12	5	83	10
	0.10	78, 92, 79, 89, 90	86	8	5		
Dried peas	0.01	92, 89, 86, 91, 87	89	3	5	90	2
	0.10	91, 89, 93, 89, 90	90	2	5		
Ion Mass Transition 145 → 95 m/z (Confirmation)							
Apples	0.01	76, 61, 74, 75, 91	75	14	5	80	12
	0.10	78, 93, 79, 88, 87	85	7	5		
Dried peas	0.01	95, 91, 85, 93, 88	90	4	5	90	3
	0.10	90, 89, 93, 89, 87	90	2	5		

RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 120% and the RSD was below 20% for flurochloridone for both transitions in all matrices, in compliance with SANCO/825/00 rev. 8.1 requirements.

Table A 38: Characteristics for the analytical method used for residue determination of flurochloridone in matrices of plant origin

	Flurochloridone
Specificity	A highly specific detection system was used (GC-MS/MS) and two mass transitions were monitored. The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No interferences above 30 % of the LOQ at the retention time of the analyte were detected in the untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of eight intermediate calibration standard solutions were prepared in hexane/acetone (70/30, v/v). The linearity was determined with seven matrix standards ranging from 1.5 ng/mL to 200 ng/mL (corresponding to 0.003 mg/kg to 0.4 mg/kg in matrix). The calibrations were found linear with coefficients of determination $R^2 \geq 0.990$ (1/X weighting): Flurochloridone in apples: 311 → 174 m/z: Y = 8666.53 x + 455.038, R^2= 0.9967 145 → 95 m/z: Y = 6467.54 x + 673.915, R^2 = 0.9974 Flurochloridone in dried peas: 311 → 174 m/z: Y = 19308.7 x + 4649.65, R^2= 0.9960

	Flurochloridone
	145 → 95 m/z: Y = 11142.1 x + 2775.26, R^2 = 0.9963
Assessment of matrix effects is presented	Matrix effects on the detection of Flurochloridone in extracts of matrix apples were found to be significant (≥ 20 %). Therefore, matrix-matched standards were used for quantification. Matrix effects on the detection of Flurochloridone in extracts of matrix dry peas were found to be insignificant (< 20 %). However, matrix-matched standards were used for quantification.
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.01 mg/kg. The limit of detection (LOD) was set at 0.003 mg/kg, which is 30% of the LOQ.
Extract Stability	Flurochloridone was found to be stable in final extracts of apples for at least 7 days and of dry peas for at least 10 days when stored at 1 °C to 10 °C in the dark.

Conclusion

The method was found to be valid for the determination of flurochloridone in plant commodities (apples and dried peas) with a limit of quantification (LOQ) of 0.01 mg/kg.

Hauler, C. (2018a)

A 2.1.2.1.1.3 Confirmatory method (if required)

The method of Garrigue, P. (2017a), validated independently by Hauler, C. (2018a), includes GC-MS/MS, which achieves a high level of specificity. Thus, an additional confirmatory method is not required.

A 2.1.2.1.1.4 Extraction efficiency

Not required since no residue at or above LOQ are expected in plant matrices.

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

A 2.1.2.2.1 Analytical method 1

An analytical method for the determination of the active substance flurochloridone in different animal commodities has been validated by Garrigue, P. (2017b) (see KCP 5.2/03). This method was independently validated by Hauler, C. (2018b) (see KCP 5.2/04).

A 2.1.2.2.1.1 Method validation

Comments of zRMS:	It was demonstrated (in compliance with the principles of Good Laboratory Practices (GLP)), that the validation method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries of SANCO/825/00 rev. 8.1 and is therefore applicable to correctly determine residues of flurochloridone in animal matrices (milk, fat, eggs, muscle and kidney) with a limit of quantification (LOQ) of 0.01 mg/kg. The study is acceptable.

Reference:	**2.1.2.2.1.1/01 (KCP 5.2/03)**
Report	Garrigue, P. (2017b) Validation of Residue Analytical Method for Determination of Flurochloridone in Animal Matrices Report No. BPL17-0002, Sponsor Reference No. 90020484
Guideline(s):	SANCO/825/00 rev. 8.1

Deviations: No.

GLP Yes

Acceptability: Yes

Materials and Methods

A residue analytical method for flurochloridone in animal commodities (milk, fat, eggs, muscle and kidney) in analogy to the multi-residue method QuEChERS (EN15662) was validated. The method involves extraction of flurochloridone with acetonitrile / water, followed by liquid-liquid partition using QuEChERS EN15662 salt-mixture, then C18/PSA clean-up, freezing step, and concentration into hexane / acetone, prior to analysis by gas chromatography coupled with tandem mass spectrometric detection (GC-MS/MS) using external calibration. The limit of quantification (LOQ) was set to 0.01 mg/kg, expressed as sum of isomers, for all matrices.

Specimen preparation

5 g of homogenized specimen were weighed into a 50 mL plastic centrifuge tube. 10 mL (or 5 mL for milk and egg) of water was added. 10 mL acetonitrile were added and the material was shaken vigorously for 1 min using a vortex. Thereafter, QuEChERS EN15662 salt-mixture (1 g sodium citrate, 0.5 g sodium hydrogencitrate sequihydrate, 4 g magnesium sulphate, 1 g sodium chloride) was added and shaken vertically for an additional minute. The samples were centrifuged at > 3000 rpm for 5 min at room temperature.

An aliquot of the supernatant (6 mL) was transferred into a centrifuge tube containing the QuEChERs dispersive kit (900 mg of magnesium sulphate, 150 mg of C18 and 150 mg of PSA) and shaken vertically for 1 min. The samples were centrifuged at > 3000 rpm for 5 min at room temperature. An aliquot of the supernatant (4 mL) was transferred into a centrifuge tube and stored 1 hour in a freezer. It was then transferred immediately into another centrifuge tube.

An aliquot (2 mL) was transferred into another centrifuge tube to which was added 20 µL of Protectant Solution (10g olive oil dissolved in 50 mL of hexane). The extract was evaporated to dryness under nitrogen flow at room temperature. 200 µL of lindane solution (200 ng/mL) was added and the extract was brought to a volume of 2 mL with Hexane/Acetone (70/30; v/v) prior to analysis by GC-MS/MS.

Note: Lindane was used as injection qualifier and not as internal standard for quantification

Equipment for flurochloridone determination

GC-MS/MS system	Agilent 7890A GC system with 7693 Autosampler and 7000B MS/MS and Mass Hunter software
Column:	Restek Rxi 17 Sil, 30 m x 0.250 mm x 0.25 µm
Injection:	1 µL, pulse splitless (25 psi for 0.5 min)
Injection Liner:	Agilent 2mm dimpled
Injector:	Initial 50 °C (hold for 0.05 min) Heat rate 720 °C/min to 300 °C, hold for 14 min
Oven:	Initial 50 °C (hold for 1 min) Heat rate 40 °C/min to 150 °C, hold for 0 min Heat rate 20 °C/min to 340 °C, hold for 1 min
Transfer line temperature:	340 °C
Source temperature	280 °C
Quadrupoles temperature:	150 °C
Carrier gas	Helium, 1.7 mL/min
Retention time(s)	Approx. 9.2 min (flurochloridone), approx. 8.1 min (lindane)
Ionisation type:	Electron ionization, positive mode
Ion mass transition monitored (m/z) for flurochloridone :	311 → 174, collision energy = 15 eV (Quantifier) 145 → 95, collision energy = 20 eV (Qualifier)
Ion mass transition monitored (m/z) for lindane :	225 → 189, collision energy = 5 eV

Results and discussions

Table A 39: Recovery results from method validation of flurochloridone in matrices of animal origin

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 311 → 174 m/z (Quantification)							
Milk	0.01	88, 78, 81, 79, 78	81	5.0	5	90	11.8
	0.10	98, 102, 101, 101, 97	100	2.1	5		
Fat	0.01	115, 93, 110, 101, 109	106	8.1	5	110	8.1
	0.10	118, 109, 109, 117, 124	115	5.8	5		
Eggs	0.01	89, 101, 105, 100, 110	101	7.9	5	111	11.2
	0.10	112, 122, 119, 132, 114	120	6.6	5		
Muscle	0.01	89, 86, 86, 86, 89	87	1.6		92	5.9
	0.10	100, 100, 93, 94, 96	97	3.4			
Kidney	0.01	103, 131, 98, 115, 96	109	13.1	5	109	11.0
	0.10	100, 99, 119, 122, 104	109	10.1	5		
Ion Mass Transition 145 → 95 m/z (Confirmation)							
Milk	0.01	92, 81, 81, 80, 78	82	6.9	5	94	13.2
	0.10	102, 106, 107, 106, 103	105	1.9	5		
Fat	0.01	112, 92, 109, 97, 108	103	8.2	5	110	9.0
	0.10	120, 110, 111, 118, 125	117	5.3	5		
Eggs	0.01	93, 102, 104, 100, 109	101	5.9	5	109	9.6
	0.10	112, 119, 116, 129, 109	117	6.4	5		
Muscle	0.01	89, 87, 87, 89, 90	88	1.5	5	92	4.7
	0.10	98, 98, 92, 93, 96	95	3.0	5		
Kidney	0.01	108, 133, 96, 115, 102	111	13.1	5	110	10.6
	0.10	103, 100, 119, 121, 104	109	8.9	5		

Calculation was performed on unrounded values. No correction by blank value was performed.
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 120% and the RSD was below 20% for flurochloridone for both transitions in all matrices, in compliance with SANCO/825/00 rev. 8.1 requirements.

Table A 40: Characteristics for the analytical method used for residue determination of flurochloridone in matrices of animal origin

	Flurochloridone
Specificity	A highly specific detection system was used (GC-MS/MS) and two mass transitions were monitored. The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No interferences above 30 % of the LOQ at the retention time of the analyte were detected in the untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of seven calibration standard solutions were prepared in a lindane/olive oil solution diluted in hexane/acetone (70/30, v/v). The linearity was determined with seven matrix or solvent standards ranging from 1.5 ng/mL to 200 ng/mL (corresponding to 0.003 mg/kg to 0.4 mg/kg in matrix). The calibrations were found linear with coefficients of determination $R^2 \geq 0.990$ (1/X weighting): Flurochloridone in solvent for milk, fat, muscle and kidney analysis: 311 → 174 m/z: Y = 976.429965 * x - 85.399961, R^2= 0.9981 145 → 95 m/z: Y = 809.265327 * x -18.010189, R^2 = 0.9981

	Flurochloridone
	Flurochloridone in egg: 311 → 174 m/z: Y = 773.320995 * x + 0.517884, R^2= 0.9971 145 → 95 m/z: Y = 602.150074 * x + 49.715775, R^2 = 0.9970
Assessment of matrix effects is presented	Due to non-significant matrix effect (< ±20%) on signal, quantification using standard solutions prepared in solvent was performed for milk, fat, muscle and kidney. Although non-significant matrix effect (< ±20%) on signal was found in egg, quantification using matrix matched standards was performed.
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.01 mg/kg. The limit of detection (LOD) was set at 0.003 mg/kg, which is 30% of the LOQ.
Extract Stability	Flurochloridone was found to be stable in final extracts of each matrix for at least 9 days when stored at < -18°C, except for high recoveries in fat, muscle and kidney for which no degradation was observed.

Conclusion

The method was found to be valid for the determination of flurochloridone in animal commodities (milk, fat, eggs, muscle and kidney), with a limit of quantification (LOQ) of 0.01 mg/kg, in accordance to SANCO/825/00 rev. 8.1 requirements.

Garrigue, P. (2017b)

A 2.1.2.2.1.2 Independent laboratory validation

Comments of zRMS:	The method was independently and successfully validated for the determination of flurochloridone in liver and fat according to the guidance document SANCO/825/00, rev. 8.1, with a limit of quantification (LOQ) of 0.01 mg/kg. All mean recovery values at the fortification levels of 0.01 mg/kg and 0.1 mg/kg for both mass transitions comply with the standard acceptance criteria of SANCO/825/00, rev. 8.1 since mean recoveries were in the range of 70 - 120% with relative standard deviations of ≤20% for all matrices at each level. The study is acceptable.

Reference:	**2.1.2.2.1.2/01 (KCP 5.2/04)**
Report	Hauler, C. (2018b) Independent Laboratory Validation of an Analytical Method for the Determination of Flurochloridone in Different Matrices of Animal Origin Report No. S17-08036 and amendment 1; Sponsor Reference No. 90021293
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No.
GLP	Yes
Acceptability:	Yes

Materials and Methods

The residue analytical method for flurochloridone in animal commodities validated by Garrigue, P. (2017b), report No. BPL17-0002, was independently validated by Hauler, C. (2018b) in fat and liver. The method involves extraction of flurochloridone with acetonitrile/water, followed by liquid-liquid partition using QuEChERS EN15662 salt-mixture, then C18/PSA clean-up, freezing step, and concentration into hexane / acetone, prior to analysis by gas chromatography coupled with tandem mass spectrometric detection (GC-MS/MS) using external calibration. The limit of quantification (LOQ) was set to 0.01 mg/kg, expressed as sum of isomers, for all matrices.

Primary validation and independent laboratory validation were carried out at different locations, by different study personnel, and using different instrumentation and stocks of chemicals. No addition or

modification to the original method other than optimization of instrumental parameters was done. No communication with the method developers or others familiar with the method was necessary to carry out the analysis.

Specimen preparation

5 g of homogenized specimen were weighed into a 50 mL plastic centrifuge tube. 5 mL (or 10 mL for solid matrices) of water was added. 10 mL acetonitrile were added and the material was shaken vigorously for 1 min using a vortex. Thereafter, QuEChERS EN15662 salt-mixture (1 g sodium citrate, 0.5 g sodium hydrogencitrate sequihydrate, 4 g magnesium sulphate, 1 g sodium chloride) was added and shaken vertically for an additional minute. The samples were centrifuged at 4000 rpm for 5 min at room temperature.

An aliquot of the supernatant (6 mL) was transferred into a centrifuge tube containing the QuEChERs dispersive kit (900 mg of magnesium sulphate, 150 mg of C18 and 150 mg of PSA) and shaken vertically for 1 min. The samples were centrifuged at 4000 rpm for 5 min at room temperature. An aliquot of the supernatant (4 mL) was transferred into a centrifuge tube and stored 1 hour in a freezer. It was then transferred immediately into another centrifuge tube.

An aliquot (2 mL) was transferred into another centrifuge tube to which 20 µL of Protectant Solution (10g olive oil dissolved in 50 mL of hexane) was added. The extract was evaporated to dryness under nitrogen flow at room temperature. 200 µL of lindane solution (200 ng/mL) was added and the extract was brought to a volume of 2 mL with Hexane/Acetone (70/30; v/v) prior to analysis by GC-MS/MS.

Note: Lindane was used as injection qualifier and not as internal standard for quantification

Equipment for flurochloridone determination

GC-MS/MS system	Thermo TSQ 8000 Evo GC-MS/MS system with TriPlus RSH Autosampler
Column:	VF-17-MS, 30 m x 0.250 mm x 0.25 µm
Injection:	1 µL, splitless
Injection port:	Programmable Temperature Vaporizing (PTV)
Injection temperature program:	50 °C (hold for 1.0 min), 12 °C/sec to 300 °C (hold for 14.0 min)
Oven:	Initial 50 °C (hold for 1 min) Heat rate 40 °C/min to 150 °C, hold for 0 min Heat rate 20 °C/min to 340 °C, hold for 1 min
Transfer line temperature:	280 °C
Source temperature	280 °C
Purge flow:	5 mL/min
Carrier gas	Helium, 1.7 mL/min
Retention time(s)	Approx. 9.0 min (flurochloridone), approx. 7.8 min (lindane)
Ionisation type:	Electron ionization, positive mode
Ion mass transition monitored (m/z) for flurochloridone :	311 → 174, collision energy = 15 eV (Quantifier)
	145 → 95, collision energy = 20 eV (Qualifier)
Ion mass transition monitored (m/z) for lindane :	219 → 183, collision energy = 5 eV

Results and discussions

Table A 41: Recovery results from independent laboratory validation of flurochloridone in matrices of animal origin

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 311 → 174 m/z (Quantification)							
Liver	0.01	72, 73, 83, 77, 81	77	6	5	79	5
	0.10	77, 81, 82, 82, 79	80	3	5		
Fat	0.01	73, 72, 70, 76, 77	74	4	5	73	4
	0.10	74, 78, 73, 73, 69	73	4	5		
Ion Mass Transition 145 → 95 m/z (Confirmation)							
Liver	0.01	70, 72, 80, 77, 83	76	7	5	79	6
	0.10	79, 81, 84, 82, 80	81	2	5		

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Fat	0.01	74, 75, 65, 73, 80	73	7	5	74	5
	0.10	77, 78, 74, 73, 72	75	3	5		

RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 120% and the RSD was below 20% for flurochloridone for both transitions in all matrices, in compliance with SANCO/825/00 rev. 8.1 requirements.

Table A 42: Characteristics for the analytical method used for residue determination of flurochloridone in matrices of animal origin

	Flurochloridone
Specificity	A highly specific detection system was used (GC-MS/MS) and two mass transitions were monitored. The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No interferences above 30 % of the LOQ at the retention time of the analyte were detected in the untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of eight intermediate calibration standard solutions were prepared in hexane/acetone (70/30, v/v). The linearity was determined with seven matrix standards ranging from 1.5 ng/mL to 200 ng/mL (corresponding to 0.003 mg/kg to 0.4 mg/kg in matrix). The calibrations were found linear with coefficients of determination $R^2 \geq 0.990$ (1/X weighting): Flurochloridone in liver: 311 → 174 m/z: Y = 27905.7 x + 8639.22, R^2= 0.9952 145 → 95 m/z: Y = 20874.9 x + 6069.78, R^2 = 0.9955 Flurochloridone in fat: 311 → 174 m/z: Y = 26996.9 x + 7406.59, R^2= 0.9951 145 → 95 m/z: Y = 20088.2 x + 6931.46, R^2 = 0.9952
Assessment of matrix effects is presented	Matrix effects were ≥ ± 20 % and deemed to be significant for the ion mass transition used for confirmation for fat. Therefore, matrix-matched standards were used throughout the study. Matrix suppression or enhancement was < 20 % for liver and the ion mass transition used for quantification for fat and thus deemed to be insignificant. However, matrix-matched standards were used for quantification throughout the study.
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.01 mg/kg. The limit of detection (LOD) was set at 0.003 mg/kg, which is 30% of the LOQ.
Extract Stability	Flurochloridone was found to be stable in final extracts of liver for at least 8 days and of fat for at least 8 days when stored at 1 °C to 10 °C in the dark.

Conclusion

The method was found to be valid for the determination of flurochloridone in animal commodities (fat and liver) with a limit of quantification (LOQ) of 0.01 mg/kg.

Hauler, C. (2018b)

A 2.1.2.2.1.3 Confirmatory method (if required)

The method of Garrigue, P. (2017b), validated independently by Hauler, C. (2018b), includes GC-MS/MS, which achieves a high level of specificity. Thus, an additional confirmatory method is not required.

A 2.1.2.2.1.4 Extraction efficiency

Not required since no residue at or above LOQ are expected in animal matrices.

A 2.1.2.3 Description of analytical methods for the determination of flurochloridone residues in soil

A 2.1.2.3.1 Analytical method 1

An analytical method for the determination of the active substance flurochloridone and metabolite R406639 in soil has been validated Brown, D. (2018) (see KCP 5.2/05).

A 2.1.2.3.1.1 Method validation

Comments of zRMS:	The analytical method for the determination of flurochloridone (2 isomers: FND-311-Trans and FND-311-Cis) and metabolite R406639 in three different soils has been demonstrated to be satisfactory in terms of accuracy, precision, linearity, and specificity. The method was fully validated with a limit of quantification (LOQ) of 0.01 mg/kg for flurochloridone and 0.001 mg/kg for metabolite R406639, in accordance to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 requirements. System linearity, assay accuracy and precision, system suitability, limit of detection and stability in solvent were determined by using the method AP.226834A. The study is acceptable.

Reference:	**2.1.2.3.1.1/01 (KCP 5.2/05)**
Report	Brown, D. (2018) Flurochloridone (2 isomers) and Metabolite, R406639: Non-radiolabelled Environmental Fate Method Validation in Three Soils Report No. 39322 and amendment 1, Sponsor Reference No. 90020530
Guideline(s):	SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4
Deviations:	No.
GLP	Yes
Acceptability:	Yes

Materials and Methods

A residue analytical method for flurochloridone and metabolite R406639 in 3 types of soil (see table below for details of soil characteristics) was validated. The method involves extraction of flurochloridone and metabolite R406639 with acetonitrile / water (80:20, v/v) three times, followed by centrifugation and, for flurochloridone only, by concentration into acetonitrile, prior to analysis by liquid chromatography coupled with tandem mass spectrometric detection (LC-MS/MS) using external calibration. The limit of quantification (LOQ) was set to 0.01 mg/kg for flurochloridone (2 isomers, cis and trans) and 0.001 mg/kg for metabolite R406639, for all matrices.

Specimen preparation

For study samples, 100 g (oven dried equivalent) of soil were weighed and extracted three times with 300 mL of acetonitrile:water (80:20, v/v). After adding 300 mL of extraction solvent, samples were shaken on a flatbed shaker for 15 min at 250 rpm. Samples were then centrifuged for 10 min at 3000 rpm and supernatant decanted into a 1L measuring cylinder. The three extracts were combined and the volume was made up to 1 L with acetonitrile:water (80:20, v/v).

For flurochloridone analysis, an aliquot of the final extract was ultra-centrifuged prior to analysis by LC-MS/MS

For R406639 analysis, 12 mL of the extract was ultra-centrifuged and 10 mL of the clear extract was dried down to 1mL at 40 °C. Final extract was made up to 2 mL with acetonitrile prior to analysis by LC-MS/MS.

For recovery and control samples, 10 g (oven dried equivalent) of soil were weighed and extracted three times with 30 mL of acetonitrile:water (80:20, v/v). After adding 30 mL of extraction solvent, samples were shaken on a flatbed shaker for 15 min at 250 rpm. Samples were then centrifuged for 10 min at 3000 rpm and supernatant decanted into a 100 mL measuring cylinder. The three extracts were combined and the volume was made up to 100 mL with acetonitrile:water (80:20, v/v).
For flurochloridone analysis, an aliquot of the final extract was ultra-centrifuged prior to analysis by LC-MS/MS
For R406639 analysis, 12 mL of the extract was ultra-centrifuged and 10 mL of the clear extract was dried down to 1mL at 40 °C. Final extract was made up to 2mL with acetonitrile prior to analysis by LC-MS/MS.

Table A 43: Soil characteristics

Charles River Reference		**SII 54**	**SII 52**	**SII 53**
Soil Code		B1	E1	J1
US Taxonomy		Entisol (Psamment)	Inceptisol (dystric eutrudept)	Mollisol (lithic haprendoll)
Soil Site Location		Ingleby Acid, Derbyshire	Brierlow, Derbyshire	Chapel Hill Farm, Empingham, Rutland
Soil Sampling Date		20 March 2017	10 February 2017	22 March 2017
Moisture Content (%)		17.7	44.3	42.7
pH (determined in Water)		4.1	5.5	7.8
pH (determined in 0.01M calcium Chloride)		3.7	5.1	7.4
Organic Carbon (w/w)		2.4	3.4%	7.8
Water holding Capacity at pF2(w/w)		17.8%	42.1%	43.9
Particle Size Distribution (UK)	**Sand**	71%	11%	35%
	Silt	17%	69%	33%
	Clay	12%	20%	32%
Cation Exchange Capacity (meq/100g)		9.8	20.0	33.0

Equipment for flurochloridone determination

LC-MS/MS system	Shimadzu Prominence UPLC LC pump and autosampler, Applied Biosystems Sciex API 5000 Triple Quadrupole Mass Spectrometer, and Analyst Version 1.6.2 software
Column:	Hypercarb, 3.0 x 100 mm, 5 µm
Column temperature:	40 °C
Autosampler temperature:	4 °C
Injection Volume:	30 µL
Mobile phases:	Eluent A: 0.01 % formic acid in water Eluent B: 0.01 % formic acid in acetonitrile
Gradient:	

Time [min]	Eluent A	Eluent B	Flow rate [mL/min]
0.0	40	60	1.0
5.0	40	60	1.0
15.0	1	99	1.0
16.0	1	99	1.0
16.1	40	60	1.0
18.0	40	60	1.0

Retention time(s)	Approx. 9.4 min (flurochloridone cis isomer), approx. 12.5 min (flurochloridone trans isomer)
Ionisation type:	TurboIonSpray (ESI), positive mode
Ion mass transition monitored (m/z) for flurochloridone :	312 → 292, collision energy = 32 eV (Quantifier)
	312 → 53, collision energy = 51 eV (Qualifier)
	312 → 89, collision energy = 35 eV (Qualifier)

Equipment for R406639 determination

LC-MS/MS system	Shimadzu Nexera X2 UPLC LC pump and autosampler, Applied Biosystems Sciex API

	5000 Triple Quadrupole Mass Spectrometer, and Analyst Version 1.6.2 software
Column:	ACE 3 C18 PFP 150 × 3.0 mm
Column temperature:	40 °C
Autosampler temperature:	4 °C
Injection Volume:	5 µL
Mobile phases:	Eluent A: 0.01 % formic acid in water Eluent B: 0.01 % formic acid in acetonitrile
Gradient:	

Time [min]	Eluent A	Eluent B	Flow rate [mL/min]
0.0	99	1	0.75
1.0	99	1	0.75
3.0	1	99	0.75
4.0	1	99	0.75
4.1	99	1	0.75
5.0	99	1	0.75

Retention time(s)	Approx. 1.36 min (R406639)
Ionisation type:	APCI, positive mode
Ion mass transition monitored (m/z) for R406639 :	292 → 79, collision energy = 26 eV (Quantifier) 292 → 64, collision energy = 68 eV (Qualifier)

Results and discussions

Table A 44: Recovery results from method validation of flurochloridone cis isomer in soil

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery* (%)	Overall RSD* (%)
Ion Mass Transition 312 → 292 m/z (Quantification)							
Soil SII 54	0.01	92, 89, 88, 85, 92	89	3	5	90	3
	1.00	89, 87, 94, 91, 95	91	4	5		
Soil SII 52	0.01	82, 87, 86, 101, 95	90	9	5	98	11
	1.00	106, 107, 100, 112, 108	107	4	5		
Soil SII 53	0.01	83, 85, 75, 73, 82	79	7	5	82	6
	1.00	88, 84, 83, 82, 84	84	3	5		
Ion Mass Transition 312 → 89 m/z (Confirmation)							
Soil SII 54	0.01	93, 87, 87, 85, 92	89	4	5	90	4
	1.00	87, 88, 91, 92, 97	91	4	5		
Soil SII 52	0.01	83, 92, 89, 104, 96	93	9	5	100	9
	1.00	107, 107, 102, 110, 107	107	3	5		
Soil SII 53	0.01	83, 86, 75, 77, 82	81	6	5	84	6
	1.00	90, 89, 85, 86, 88	88	2	5		
Ion Mass Transition 312 → 53 m/z (Confirmation)							
Soil SII 54	0.01	91, 89, 87, 83, 90	88	4	5	90	3
	1.00	89, 89, 93, 91, 94	91	2	5		
Soil SII 52	0.01	85, 91, 87, 103, 96	92	8	5	100	10
	1.00	106, 107, 103, 114, 106	107	4	5		
Soil SII 53	0.01	85, 88, 78, 76, 83	82	6	5	86	6
	1.00	91, 87, 89, 89, 91	89	2	5		

* Calculated from reported individual recoveries values
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 120% and the RSD was below 20% for flurochloridone cis isomer for both transitions in all matrices, in compliance with SANCO/825/00 rev. 8.1 requirements.

Table A 45: Recovery results from method validation of flurochloridone trans isomer in soil

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery* (%)	Overall RSD* (%)
Ion Mass Transition 312 → 292 m/z (Quantification)							
Soil SII 54	0.01	94, 89, 87, 84, 93	90	5	5	92	5
	1.00	88, 92, 96, 94, 98	94	4	5		
Soil SII 52	0.01	81, 86, 85, 95, 86	87	6	5	93	8
	1.00	96, 98, 94, 104, 100	98	4	5		
Soil SII 53	0.01	83, 85, 76, 75, 82	80	6	5	83	5
	1.00	87, 86, 84, 82, 87	85	2	5		
Ion Mass Transition 312 → 89 m/z (Confirmation)							
Soil SII 54	0.01	91, 88, 87, 86, 92	89	3	5	91	5
	1.00	89, 91, 95, 93, 101	94	5	5		
Soil SII 52	0.01	82, 88, 87, 99, 86	88	7	5	93	8
	1.00	97, 98, 92, 103, 99	98	4	5		
Soil SII 53	0.01	84, 88, 79, 78, 82	82	5	5	86	6
	1.00	93, 90, 88, 86, 91	90	3	5		
Ion Mass Transition 312 → 53 m/z (Confirmation)							
Soil SII 54	0.01	91, 88, 84, 82, 92	87	5	5	90	5
	1.00	88, 91, 94, 93, 99	93	5	5		
Soil SII 52	0.01	84, 89, 88, 96, 86	89	5	5	93	6
	1.00	95, 98, 92, 102, 98	97	4	5		
Soil SII 53	0.01	86, 88, 79, 78, 84	83	5	5	86	5
	1.00	91, 89, 90, 86, 91	89	2	5		

* Calculated from reported individual recoveries values
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 120% and the RSD was below 20% for flurochloridone trans isomer for both transitions in all matrices, in compliance with SANCO/825/00 rev. 8.1 requirements.

Table A 46: Recovery results from method validation of R406639 in soil

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery* (%)	Overall RSD* (%)
Ion Mass Transition 292 → 79 m/z (Quantification)							
Soil SII 54	0.001	96, 85, 89, 83, 88	88	6	5	85	7
	0.10	78, 85, 83, 82, 77	81	4	5		
Soil SII 52	0.001	92, 102, 91, 91, 100	95	6	5	95	5
	0.10	90, 101, 94, 94, 96	95	4	5		
Soil SII 53	0.001	95, 89, 98, 92, 95	94	4	5	93	4
	0.10	97, 90, 87, 94, 89	91	4	5		
Ion Mass Transition 292 → 64 m/z (Confirmation)							

Matrix	Fortification level (mg/kg)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery* (%)	Overall RSD* (%)
Soil SII 54	0.001	91, 90, 91, 81, 84	88	5	5	85	6
	0.10	88, 83, 83, 79, 76	82	5	5		
Soil SII 52	0.001	89, 99, 93, 97, 98	95	4	5	93	4
	0.10	88, 97, 91, 89, 92	92	4	5		
Soil SII 53	0.001	94, 92, 92, 89, 98	93	3	5	93	5
	0.10	95, 95, 92, 100, 83	93	7	5		

* Calculated from reported individual recoveries values
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 120% and the RSD was below 20% for R406639 for both transitions in all matrices, in compliance with SANCO/825/00 rev. 8.1 requirements.

Table A 47: Characteristics for the analytical method used for residue determination of flurochloridone (cis and trans isomers) and metabolite R406639 in soil

	Flurochloridone (2isomers)	R406639 (metabolite)
Specificity	A highly specific detection system was used (LC-MS/MS) and three mass transitions were monitored. The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No interferences above 30 % of the LOQ at the retention time of the analyte were detected in the untreated control samples.	A highly specific detection system was used (LC-MS/MS) and two mass transitions were monitored. The retention time of R406639 in the samples matches the retention time in the standard solutions. No interferences above 30 % of the LOQ at the retention time of the analyte were detected in the untreated control samples.
Calibration (type, number of data points) Calibration range	A series of intermediate calibration standard solutions were prepared in acetonitrile/water (80/20, v/v). The linearity was determined with eight matrix standards ranging from 1.5 ng/mL (30% of LOQ) to 20 ng/mL. In order to be within the linear calibration range extracts of samples fortified at 100x LOQ were diluted with control extract 100 fold. The calibrations were found linear with correlation coefficients r ≥ 0.995 (1/X weighting): Flurochloridone cis in soil: 312 → 292 m/z: $Y = 1.03\ e^{+004} * x - 1.47\ e^{+003}$, r = 0.9996 312 → 89 m/z: $Y = 1.8\ e^{+004} * x - 3.72\ e^{+003}$, r = 0.9992 312 → 53 m/z: $Y = 2.41\ e^{+004} * x - 3.94\ e^{+003}$, r = 0.9993 Flurochloridone trans in soil: 312 → 292 m/z: $Y = 1.46\ e^{+004} * x - 1.87\ e^{+003}$, r = 0.9994 312 → 89 m/z: $Y = 2.15\ e^{+004} * x - 3.12\ e^{+003}$, r = 0.9992 312 → 53 m/z: $Y = 3.09\ e^{+004} * x - 5.6\ e^{+003}$, r = 0.9995	A series of intermediate calibration standard solutions were prepared in acetonitrile/water (80/20, v/v). The linearity was determined with seven matrix standards ranging from 0.03 ng/mL (30% of LOQ) to 1.5 ng/mL. In order to be within the linear calibration range extracts of samples fortified at 100x LOQ were diluted with control extract 100 fold. The calibrations were found linear with correlation coefficients r ≥ 0.995 (1/X weighting): R406639 trans in soil: 292 → 79 m/z: $Y = 3.92\ e^{+005} * x + 8.78\ e^{+003}$, r = 0.9988 292 → 64 m/z: $Y = 1.19\ e^{+005} * x + 1.99\ e^{+003}$, r = 0.9997
Assessment of matrix effects is presented	Not assessed, however matrix matched standards were used for quantitation.	
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.01 mg/kg. The limit of detection (LOD) was set at 0.003 mg/kg, which is 30% of the LOQ.	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.001 mg/kg. The limit of detection (LOD) was set at 0.0003 mg/kg, which is 30% of the LOQ.
Extract Stability	Not assessed. Extracts were analysed against freshly prepared matrix matched standards for each analysis.	
Standard stability	Stocks solutions at 0.1 ug/mL to 100 ug/mL are stable for at least 59 days when stored at -20°C	Stocks solutions at 0.001 ug/mL to 100 ug/mL are stable for at least 57 days when stored at -20°C

Conclusion

The method was found to be valid for the determination of flurochloridone (cis and trans isomers) and metabolite R406639 in soil, with a limit of quantification (LOQ) of 0.01 mg/kg for flurochloridone and 0.001 mg/kg for metabolite R406639, in accordance to SANCO/825/00 rev. 8.1 requirements.

Brown, D. (2018)

A 2.1.2.3.1.2 Confirmatory method

The method of Brown, D. (2018) includes LC-MS/MS, which achieves a high level of specificity. Thus, an additional confirmatory method is not required.

A 2.1.2.4 Description of analytical methods for the determination of flurochloridone in water (KCP 5.2)

A 2.1.2.4.1 Analytical method 1

An analytical method for the determination of the active substance flurochloridone in surface and drinking water has been validated by Garrigue, P. (2017c) (see KCP 5.2/06). This method was independently validated by Hauler, C. (2018c) (see KCP 5.2/07).

A 2.1.2.4.1.1 Method validation

Comments of zRMS:	It was demonstrated that the validation method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries of SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 and is therefore applicable to correctly determine residues of flurochloridone in drinking water and surface water with a limit of quantification (LOQ) of 0.05 µg/L. Mean recoveries at each fortification level were between 70 and 120%, and the RSD was below 20% for flurochloridone for both transitions in drinking water and surface water The study is acceptable.

Reference:	**2.1.2.4.1.1/01 (KCP 5.2/06)**
Report	Garrigue, P. (2017c) Validation of Residue Analytical Method for Determination of Flurochloridone in Water Report No. BPL17-0004 and amendment 1, Sponsor Reference No. 90020486
Guideline(s):	SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4
Deviations:	No.
GLP	Yes
Acceptability:	Yes

Materials and Methods

A residue analytical method for flurochloridone in water (drinking water and surface water) was validated. The method involves extraction of flurochloridone by liquid-liquid partition using hexane, followed by evaporation and concentration into hexane / acetone, prior to analysis by gas chromatography coupled with tandem mass spectrometric detection (GC-MS/MS) using external calibration. The limit of quantification (LOQ) was set to 0.05 µg/L, expressed as sum of isomers, for all matrices.

Specimen preparation

200 mL of homogenized specimen were weighed into a 500 mL glass bottle. 20 mL of hexane was added and the material was shaken vigorously on a reciprocating flatbed shaker for 30 min.

An aliquot of the supernatant (15 mL) was transferred into a 15 mL centrifuge tube to which was added 20 µL of Protectant Solution (15g olive oil dissolved in 50 mL of hexane). The extract was evaporated to dryness under nitrogen flow at room temperature. 150 µL of lindane solution (200 ng/mL) was added and the extract was brought to a volume of 1.5 mL with Hexane/Acetone (70/30; v/v) prior to analysis by GC-MS/MS.

Note: Lindane was used as injection qualifier and not as internal standard for quantification

Equipment for flurochloridone determination

GC-MS/MS system	Agilent 7890A GC system with 7693 Autosampler and 7000B MS/MS and Mass Hunter software
Column:	Restek Rxi 17 Sil, 30 m x 0.250 mm x 0.25 µm
Injection:	1 µL, pulse splitless (25 psi for 0.5 min)
Injection Liner:	Agilent 2mm dimpled
Injector:	Initial 50 °C (hold for 0.05 min) Heat rate 720 °C/min to 300 °C, hold for 14 min
Oven:	Initial 50 °C (hold for 1 min) Heat rate 40 °C/min to 150 °C, hold for 0 min Heat rate 20 °C/min to 340 °C, hold for 1 min
Transfer line temperature:	340 °C
Source temperature	280 °C
Quadrupoles temperature:	150 °C
Carrier gas	Helium, 1.7 mL/min
Retention time(s)	Approx. 9.2 min (flurochloridone), approx. 8.1 min (lindane)
Ionisation type:	Electron ionization, positive mode
Ion mass transition monitored (m/z) for flurochloridone :	311 → 174, collision energy = 15 eV (Quantifier)
	145 → 95, collision energy = 20 eV (Qualifier)
Ion mass transition monitored (m/z) for lindane :	225 → 189, collision energy = 5 eV

Results and discussions

Table A 48: Recovery results from method validation of flurochloridone in water (drinking water and surface water)

Matrix	Fortification level (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 311 → 174 m/z (Quantification)							
Drinking water	0.05	97, 100, 106, 111, 106	104	5.3	5	111	7.5
	0.5	118, 116, 117, 118, 120	118	1.3	5		
Surface water	0.05	101, 96, 97, 98, 111	101	5.9	5	105	6.5
	0.5	110, 111, 116, 107, 104	110	4.0	5		
Ion Mass Transition 145 → 95 m/z (Confirmation)							
Drinking water	0.05	95, 104, 105, 107, 101	102	4.7	5	109	7.1
	0.5	117, 115, 113, 115, 118	116	1.6	5		
Surface water	0.05	106, 101, 103, 101, 110	104	3.7	5	107	4.7
	0.5	111, 114, 115, 109, 105	111	3.6	5		

No correction by blank value was performed.
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 120% and the RSD was below 20% for flurochloridone for both transitions in all matrices, in compliance with SANCO/825/00 rev. 8.1 requirements.

Table A 49: Characteristics for the analytical method used for residue determination of flurochloridone in water (drinking water and surface water)

	Flurochloridone
Specificity	A highly specific detection system was used (GC-MS/MS) and two mass transitions were monitored. The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No interferences above 30 % of the LOQ at the retention time of the analyte were detected in the untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of seven calibration standard solutions were prepared in a lindane/olive oil solution diluted in hexane/acetone (70/30, v/v). The linearity was determined with seven matrix standards ranging from 1.5 ng/mL to 200 ng/mL (corresponding to 0.015 µg/L to 0.2 µg/L in matrix). The calibrations were found linear with coefficients of determination $R^2 \geq 0.990$ (1/X weighting): Flurochloridone in drinking water: 311 → 174 m/z: Y = 464.579566 x + 18.067203, R^2 = 0.9998 145 → 95 m/z: Y = 333.713940 x + 53.233062, R^2 = 0.9999 Flurochloridone in surface water: 311 → 174 m/z: Y = 500.538869 x + 103.214357, R^2 = 0.9992 145 → 95 m/z: Y = 347.770938 x + 62.688404, R^2 = 0.9989
Assessment of matrix effects is presented	Although non-significant matrix effect (< ±20%) on signal was found, quantification using matrix matched standards was performed for each matrix.
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.05 µg/L. The limit of detection (LOD) was set at 0.015 µg/L, which is 30% of the LOQ.
Extract Stability	Flurochloridone was found to be stable in final extracts of each matrix for at least 11 days when stored at < -18°C.

Conclusion

The method was found to be valid for the determination of flurochloridone in water (drinking water and surface water) with a limit of quantification (LOQ) of 0.05 µg/L in accordance to SANCO/825/00 rev. 8.1 requirements.

Garrigue, P. (2017c)

A 2.1.2.4.1.2 Independent laboratory validation

Comments of zRMS:	The method was independly and successfully validated for the determination of flurochloridone in drinking water with a limit of quantification (LOQ) of 0.05 µg/L in accordance to SANCO/825/00 rev. 8.1 requirements. The study is acceptable.

Reference:	**2.1.2.4.1.2/01 (KCP 5.2/07)**
Report	Hauler, C. (2018c) Independent Laboratory Validation of an Analytical Method for the Determination of Flurochloridone in Water Report No. S17-08037; Sponsor Reference No. 90021294
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No.
GLP	Yes
Acceptability:	Yes

Materials and Methods

The residue analytical method for flurochloridone in water (drinking water and surface water) validated by Garrigue, P. (2017c), report No. BPL17-0004, was independently validated by Hauler, C. (2018c) in drinking water. The method involves extraction of flurochloridone by liquid-liquid partition using hexane, followed by evaporation and concentration into hexane / acetone, prior to analysis by gas chromatography coupled with tandem mass spectrometric detection (GC-MS/MS) using external calibration. The limit of quantification (LOQ) was set to 0.05 µg/L, expressed as sum of isomers, for all matrices.

Primary validation and independent laboratory validation were carried out at different locations, by different study personnel, and using different instrumentation and stocks of chemicals. No addition or modification to the original method other than optimization of instrumental parameters was done. No communication with the method developers or others familiar with the method was necessary to carry out the analysis.

Specimen preparation

200 mL of homogenized specimen were weighed into a 500 mL glass bottle. 20 mL of hexane was added and the material was shaken vigorously on a reciprocating flatbed shaker for 30 min.
An aliquot of the supernatant (15 mL) was transferred into a 15 mL centrifuge tube to which was added 20 µL of Protectant Solution (15g olive oil dissolved in 50 mL of hexane). The extract was evaporated to dryness under nitrogen flow at room temperature. 150 µL of lindane solution (200 ng/mL) was added and the extract was brought to a volume of 1.5 mL with Hexane/Acetone (70/30; v/v), prior to analysis by GC-MS/MS.
Note: Lindane was used as injection qualifier and not as internal standard for quantification

Equipment for flurochloridone determination

GC-MS/MS system	Thermo TSQ 8000 Evo GC-MS/MS system with TriPlus RSH Autosampler
Column:	VF-17-MS, 30 m x 0.250 mm x 0.25 µm
Injection:	1 µL, splitless
Injection port:	Programmable Temperature Vaporizing (PTV)
Injection temperature program:	50 °C (hold for 1.0 min), 12 °C/sec to 300 °C (hold for 14.0 min)
Oven:	Initial 50 °C (hold for 1 min) Heat rate 40 °C/min to 150 °C, hold for 0 min Heat rate 20 °C/min to 340 °C, hold for 1 min
Transfer line temperature:	280 °C
Source temperature	280 °C
Purge flow:	5 mL/min
Carrier gas	Helium, 1.7 mL/min
Retention time(s)	Approx. 9.0 min (flurochloridone), approx. 7.8 min (lindane)
Ionisation type:	Electron ionization, positive mode
Ion mass transition monitored (m/z) for flurochloridone :	311 → 174, collision energy = 15 eV (Quantifier) 145 → 95, collision energy = 20 eV (Qualifier)
Ion mass transition monitored (m/z) for lindane :	219 → 183, collision energy = 5 eV

Results and discussions

Table A 50: Recovery results from independent laboratory validation of flurochloridone in drinking water

Matrix	Fortification level (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 311 → 174 m/z (Quantification)							
Drinking water	0.05	97, 119, 106, 110, 117	110	8	5	105	10
	0.5	86, 98, 102, 113, 105	101	10	5		
Ion Mass Transition 145 → 95 m/z (Confirmation)							
Drinking water	0.05	105, 120, 96, 102, 113	107	9	5	102	10
	0.5	87, 92, 97, 108, 100	97	8	5		

RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 120% and the RSD was below 20% for flurochloridone for both transitions, in compliance with SANCO/825/00 rev. 8.1 requirements.

Table A 51: Characteristics for the analytical method used for residue determination of flurochloridone in drinking water

	Flurochloridone
Specificity	A highly specific detection system was used (GC-MS/MS) and two mass transitions were monitored. The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No interferences above 30 % of the LOQ at the retention time of the analyte were detected in the untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of eight intermediate calibration standard solutions were prepared in hexane/acetone (70/30, v/v). The linearity was determined with seven matrix standards ranging from 1.5 ng/mL to 200 ng/mL (corresponding to 0.015 µg/L to 2 µg/L in matrix). The calibrations were found linear with coefficients of determination $R^2 \geq 0.990$ (1/X weighting): Flurochloridone in drinking water: 311 → 174 m/z: Y = 6548.5 x + 2668.37, R^2 = 0.9951 145 → 95 m/z: Y = 5223.61 x + 1661.99, R^2 = 0.9954
Assessment of matrix effects is presented	Matrix suppression or enhancement was < 20 % for drinking water and thus deemed to be insignificant. However, matrix-matched standards were used for quantification throughout the study.
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.05 µg/L. The limit of detection (LOD) was set at 0.015 µg/L, which is 30% of the LOQ.
Extract Stability	Flurochloridone was found to be stable in final extracts of drinking water for at least 7 days when stored at 1 °C to 10 °C in the dark.

Conclusion

The method was found to be valid for the determination of flurochloridone in drinking water with a limit of quantification (LOQ) of 0.05 µg/L.

Hauler, C. (2018c)

A 2.1.2.4.1.3 Confirmatory method

The method of Garrigue, P. (2017c), validated independently by Hauler, C. (2018c), includes GC-MS/MS, which achieves a high level of specificity. Thus, an additional confirmatory method is not required.

A 2.1.2.4.2 Analytical method 2

An analytical method for the determination of the active substance flurochloridone metabolites R42819 and R406639 in surface and drinking water has been validated by Garrigue, P. (2017d) (see KCP 5.2/08).

A 2.1.2.4.2.1 Method validation

Comments of zRMS:	It was demonstrated that the validation method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries of SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 and is therefore applicable to correctly determine residues of R42819 and R406639 in drinking and surface water with a limit of quantification (LOQ) of 0.05 µg/L. The study is acceptable.

Reference: **2.1.2.4.2.1/01 (KCP 5.2/08)**

Report Garrigue, P. (2017d)

	Validation of Residue Analytical Method for Determination of Flurochloridone Metabolites R42819 and R406639 in Water Report No. BPL17-0005 and amendment 1, Sponsor Reference No. 90020487
Guideline(s):	SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4
Deviations:	No.
GLP	Yes
Acceptability:	Yes

Materials and Methods

A residue analytical method for flurochloridone metabolites R42819 and R406639 in water (drinking water and surface water) in analogy to the multi-residue method QuEChERS (EN15662) was validated. The method involves extraction of flurochloridone metabolites R42819 and R406639 with acetonitrile, followed by liquid-liquid partition using QuEChERS EN15662 salt-mixture and concentration into acetonitrile, prior to analysis by liquid chromatography coupled with tandem mass spectrometric detection (LC-MS/MS) using external calibration. The limit of quantification (LOQ) was set to 0.05 µg/L for both metabolites.

Specimen preparation

10 mL of homogenized specimen were weighed into a 50 mL plastic centrifuge tube. 10 mL acetonitrile were added and the material was shaken vigorously by hand for 1 min. Thereafter, QuEChERS EN15662 salt-mixture (1 g sodium citrate, 0.5 g sodium hydrogencitrate sequihydrate, 4 g magnesium sulphate, 1 g sodium chloride) was added and shaken by hand for an additional minute. The samples were centrifuged at 4700 rpm for 5 min at room temperature.
An aliquot (5 mL) was transferred into another centrifuge tube. The extract was evaporated to dryness under nitrogen flow at room temperature. The extract was brought to a volume of 1 mL with acetonitrile prior to analysis by LC-MS/MS.

Equipment for flurochloridone metabolites R42819 and R406639 determination

LC-MS/MS system	Shimadzu LC-30AD LC pump, CTO-20AC Oven, SIL-30ACMP Autosampler and AB Sciex API 5500 Qtrap MS Detector
Column:	Kinetex 2,6µm XB-C18 100 A, 100 X 4,6mm
Column temperature:	30 °C
Autosampler temperature:	10 °C
Injection Volume:	20 µL
Mobile phases:	Eluent A: 0.1% of formic acid in water containing 2mM of ammonium acetate Eluent B: acetonitrile
Gradient:	

Time [min]	Eluent A	Eluent B	Flow rate [mL/min]
0.0	20	80	0.8
0.5	20	80	0.8
2.5	2	98	0.8
5.0	2	98	0.8
5.5	20	80	0.8
7.0	20	80	0.8

Retention time(s)	Approx. 1.6 min (R42819), approx. 1.3 min (R406639),
Ionisation type:	TurboIonSpray (ESI), positive mode
Ion mass transition monitored (m/z) for R42819 :	278 → 258, collision energy = 31 eV (Quantifier)
	278 → 127, collision energy = 53 eV (Qualifier)
Ion mass transition monitored (m/z) for R406639 :	294 → 97, collision energy = 21 eV (Quantifier)
	294 → 69**, collision energy = 29 eV

** Not Validated due to peak interference, see below for equipment parameters for confirmatory analysis of R406639

Equipment for flurochloridone metabolite R406639 confirmatory analysis

LC-MS/MS system	Shimadzu LC-30AD LC pump, CTO-20AC Oven, SIL-30ACMP Autosampler and AB Sciex API 5500 Qtrap MS Detector

Column:	Kinetex 2,6µm XB-C18 100 A, 100 X 4,6mm
Column temperature:	30 °C
Autosampler temperature:	10 °C
Injection Volume:	20 µL
Mobile phases:	Eluent A: 0.5% of formic acid in water Eluent B: acetonitrile
Gradient:	

Time [min]	Eluent A	Eluent B	Flow rate [mL/min]
0.00	85	15	0.45
0.21	85	15	0.45
1.00	40	60	0.45
3.80	2	98	0.45
5.00	2	98	0.45
5.25	85	15	0.45
7.00	85	15	0.45

Retention time(s)	Approx. 4.1-4.2 min (R406639),
Ionisation type:	TurboIonSpray (ESI), positive mode
Ion mass transition monitored (m/z) for R406639 :	294 → 97, collision energy = 21 eV 294 → 240**, collision energy = 27 eV

** Proposed for confirmation

Results and discussions

Table A 52: Recovery results from method validation of R42819 in water (drinking water and surface water)

Matrix	Fortification level (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 278 → 258 m/z (Quantification)							
Drinking water	0.05	85, 94, 101, 110, 88	95	10.7	5	93	8.5
	0.5	95, 92, 94, 83, 90	91	5.2	5		
Surface water	0.05	91, 89, 96, 92, 93	92	3.1	5	92	2.4
	0.5	92, 92, 92, 91, 89	91	1.7	5		
Ion Mass Transition 278 → 127 m/z (Confirmation)							
Drinking water	0.05	80, 80, 96, 102, 81	88	12	5	89	8.4
	0.5	93, 92, 89, 85, 92	90	3.7	5		
Surface water	0.05	95, 88, 91, 93, 87	91	3.7	5	91	2.9
	0.5	92, 93, 89, 90, 88	90	2.3	5		

No correction by blank value was performed.
RSD = Relative Standard Deviation

Table A 53: Recovery results from method validation of R406639 in water (drinking water and surface water)

Matrix	Fortification level (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Ion Mass Transition 294 → 97 m/z (Quantification)							
Drinking water	0.05	88, 82, 88, 87, 86	86	3.0	5	87	4.4
	0.5	88, 92, 88, 80, 92	88	5.7	5		
Surface water	0.05	88, 93, 91, 91, 88	90	2.3	5	90	2.7
	0.5	89, 92, 84, 90, 88	89	3.1	5		
Ion Mass Transition 294 → 240 m/z (Confirmation)							

Matrix	Fortification level (µg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates	Overall Mean Recovery (%)	Overall RSD (%)
Drinking water	0.05	93, 84, 92, 74, 87	86	9	5	86	7.1
	0.5	84, 92, 89, 80, 84		5,5	5		
Surface water	0.05	93, 100, 87, 88, 89	91	5,6	5	92	4.3
	0.5	89, 93, 93, 96, 89	92	3,2	5		

No correction by blank value was performed.
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 120% and the RSD was below 20% for flurochloridone metabolites R42819 and R406639 for both transitions in all matrices, in compliance with SANCO/825/00 rev. 8.1 requirements.

Table A 54: Characteristics for the analytical method used for residue determination of flurochloridone metabolites R42819 and R406639 in water (drinking water and surface water)

	Flurochloridone metabolites R42819 and R406639
Specificity	A highly specific detection system was used (LC-MS/MS) and two mass transitions were monitored. The retention time of flurochloridone metabolites R42819 and R406639 in the samples matches the retention time in the standard solutions. No interferences above 30 % of the LOQ at the retention time of the analyte were detected in the untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of eight intermediate calibration standard solutions were prepared in acetonitrile. The linearity was determined with seven matrix standards ranging from 0.075 ng/mL to 10 ng/mL (corresponding to 0.015 µg/L to 2 µg/L in matrix). The calibrations were found linear with correlation coefficients r ≥ 0.995 (1/X weighting): R42819 in drinking water: 278 → 258 m/z: Y = 3.78e+005 x + 1.06e+004, r = 0.9998 278 → 127 m/z: Y = 4.81e+004 x + 1.48e+003, r = 0.9997 R42819 in surface water: 278 → 258 m/z: Y = 3.58e+005 x + 6.6e+003, r = 0.9999 278 → 127 m/z: Y = 4.6e+004 x + 177, r = 0.9998 R406639 in drinking water: 294 → 97 m/z: Y = 1.59e+005 x + 1.16e+004, r = 0.9999 294 → 240 m/z: Y = 3.48e+004 x + 361, r = 0.9967 R406639 in surface water: 294 → 97 m/z: Y = 1.54e+005 x + 9.89e+003, r = 0.9998 294 → 240 m/z: Y = 3.52e+004 x + 332, r = 0.9999
Assessment of matrix effects is presented	Due to significant matrix effect (≥ ±20%) on signal, quantification using matrix matched standards was performed on both transition of R42819 and on quantification method of R406639 for each matrix. Although non-significant matrix effect (< ±20%) on signal was found, quantification using matrix matched standards was performed on confirmation method of R406639 for each matrix.
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.05 µg/L. The limit of detection (LOD) was set at 0.015 µg/L, which is 30% of the LOQ.
Extract Stability	Metabolites R42819 and R406639 were found to be stable in final extracts of each matrix for at least 9 days when stored at 4°C.

Conclusion

The method was found to be valid for the determination of flurochloridone metabolites R42819 and R406639 in water (drinking water and surface water), with a limit of quantification (LOQ) of 0.05 µg/L, in accordance to SANCO/825/00 rev. 8.1 requirements.

Garrigue, P. (2017d)

A 2.1.2.4.2.2 Confirmatory method

The method of Garrigue, P. (2017d) includes LC-MS/MS, which achieves a high level of specificity. Thus, an additional confirmatory method is not required.

A 2.1.2.5 Description of analytical methods for the determination of flurochloridone in air (KCP 5.2)

No new method validations will be submitted for the analysis of flurochloridone in air.

A 2.1.2.6 Description of analytical methods for the determination of flurochloridone in Body Fluids and Tissues (KCP 5.2)

A 2.1.2.6.1 Analytical method 1

An analytical method for the determination of the active substance flurochloridone in body fluids matrices has been validated by Garrigue, P. (2017e) (see KCP 5.2/09). The method validation for flurochloridone residues in body tissues is presented under KCP 5.2/03 and its respective ILV under KCP 5.2/04.

A 2.1.2.6.1.1 Method validation

Comments of zRMS:	It was demonstrated that the validation method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries of SANCO/825/00 rev. 8.1 and is therefore applicable to correctly determine residues of flurochloridone in body fluids (human urine and whole blood) with a limit of quantification (LOQ) of 0.01 mg/L. The study is acceptable.

Reference:	**2.1.2.6.1.1/01 (KCP 5.2/09)**
Report	Garrigue, P. (2017e) Validation of Residue Analytical Method for Determination of Flurochloridone in Body Fluid Matrices Report No. BPL17-0003, Sponsor Reference No. 90020485
Guideline(s):	SANCO/825/00 rev. 8.1
Deviations:	No.
GLP	Yes
Acceptability:	Yes

Materials and Methods

A residue analytical method for flurochloridone in body fluid matrices (blood and urine) in analogy to the multi-residue method QuEChERS (EN15662) was validated. The method involves extraction of flurochloridone with acetonitrile / water, followed by liquid-liquid partition using QuEChERS EN15662 salt-mixture, then C18/PSA clean-up, freezing step, and concentration into hexane / acetone, prior to analysis by gas chromatography coupled with tandem mass spectrometric detection (GC-MS/MS) using external calibration. The limit of quantification (LOQ) was set to 0.01 mg/L, expressed as sum of isomers, for all matrices.

Specimen preparation
5 mL of homogenized specimen were weighed into a 50 mL plastic centrifuge tube. 5 mL of water was added. 10 mL acetonitrile were added and the material was shaken vigorously for 1 min using a vortex.

Thereafter, QuEChERS EN15662 salt-mixture (1 g sodium citrate, 0.5 g sodium hydrogencitrate sequihydrate, 4 g magnesium sulphate, 1 g sodium chloride) was added and shaken vertically for an additional minute. The samples were centrifuged at > 3000 rpm for 5 min at room temperature.
An aliquot of the supernatant (6 mL) was transferred into a centrifuge tube containing the QuEChERs dispersive kit (900 mg of magnesium sulphate, 150 mg of C18 and 150 mg of PSA) and shaken vertically for 1 min. The samples were centrifuged at > 3000 rpm for 5 min at room temperature. An aliquot of the supernatant (4 mL) was transferred into a centrifuge tube and stored 1 hour in a freezer. It was then transferred immediately into another centrifuge tube.
An aliquot (2 mL) was transferred into another centrifuge tube to which was added 20 µL of Protectant Solution (10g olive oil dissolved in 50 mL of hexane). The extract was evaporated to dryness under nitrogen flow at room temperature. 200 µL of lindane solution (200 ng/mL) was added and the extract was brought to a volume of 2 mL with Hexane/Acetone (70/30; v/v) prior to analysis by GC-MS/MS.
Note: Lindane was used as injection qualifier and not as internal standard for quantification

Equipment for flurochloridone determination

GC-MS/MS system	Agilent 7890A GC system with 7693 Autosampler and 7000B MS/MS and Mass Hunter software
Column:	Restek Rxi 17 Sil, 30 m x 0.250 mm x 0.25 µm
Injection:	1 µL, pulse splitless (25 psi for 0.5 min)
Injection Liner:	Agilent 2mm dimpled
Injector:	Initial 50 °C (hold for 0.05 min) Heat rate 720 °C/min to 300 °C, hold for 14 min
Oven:	Initial 50 °C (hold for 1 min) Heat rate 40 °C/min to 150 °C, hold for 0 min Heat rate 20 °C/min to 340 °C, hold for 1 min
Transfer line temperature:	340 °C
Source temperature	280 °C
Quadrupoles temperature:	150 °C
Carrier gas	Helium, 1.7 mL/min
Retention time(s)	Approx. 9.2 min (flurochloridone), approx. 8.1 min (lindane)
Ionisation type:	Electron ionization, positive mode
Ion mass transition monitored (m/z) for flurochloridone :	311 → 174, collision energy = 15 eV (Quantifier)
	145 → 95, collision energy = 20 eV (Qualifier)
Ion mass transition monitored (m/z) for lindane :	225 → 189, collision energy = 5 eV

Results and discussions

Table A 55: Recovery results from method validation of flurochloridone in body fluid matrices (blood and urine)

Matrix	Fortification level (mg/L)	Recovery (%)	Mean recovery (%)	RSD (%)	Replicates
Ion Mass Transition 311 → 174 m/z (Quantification)					
Drinking water	0.01	85, 84, 102, 82, 81	87	9.5	5
Surface water	0.01	104, 95, 105, 101, 103	102	4.0	5
Ion Mass Transition 145 → 95 m/z (Confirmation)					
Drinking water	0.01	85, 83, 100, 80, 77	85	10.5	5
Surface water	0.01	107, 97, 101, 112, 103	104	5.5	5

Calculation was performed on unrounded values. No correction by blank value was performed.
RSD = Relative Standard Deviation

Mean recoveries at each fortification level were between 70 and 120% and the RSD was below 20% for flurochloridone for both transitions in all matrices, in compliance with SANCO/825/00 rev. 8.1 requirements.

Table A 56: Characteristics for the analytical method used for residue determination of flurochloridone in body fluid matrices (blood and urine)

	Flurochloridone
Specificity	A highly specific detection system was used (GC-MS/MS) and two mass transitions were monitored. The retention time of flurochloridone in the samples matches the retention time in the standard solutions. No interferences above 30 % of the LOQ at the retention time of the analyte were detected in the untreated control samples.
Calibration (type, number of data points) **Calibration range**	A series of seven calibration standard solutions were prepared in a lindane/olive oil solution diluted in hexane/acetone (70/30, v/v). The linearity was determined with seven matrix standards ranging from 1.5 ng/mL to 200 ng/mL (corresponding to 0.003 mg/L to 0.4 mg/L in matrix). The calibrations were found linear with coefficients of determination $R^2 \geq 0.990$ (1/X weighting): Flurochloridone in urine: 311 → 174 m/z: Y = 850.917218 x - 259.445695, $R^2 = 0.9997$ 145 → 95 m/z: Y = 586.089336 x - 91.106581, $R^2 = 0.9998$ Flurochloridone in blood: 311 → 174 m/z: Y = 902.565236 x - 124.156074, $R^2 = 0.9971$ 145 → 95 m/z: Y = 613.794411 x - 92.911808, $R^2 = 0.9969$
Assessment of matrix effects is presented	Even if non-significant matrix effect (< ±20%) on signal was found in both matrices, quantification using matrix matched standards was performed.
Limit of determination / quantification	The limit of quantification (LOQ) is defined as the lowest validated level and was successfully established at 0.01 mg/L. The limit of detection (LOD) was set at 0.003 mg/L, which is 30% of the LOQ.
Extract Stability	Flurochloridone was found to be stable in final extracts of each matrix for at least 10 days when stored at < -18°C.

Conclusion

The method was found to be valid for the determination of flurochloridone in body fluid matrices (blood and urine), with a limit of quantification (LOQ) of 0.01 mg/L, in accordance to SANCO/825/00 rev. 8.1 requirements.

Garrigue, P. (2017e)

A 2.1.2.6.1.2 Confirmatory method

The method of Garrigue, P. (2017d) includes GC-MS/MS, which achieves a high level of specificity. Thus, an additional confirmatory method is not required.

A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted.