REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: 102000007779

Product name: Flufenacet SC 508.8 G

Chemical active substance(s):

Flufenacet 508.8 g/L

Central Zone

Zonal Rapporteur Member State: POLAND

CORE ASSESSMENT

Applicant: Bayer Crop Science Division

Submission date: 30 June 2021

MS Finalisation date: February 2023 (initial Core Assessment)

June 2023 (final Core Assessment)

Version history

When	What
June 2021	Original Bayer Crop Science Division submission
February 2023	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~~.
June 2023	Final report (Core Assessment updated following the commenting period) Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Information no longer relevant ~~is struck through and shaded~~.

OECD Statement on Confidentiality

The summaries and evaluations contained in this monograph or review report may be based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority that prepared it. Other registration authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this Monograph or review report unless they have received the data on which the summaries and evaluation are based, either:

- From the owner of the data; or
- From a second party that has obtained permission from the owner of the data for this purpose or, alternatively, the applicant has received permission from the data owner that the summaries and evaluation contained in this Monograph or review report may be used in lieu of the data; or
- Following expiry of any period of exclusive use, by offering – in certain jurisdictions – mandatory compensation;

unless the period of protection of the proprietary data concerned has expired.

Applicants wishing to avail of information in this Monograph or review report should seek advice from the regulatory authority to which application is made concerning the requirements in their country.

Table of Contents

5 Analytical methods

5.1 Conclusion and summary of assessment

zRMS conclusions:

In EFSA Journal 2012;10(4):2689 it is stated that *'total residue' approach has been proposed and the current residue definition for risk assessment and enforcement is the sum of all compounds containing the N-fluorophenyl-N-isopropyl moiety expressed as flufenacet equivalent. A validated analytical method for enforcement of the residue definition in food of plant origin is available, with an overall LOQ of 0.05 mg/kg in dry, high fat and high water commodities. Further validation of the analytical method in acidic commodities is still required.*

Excerpt from EFSA Journal 2012;10(4):2689:
Methods for enforcement of residues in food of plant origin
"During the peer review under Directive 91/414/EEC a single–residue method (GC-MS), presented as Method 00346, and its ILV were evaluated (France, 1997). The method relies on a single ion (m/z 207) for quantitation. The extraction procedure includes oxidation and hydrolysis, followed by steam distillation and derivatisation with trifluoroacetic anhydride.
Validation data for the primary method was evaluated for various materials of a range of crops including cereals, maize, sunflower and soya bean. Recoveries were determined by fortification with flufenacet, flufenacet oxalate, flufenacet thioglycolate sulfoxide, flufenacet sulfonic acid and a mixture of these four substances. In all cases the primary method was fully validated for the determination of residues as 4-fluoro-N-methylethyl benzenamin trifluoroacetamide (to which all compounds containing the N-fluorophenyl-N-isopropyl moiety are derivatised) in cereals and other dry crops as well as high fat commodities with a LOQ of 0.05 mg/kg for cereal grain, maize, sunflower and soya bean and 0.1 mg/kg for straw (France, 1997).
Following the peer review the same primary method (amendment E004) was validated in high water commodities with a LOQ of 0.01 mg/kg for apples (France, 2012). In the primary method and ILV two ions were used for confirmation. Following the review the RMS also provided evidence that the ILV is also validated for wheat (France, 2012).

The multi-residue method using diatomaceous earth for clean-up in combination with HPLC-MS/MS, described in the European Standard EN 15637:2008 (CEN, 2008a), is available for the determination of flufenacet in high water commodities. However, this method does not include other metabolites containing the N-fluorophenyl-N-isopropyl moiety; it is therefore not suitable for enforcement of this substance according to its complete residue definition.

In addition, the multi-residue QuEChERS method in combination with HPLC-MS/MS, described in the European Standard EN 15662:2008 (CEN, 2008b), is available for the determination of flufenacet in high acid, dry, high sugar and high water commodities, see Table 1-2. However, this method does not include other metabolites containing the N-fluorophenyl-N-isopropyl moiety; it is therefore not suitable for enforcement of this substance according to its complete residue definition.

Hence it is concluded that levels of all compounds containing the N-fluorophenyl-N-isopropyl moiety can be enforced in food of plant origin with a LOQ of 0.05 mg/kg in high fat and dry commodities (based on the method of analysis validated during the peer review) and a LOQ of 0.01 mg/kg in high water commodities (based on the method of analysis validated after the peer review). Considering that most of the residues trials were carried out with a LOQ of 0.05 mg/kg, an overall LOQ of 0.05 mg/kg is considered adequate noting that further validation of the analytical method in acidic commodities is still required.

During commenting period Applicant submitted additional explanation:
As a method for post-authorization control and monitoring purposes that is validated in acidic commodities, Method 01100 can be used. This method is comparable to Method 00346 (cf expert statement in A 2.1.2.1.3.5), has been validated in orange fruit and is currently evaluated in the ongoing AIR dossier for renewal of flufenacet. Therefore, it has not been evaluated in this dossier.

Methods for enforcement of residues in food of animal origin
During the peer review under Directive 91/414/EEC a single–residue method (GC-MS), referred to as Method 00418, and its ILV were evaluated and considered validated (France, 1997). The method is essentially the same as Method 00346, for plants and described above, but quantitation is achieved using three ions, whereas in the plant method one ion is used.
Validation data for the primary method was evaluated for various animal tissues, milk and eggs. Recoveries were determined by fortification with flufenacet, flufenacet oxalate, flufenacet thioglycolate sulfoxide, flufenacet

sulfonic acid and a mixture of these four substances. In all cases the primary method was considered validated. In the ILV two ions were used for confirmation and the method was considered validated for beef liver.
The primary method determines residues as 4-fluoro-N-methylethyl benzenamin trifluoroacetamide (to which all compounds containing the N-fluorophenyl-N-isopropyl moiety are derivatised) in products of animal origin with a LOQ of 0.01 mg/kg for milk, 0.02 mg/kg for liver (ILV: 0.05 mg/kg for beef liver) and 0.05 mg/kg for kidney, fat, muscle and eggs.
Hence it is concluded that levels of all compounds containing the N-fluorophenyl-N-isopropyl moiety can be enforced in food of animal origin with a LOQ of 0.01 mg/kg in milk, 0.02 mg/kg for liver and 0.05 mg/kg for kidney, fat, muscle and eggs.

The Applicant submitted a number of methods for analysis of residues of flufenacet for the generation of pre-authorization data and methods for post-authorization control and monitoring purposes.
Many analytical methods for the determination of flufenacet in different matrices were submitted in the ongoing AIR dossier for renewal of flufenacet and was evaluated by RMS-Poland in the frame of the re-approval (RAR for Flufenacet; Vol. 3 – B.5, April 2022). Therefore, the studies are not evaluated in this dossier.
The details of the evaluation of additional studies are referred in Appendix 2.

Sufficiently sensitive and selective analytical methods are available for the active substance 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
Cereals (winter wheat, winter triticale, winter barley, rye, durum wheat, spelt)	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 flufenacet in plant protection product FFA SC 508.8 G is provided as follows:

Comments of zRMS:	The method is considered to be sufficient for the determination of flufenacet in the FFA SC 508.8 G formulation - the method has been validated according to the SANCO/3030/99 rev. 5.

Additionally, to the method(s) previously submitted and reviewed at European level, X new methods has/have been developed and validated.

Analytical method AM036120MF1

Reference:	**KCP 5.1.1/01**
Title:	Determination of flufenacet in formulations - HPLC-UV, external standard
Report:	Hoffmann, D.; 2020; M-688742-01-1
Authority registration No:	--
Guideline(s):	Commission Regulation (EU) 284/2013 in accordance with Regulation (EC) No 1107/2009 (10/2009), US EPA OCSPP Test Guideline No. 830.1800 (08/1996)
Deviations:	None
GLP/GEP:	No
Acceptability:	Yes
Duplication (if vertebrate study):	--

Reference:	**KCP 5.1.1/02**
Title:	Validation of analytical method AM036120MF1 - Determination of flufenacet in the formulation flufenacet SC 508.8 (508.8 g/L)
Report:	Hoffmann, D.; Garcia Sanchez, M. T.; 2020; FM0415(MVF00)G01; M-758632-01-1
Authority registration No:	--
Guideline(s):	SANCO/3030/99 rev. 5 (03/2019), Commission Regulation (EU) 284/2013 (03/2013) in accordance with Regulation (EC) No 1107/2009 (10/2009), US EPA OCSPP Test Guideline No. 830.1800 (08/1996)
Deviations:	None
GLP/GEP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study):	--

Materials and methods

The analytical method AM036120MF1 was developed for the determination of flufenacet in formulations. The active substance flufenacet is separated from formulation components on a reversed phase column using isocratic elution. After UV detection, the quantitative evaluation is carried out by comparing the peak areas with those of reference item, using either a single-point calibration or a standard curve.

Equipment and operating conditions are as follows:

Chromatographic conditions:

Column: Kinetex C18; 2.6 µm, 50 x 4.6 mm +
Supplier: Phenomenex +
Guard: -- +
+ or equivalent apparatus/configuration

Flow rate: 2.0 mL/min
Temperature: 40 °C
Injection volume: 3 µL
Eluent A: phosphoric acid 0.01 mol/L
Eluent B: acetonitrile
Gradient program:

	Time (min)*	% A	% B	Flow rate (mL/min)
Separation	0.0	60	40	2.0
	5.5	60	40	2.0
Rinsing gradient	5.6	5	95	2.0
	6.5	5	95	2.0
	6.6	60	40	2.0
	8.0	60	40	2.0

*adjust the equilibration time according to the pump- and injection system

Total run time: 8.0 min

Retention time: Flufenacet approx. 3.8 min
Measurement wavelength:240 nm

Validation of the analytical method AM036120MF1

The analytical method AM036120MF1 was validated for the determination of flufenacet in the test item Flufenacet SC 508.8 (508.8 g/L). This was accomplished by evaluating specificity, linearity, accuracy, and precision.

Validation - Results and discussions

Table 5.2-1: Method suitable for the determination of the active substance flufenacet in the plant protection product Flufenacet SC 508.8 (508.8 g/L)

Analyte	**flufenacet**
Author(s), year	Hoffmann, D.; Garcia Sanchez, M. T.; 2020
Principle of method	HPLC-UV
Linearity I (50-150%)	n = 6
regression equation	y = 0.080939 + 0.24593 x
correlation coefficient	0.99988
type of regression function	linear (1st order)
concentration range [mg/100 mL]	29.87- 90.53
concentration range [% (w/w)][1]	21.04– 63.75
concentration range [% DC][2]	49.8– 151
Linearity II (5-50%)	n = 7
regression equation	y = -0.0024198 + 0.24992 x
correlation coefficient	1.0000
type of regression function	linear (1st order)
concentration range [mg/100 mL]	2.987-29.87
concentration range [% (w/w)][1]	2.104-21.04
concentration range [% DC][2]	4.98-49.8
Precision	n = 6
mean value [% (w/w)]	43.05
RSD [%]	0.20
outliers detected	No
Horwitz-Value RSDr(max) [%]	1.52
Horrat value (Horwitz ratio) Hr	0.13 The Horrat value (Horwitz ratio, Hr) is ≤ 1 and thus, the precision of the analytical method is assessed acceptable.
Accuracy	n = 6
mean recovery [%]	100.5
confidence interval [95%ile]	± 0.185
RSD [%]	0.18
concentration range [mg/100 mL]	30.25-90.30
concentration range [% (w/w)][1]	21.30-63.59
concentration range [% DC][2]	50.4-151
Specificity	
identity	Identity of flufenacet in test item was confirmed by comparison of UV spectra and retention time with those of a certified reference item.
identity confirmed	yes
Interferences	Comparison of chromatograms of reference item, test item and empty formulation with regard to interferences
interferences identified	no
Comment	No deviation from guideline SANCO/3030/99 rev.5.

1 Referred to a nominal test item concentration of 142 mg/100 mL.
2 Referred to the declared contents of the active substances acc. to specification (flufenacet declared content 508.8 g/L, corresponds to 42.4 % (w/w))

Conclusion

The analytical method AM036120MF1 was validated with success for the determination of flufenacet in the test item Flufenacet SC 508.8 (508.8 g/L) according to the requirements laid down by SANCO/3030/99 rev.5, all criteria were met.

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

FFA SC 508.8 G does not contain any relevant impurities.

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

With respect to toxicological, eco-toxicological or environmental aspects the product FFA SC 508.8 G does not contain any relevant formulants. Therefore, a special analytical method and validation is not needed.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There is no CIPAC method available for the determination of flufenacet in formulations.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

Flufenacet
An overview on the acceptable methods for analysis of residues of flufenacet for the generation of pre-authorization data is given in the following table.

The residue analytical methods for the determination of flufenacet in plants and animal matrices were evaluated in the Monograph (France 1997) and by EFSA (2012). The crops supported for Annex I inclusion were cereals, corn, soybean and sunflower. All methods were considered adequate in the EU peer review.
In the table below, only those methods (EU peer reviewed methods and new methods) were included which were used for data generation in studies reported in section B7. Method 00346 (evaluated in the EU peer review) and method 0418 and 0418/M001 (EU peer reviewed, for animal commodities) were considered suitable for both data generation and monitoring purposes. Therefore, they are also tabulated under 5.3.
In the table below, a new extension (E004) to data generation method 00346 is reported, which was not evaluated yet on EU level. The extension E004 was performed to validate the sample material small cereal grain at a lower LOQ of 0.01 mg/kg and was used for the analysis of cereal grain from the field rotational crop study (cf. B7; 7.2.6.1.). The extension E004 to method 00346 has been evaluated on Member State level and has been reviewed in the DRAR for renewal of the active substance (DRAR Poland, 2017).
For the detailed evaluation of new studies, it is referred to Appendix 2.

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

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Component of residue definition: flufenacet including all metabolites containing the N-fluorophenyl-N-isopropyl moiety, expressed as flufenacet equivalents				
Plants High water content High protein/starch content (dry) High oil content **(Residues)**	00346 / Primary and confirmatory	0.05 mg/kg in cereals (wheat, barley, rye): - grain, green material, corn - green material - corn grain; sunflower seed; soya seed 0.1 mg/kg in straw	GC/MS	Seym, M., 1995, EU agreed report of ECCO 73 (BBA 1999), Annex 2, Complete List of Endpoints
New data				
Plants High water content High protein/starch content (dry)	00346 / Primary and confirmatory Additional validation data in	0.05 mg/kg in green plant material (wheat, barley, rye) 0.05 mg/kg in grain	GC-MS	Residue report RA-2010/94 (Seym, M.; 1996; M-004451-01-2) EFSA 2012

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
(Residues)	residue study RA-2010/94	(wheat, barley, rye) 0.1 mg/kg in straw (wheat, barley, rye)		Appendix 2
Plants High water content High protein/starch content (dry) **(Residues)**	00346 / Primary and confirmatory Additional validation data in residue study RA-2144/00	0.05 mg/kg in green plant material (wheat, barley, rye) 0.05 mg/kg in grain (wheat, barley, rye) 0.1 mg/kg in straw (wheat, barley, rye)	GC-MS	Residue report RA-2144/00 (Hoffmann, M.; 2002; M-058156-01-1) EFSA 2012 Appendix 2
Plants Rice grain (validated for cereal grain in residue study RA-2020/06) (Residues)	00346 / E004 /Primary	0.01 mg/kg rice grain	GC-MS	Rzepka, S., 2006, M-277805-01-1, DRAR Poland 2017 Appendix 2
Plants High starch content (dry)	00346 / E004 /Primary Additional validation data in residue study RA-2020/06	0.01 mg/kg Wheatl grain	GC-MS	Residue report RA-2020/06 (Melrose, I.; Erler, S.; 2008; M-306269-01-1) ~~DRAR Poland 2017~~ Appendix 2
Plants High water content Dry (straw) **(Residues)**	00346 / Primary Additional validation data in residue study RA-2020/06	0.05 mg/kg in green plant material (wheat, barley) 0.1 mg/kg in straw (wheat, barley	GC-MS	Residue report RA-2020/06 (Melrose, I.; Erler, S.; 2008; M-306269-01-1) ~~DRAR Poland 2017~~ Appendix 2
Component of residue definition: flufenacet including all metabolites containing the N-fluorophenyl-N-isopropyl moiety, expressed as flufenacet equivalents				
Animal products, food of animal origin **(Residues)**	method 00418 primary & confirmatory	Milk: 0.01 mg/kg Bovine liver: 0.02 mg/kg, Bovine kidney, muscle, fat: 0.05 mg/kg	GC-MS	Xxx 1995 EU agreed Report of ECCO 73, Annex 2, Complete List of Endpoints (BBA 1999)
Animal products, food of animal origin **(Residues)**	method 00418/M001 / primary & confirmatory	0.05 mg/kg eggs	GC-MS	M-019614-01-1 xxx 1995 EU agreed MR-1118/95* (MR-118/95 in reference list) Monograph France, 1997
Component of residue definition: flufenacet and the metabolite flufenacet-oxalate				
Soil (Environmental fate)	Not relevant			
Soil, water,... (Efficacy)	Not relevant			
Feed, body fluids,... (Toxicology)	Not relevant			
Body fluids, air,.... (Exposure)	Not relevant			
Sediment (Ecotoxicology)	Not relevant			
Soil (Ecotoxicology)	Primary **(Method 01080)**	4 µg/kg	HPLC-MS/MS	Brumhard, B.; 2009; M-357296-01-1; Appendix 2

Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Soil (Ecotoxicology)	Primary **(Method 01080, supplementary validation)**	4 µg/kg	HPLC-MS/MS	Leicher, T.; 2008; M-307211-01-1; Appendix 2 In support of Brumhard, B.; 2009; M-357296-01-1
Water (Ecotoxicology)	Primary **(Method 01169)**	flufenacet-oxalate: 0.91 µg/L	HPLC-MS/MS	Krebber, R.; Leppelt, L.; 2009; M-357278-01-1; Appendix 2
Water (Ecotoxicology)	Primary **(Method 01169, supplementary validation)**	flufenacet-oxalate: 0.91 µg/L	HPLC-MS/MS	Bruns, E.; 2009; M-358823-01-1; Appendix 2 In support of Krebber, R.; Leppelt, L.; 2009; M-357278-01-1
Water (Ecotoxicology)	Primary **(Method 01169, supplementary validation)**	flufenacet-oxalate: 0.91 µg/L	HPLC-MS/MS	Bruns, E.; 2009; M-359515-02-1; Appendix 2 In support of Krebber, R.; Leppelt, L.; 2009; M-357278-01-1
Water (Ecotoxicology)	Primary **(Report 796364)**	0.01 µg/L	HPLC-MS	Baetscher, R.; 2001; M-055471-01-1; Appendix 2
Water (Ecotoxicology)	Primary **(Report 796342)**	0.4 µg/L	HPLC-MS	Baetscher, R.; 2001; M-055476-01-1; Appendix 2
Feeding solution (Ecotoxicology)	Primary **(Method 01080, supplementary validation)**	0.004 mg/kg	HPLC-MS/MS	Kling, A.; 2014; M-477339-01-1; Appendix 2
Feeding solution (Ecotoxicology)	Primary **(Report EBFO0015)**	1.00 µg/g	HPLC-MS/MS	Rathjen, K. A.; 2018; M-615473-01-1; Appendix 2
Spray Solution (Ecotoxicology)	Primary **(Method 00372, supersedes original method validation)**	0.038 µg/mL	HPLC-UV	Friedrich, S.; 2005; M-248250-01-1; Appendix 2
Spray Solution (Ecotoxicology)	Primary **(Method 00372, supersedes original method validation)**	0.038 µg/mL	HPLC-UV	Friedrich, S.; 2005; M-248251-01-1; Appendix 2
Water, buffer solutions,... (Properties)	Not relevant			

1) Report of ECCO, 1999. Complete List of Endpoints: flufenacet. Annex 2, p. 37-57
2) Monograph, France, 1997. Draft assessment report (monograph) on the active substance flufenacet prepared by the rapporteur Member State France in the framework of Council Directive 91/414/EEC, Annex B, B2, August 1997.

*Methods have been evaluated as monitoring and data generation methods for plant and animal matrices in the EU peer review process and are also referred to in Section 5.3. Method 01179 was evaluated in the DRAR (Poland 2017)

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

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

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

Compared to the residue definition proposed in the Monograph (France 1997) and established in the Report of ECCO 73 (BBA 1999) the current legal residue definition is identical.

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

Matrix	Residue definition	MRL / limit	Reference MRL level Remarks
Plant, high water content	Flufenacet (sum of all compounds containing the N fluorophenyl-N-isopropyl moiety expressed as flufenacet equivalent)	0.05* mg/kg (lowest MRL) 0.01 mg/kg (LOQ of new methods)	Reg. (EC) 1127/2014
Plant, high acid content		0.05* mg/kg (lowest MRL) 0.01 mg/kg (LOQ of new methods)	
Plant, high protein/high starch content (dry commodities)		0.05* mg/kg (lowest MRL) 0.01 mg/kg (LOQ of new methods)	
Plant, high oil content		0.05* mg/kg (lowest MRL) 0.01 mg/kg (LOQ of new methods)	
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg (lowest MRL)	
Muscle	Flufenacet (sum of all compounds containing the N fluorophenyl-N-isopropyl moiety expressed as flufenacet equivalent)	0.05* mg/kg (lowest MRL) 0.05 mg/kg (LOQ)	
Milk		0.01* mg/kg (lowest MRL) 0.01 mg/kg (LOQ)	
Eggs		0.05* mg/kg (lowest MRL) 0.05 mg/kg (LOQ)	
Fat		0.05* mg/kg (lowest MRL) 0.05 mg/kg (LOQ)	
Liver		0.02* mg/kg (lowest MRL) 0.02 mg/kg (LOQ)	
Kidney		0.05* mg/kg (lowest MRL) 0.05 mg/kg (LOQ)	
Soil (Ecotoxicology)	flufenacet	0.05 mg/kg	common limit
Drinking water (Human toxicology)	flufenacet	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	flufenacet	0.00204 mg/l	*Lemna gibba*, EC_{50}
Air	flufenacet	LOQ = 2.2 µg/m^3	AOEL:0.017 mg/kg bw/d
Animal tissues	Flufenacet (sum of all compounds containing the N fluorophenyl-N-isopropyl moiety expressed as flufenacet equivalent)	0.05 mg/kg (muscle, fat, kidney)* 0.02 mg/kg (liver)*	Reg. (EC) 1127/2014

Matrix	Residue definition	MRL / limit	Reference MRL level Remarks
Body fluids (plasma)	No information	MRL not required Limit 50µg/L	SANCO/825/00 rev 8.1**

*SANTE/2020/12830 rev 1: Suitable methods for body tissues are available from methods for food of animal origin covering the residue definition
**Validation was done at the LOQ of 0.05 mg/L according to the requirements set out in SANCO/825/00 rev 8.1 before SANTE/2020/12830 rev 1 became effective. The method is still considered fit for purpose.

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

An overview on the acceptable methods for analysis of flufenacet in plant matrices is given in the following tables. For the detailed evaluation of additional studies, it is referred to Appendix 2.
The new methods (method no 01179, 01100, 01100/M001) reflect state of science technologies using HPLC-MS/MS and are less complex due to omission of the derivatisation step included in the former enforcement method.The analytical methods fulfil the requirements detailed in the EC Guidance documents on residue analytical methods (SANCO/825/00 rev. 8.1) and in OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13). However, it might be up to the descretion of the authority to decide on the need to evaluate.
Methods are considered to also fulfil the quality criteria set out in SANTE/2020/12830 rev 1.

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: flufenacet including all metabolites containing the N-fluorophenyl-N-isopropyl moiety, expressed as flufenacet equivalents exemplified with analytical targets flufenacet, FOE 5043 oxalate (hydrate), FOE 5043 sulfonic acid (sodium salt), FOE 5043 thioglycolate sulfoxide				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary and confirmatory method 00346	0.05 mg/kg cereals (wheat, barley, rye): - green material, corn - green material	GC-MS	Seym, M., 1995 Report N° MR-981/95* Report of ECCO 73 (BBA 1999), Annex 2, Complete List of Endpoints EU agreed
	ILV to method 00346	0.05 mg/kg (corn forage)	GC-MS	Seym, M., 1994 106907* Report of ECCO 73 (BBA 1999), Annex 2, Complete List of Endpoints EU agreed
	Primary and confirmatory method 01179	0.01 mg/kg cereal green material	HPLC-MS/MS	Class, Th.; Merdian, H.; 2010 M-362716-01-1 DRAR Poland 2017 Appendix 2
	Primary and confirmatory method 01100/M001	0.01 mg/kg cereal green material	HPLC-MS/MS	Stuke, S., Bauer, J.; Ruhl, S.; 2012; M-433720-02-1 DRAR Poland 2017 Appendix 2
	ILV to 01100, 01100/M001 and 01179	0.01 mg/kg cereal green material	HPLC-MS/MS	Meyer, M.; 2011; M-405654-01-1 DRAR Poland 2017 Appendix 2
High protein/starch content and dry	Primary and confirmatory method 00346	0.05 mg/kg in corn grain 0.1 mg/kg in straw	GC-MS	Seym, M., 1995, Report N° MR-981/95* Report of ECCO 73 (BBA 1999), Annex 2, Complete List of Endpoints

Component of residue definition: flufenacet including all metabolites containing the N-fluorophenyl-N-isopropyl moiety, expressed as flufenacet equivalents exemplified with analytical targets flufenacet, FOE 5043 oxalate (hydrate), FOE 5043 sulfonic acid (sodium salt), FOE 5043 thioglycolate sulfoxide				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				EU agreed
	Primary and confirmatory method 01179 /	0.01 mg/kg cereal grain 0.05 mg/kg cereal straw	HPLC-MS/MS	Class, Th.; Merdian, H.; 2010 M-362716-01-1 DRAR Poland 2017 Appendix 2
	Primary and confirmatory method 01100	0.01 mg/kg dry bean seed	HPLC-MS/MS	Billian, P.; 2010; M-362575-03-1 DRAR Poland 2017 Appendix 2
	Primary and confirmatory method 01100/M001	0.01 mg/kg cereal grain 0.05 mg/kg cereal straw	HPLC-MS/MS	Stuke, S., Bauer, J.; Ruhl, S.; 2012; M-433720-02-1 DRAR Poland 2017 Appendix 2
	ILV to 01100, 01100/M001 and 01179	0.01 mg/kg dry bean seed	HPLC-MS/MS	Meyer, M.; 2011; M-405654-01-1 DRAR Poland 2017 Appendix 2
High oil content	Primary and confirmatory method 00346	0.05 mg/kg sunflower seed; soya seed	GC-MS	Seym, M., 1995, Report N° MR-981/95* Report of ECCO 73 (BBA 1999), Annex 2, Complete List of Endpoints EU agreed
	Primary and confirmatory method 01100	0.01 mg/kg rape seed	HPLC-MS/MS	Billian, P.; 2010; amended by Stuke. S. 2018 Stuke, S.; 2018; M-362575-03-1 DRAR Poland 2017 Appendix 2
	ILV to 01100, 01100/M001 and 01179	0.01 mg/kg rape seed	HPLC-MS/MS	Meyer, M.; 2011; M-405654-01-1 DRAR Poland 2017 Appendix 2
High acid content	Primary & confirmatory method 01100	0.01 mg/kg orange fruit	HPLC-MS/MS	Billian, P.; 2010; amended by Stuke. S. 2018 Stuke, S.; 2018; M-362575-03-1 DRAR Poland 2017 Appendix 2
	ILV to 01100, 01100/M001 and 01179	0.01 mg/kg orange fruit	HPLC-MS/MS	Meyer, M.; 2011; M-405654-01-1 DRAR Poland 2017 Appendix 2

* In the Monograph the report no. is used

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

These studies have been submitted here and in the AIR dossier for renewal of flufenacet and have been evaluated in the frame of the re-approval. FR, as co-RMS of the RAR of flufenacet, has commented these studies and has stated that adequate GC-MS and HPLC-MS/MS methods and corresponding ILV are available to monitor flufenacet residues in the four groups of plant matrices.

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Information on extraction efficiency is available from the following studies: Extraction efficiency of the residue analytical methods is compared to the relative amount extracted from the metabolism studies: maize and soybean commodities, i.e. fodder, forage and seeds (Gould , T. J.; 1995); and wheat grain and straw (Krolski, M. E.; Bosnak, L. L.; 1997; M-002275-01-1, appendix 2). Since the extraction conditions are the same in data generation methods and methods for post registration control the findings reported in Appendix 2 are evenly valid for data generation methods. Extraction efficiency is only reported for cereal (wheat) matrices in the present dossier.
Not required, because:	--

For the detailed evaluation of (additional) studies on extraction efficiency, it is referred to Appendix 2.

These studies have been submitted here and in the dossier for renewal of flufenacet and have been evaluated in the frame of the re-approval. FR, as co-RMS of the RAR of flufenacet, has evaluated these studies and concluded that the extraction efficiency of the residues in plant matrices is considered acceptable.

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

An overview on the acceptable methods and possible data gaps for analysis of flufenacet in animal matrices is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2. The new ILV provides validation data for all animal tissues, milk and eggs fulfilling the data requirements of SANCO 825/00 rev 8.1. The methods including the new ILV were evaluated in the DRAR (Poland 2017) for renewal of approval of flufenacet by France acting as co-RMS.
Methods are considered to also fulfil the quality criteria set out in SANTE/2020/12830 rev 1.

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

Component of residue definition: flufenacet including all metabolites containing the N-fluorophenyl-N-isopropyl moiety, expressed as flufenacet equivalents exemplified with analytical targets flufenacet, FOE 5043 oxalate (hydrate), FOE 5043 sulfonic acid (sodium salt), FOE 5043 thioglycolate sulfoxide				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary & confirmatory method 00418 /	0.01 mg/kg	GC-MS	xxx, 1995, Report 106773* Report of ECCO 73, Annex 2, Complete List of Endpoints (BBA 1999) EU agreed
	ILV to method 00418 and 00418/M001	0.01 mg/kg	GC-MS	xxx M-461242-01-1 DRAR Poland 2017 Appendix 2
Muscle	Primary & confirmatory method 00418	0.05 mg/kg	GC-MS	xxx, 1995, Report 106773* Report of ECCO 73, Annex 2, Complete List of Endpoints (BBA 1999) [1)] EU agreed
	ILV to method 00418 and 00418/M001	0.05 mg/kg	GC-MS	xxx 2013; M-461242-01-1 DRAR Poland 2017 Appendix 2

Component of residue definition: flufenacet including all metabolites containing the N-fluorophenyl-N-isopropyl moiety, expressed as flufenacet equivalents exemplified with analytical targets flufenacet, FOE 5043 oxalate (hydrate), FOE 5043 sulfonic acid (sodium salt), FOE 5043 thioglycolate sulfoxide				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Fat	Primary & confirmatory method 00418 /	0.05 mg/kg	GC-MS	xxx 1995, Report 106773* Report of ECCO 73, Annex 2, Complete List of Endpoints (BBA 1999) [1)] EU agreed
	ILV to method 00418 and 00418/M001	0.05 mg/kg	GC-MS	xxx 2013; M-461242-01-1 DRAR Poland 2017 Appendix 2
Kidney	Primary & confirmatory method 00418	0.05 mg/kg	GC-MS	xxx 1995, Report 106773* Report of ECCO 73, Annex 2, Complete List of Endpoints (BBA 1999) [1)] EU agreed
Liver	Primary & confirmatory method 00418	0.02 mg/kg	GC-MS	Xxx 1995, Report 106773* Report of ECCO 73, Annex 2, Complete List of Endpoints (BBA 1999) [1)] EU agreed
	ILV to method 00418 and 00418/M001	0.05 mg/kg	GC-MS	xxx 1995, Report 106913* Report of ECCO 73, Annex 2, Complete List of Endpoints (BBA 1999) [1)] EU agreed
	ILV to method 00418 and 00418/M001	0.02 mg/kg	GC-MS	xxx.; 2013; M-461242-01-1 DRAR Poland 2017 Appendix 2
Eggs	Primary & confirmatory method 00418/M001	0.05 mg/kg	GC-MS	Seym, M., 1995, MR-1118/95* (MR-118/95 in reference list) Monograph France, 1997[2)] EU agreed
	ILV to method 00418 and 00418/M001 /	0.05 mg/kg	GC-MS	xxx; 2013; M-461242-01-1 DRAR Poland 2017 Appendix 2

* In the Monograph the report no. is used

1) Report of ECCO, 1999. Complete List of Endpoints: flufenacet. Annex 2, p. 37-57

2) Monograph, France, 1997. Draft assessment report (monograph) on the active substance flufenacet prepared by the rapporteur Member State France in the framework of Council Directive 91/414/EEC, Annex B, B2, August 1997.

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

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	--

	Method for products of animal origin
Not required, because:	Since residues ≥LOQ are not anticipated in commodities of animal origin data on extraction efficiency in products of animal origin are not reported in the present submission which is in accordance with the requirements outlined in SANCO/825/00 rev. 8.1. and SANTE/2017/10632 rev 3 (Technical Guideline on the Evaluation of Extraction Efficiency) MRLs for animal commodities are established at the LOQ level. EFSA, 2012[1)]: "*On the basis of the animal metabolism studies it is concluded that, after exposure to the maximum dietary burden (about 200 times lower than the dose level in the metabolism studies, [5 mg/kg bw/d]), residue levels in livestock commodities are expected to remain below the enforcement LOQ."*

1) EFSA(European Food Safety Authority), 2012. Reasoned opinion on the review of the existing maximum residue levels (MRLs) for flufenacet according to Article 12 of Regulation (EC) No 396/2005, EFSA Journal 2012; 10(4):2689

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

The purpose of such analytical method for analysis of the active substance and relevant metabolites is the detection of intoxications in humans and animals or for biomonitoring purposes.
Relevant criteria for provision of a method as outlined in SANCO/825/00 rev. 8.1 are classification of the active substance or a relevant metabolite as toxic or very toxic, or classification according to GHS as follows: Acute toxicity (Cat. 1-3), CMR (Cat. 1) or STOT (Cat. 1). Flufenacet or any of its metabolites is not classified according to those categories.
With SANTE/2020/12830 rev 1 repealing SANCO/825/00 rev 8.1 the requirement for an analytical method in body fluids is set out irrespective of the toxicological classification of the active substance.
No recommendation was provided during the EU peer review for analytes relevant for monitoring in body fluids.
A validated method for body fluids is available and summarised in A 2.1.2.3.1. The method has been evaluated in the DRAR (Poland 2017).
Enforcement methods relevant to animal tissues are available covering the residue definition for animal matrices and are included in Table 5.3-4.

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

An overview on the acceptable methods and possible data gaps for analysis of flufenacet in soil is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

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

Component of residue definition: flufenacet			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary **(Method 00359)**	0.01 mg/kg	HPLC-MS/MS	Bachlechner, G.; Allmendinger, H.;1994; M-019071-01-2 EU agreed Monograph, Annex B4, France, 1997
Confirmatory **(Method 00359/M001)**	0.004 mg/kg	HPLC-MS/MS	Brumhard, B.; 2005; M-248543-01-1 A.2.1.2.4

[1]Review Report (7469/VI/98-Final – 3rd July 2003).

The analytical method 00359 was evaluated during the Annex I Inclusion and was accepted by the European Commission as an adequate monitoring/enforcement method for the determination of flufenacet in soil. No confirmatory data were requested on that topic.
However, if flufenacet is detected using this enforcement method it may be desired to confirm these findings to avoid false-positive results. In case of an accidental event or other reasons, which necessitates soil

investigations, it is recommended to use the analytical method 00359 (HPLC-MS/MS) for a first analysis of flufenacet. If flufenacet is detected with method 00359 it is suggested to use the analytical method 00359/M001 as confirmatory method, to avoid false-positive results.

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

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

An overview on the acceptable methods and possible data gaps for analysis of flufenacet in surface and drinking water is given in the following tables. For the detailed valuation of new/additional studies it is referred to Appendix 2.

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

Component of residue definition: flufenacet				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary **(Method AMFOE3)**	0.1 µg/L	HPLC-MS/MS	Bethem, R. A.; Peterson, R. G.; Leimkuehler, W. A.; Mattern, G. C.; 1995; EU agreed Monograph, Annex B4, France, 1997
	Primary **(Method 01387)**	0.05 µg/L	HPLC-MS/MS	Krebber, R.; Braune, M.; 2013; M-466732-01-1 A.2.1.2.5.1.1
	ILV **(Method 01387)**	0.05 µg/L	DI-HPLC-MS/MS	Stanislowski, T.; 2013; M-470714-02-1 A.2.1.2.5.1.2
Surface water	Primary **(Method AMFOE3)**	0.1 µg/L	HPLC-MS/MS	Bethem, R. A.; Peterson, R. G.; Leimkuehler, W. A.; Mattern, G. C.; 1995; EU agreed Monograph, Annex B4, France, 1997
	Primary **(Method 01387)**	0.05 µg/L	HPLC-MS/MS	Krebber, R.; Braune, M.; 2013; M-466732-01-1 A.2.1.2.5.1.1
	ILV **(Method 01387)**	0.05 µg/L	DI-HPLC-MS/MS	Stanislowski, T.; 2013; M-470714-02-1 A.2.1.2.5.1.2

The analytical method AMFOE3 was evaluated during the Annex I Inclusion and was accepted by the European Commission as an adequate monitoring/enforcement method for the determination of flufenacet in drinking and surface water. No confirmatory data were requested on that topic.
However, a new highly selective analytical method for the determination of flufenacet in water was recently developed in preparation of the renewal of approval of flufenacet (Annex I renewal process). The new method fulfils the current requirements on enforcement methods according to SANCO 825/00 rev 8.1; e.g. using two mass transitions for the detection of flufenacet - one for the quantitation and one for the confirmation – and thus, requires no separate confirmatory method. As required, an independent laboratory validation (ILV) is also available for this new analytical method.

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

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

An overview on the acceptable methods and possible data gaps for analysis of flufenacet in air is given in the following tables. For the detailed evaluation of new/additional studies please refer to Appendix 2.

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

Component of residue definition: flufenacet			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary **(Method 00410)**	2.2 µg/m^3	HPLC-UV	Riegner, K.; 1995; M-012833-01-2 EU agreed Monograph, Annex B4, France, 1997
Confirmatory **(Method 00410C)**	2.2 µg/m^3	HPLC-UV	Hellpointner, E.; 2000; M-048783-01-1 A.2.1.2.6.1

The analytical method 00410 was evaluated during the Annex I Inclusion and was accepted by the European Commission as an adequate monitoring/enforcement method for the determination of flufenacet in air. No confirmatory data were requested on that topic.
However, if flufenacet is detected using this enforcement method it may be desired to confirm these findings to avoid false-positive results. In case of an accidental event or other reasons, which necessitates air investigations, it is recommended to use the analytical method 00410 (HPLC-UV) for a first analysis of flufenacet. If flufenacet is detected with method 00410 it is suggested to use the analytical method 00410C as confirmatory method, to avoid false-positive results.

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

5.3.2.8 Other studies/ information

None.

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** **GLP or GEP status** **published or not**	**Vertebrate study Y/N**	**Owner**
KCP 5.1.1 / 01	Hoffmann, D.	2020	Determination of flufenacet in formulations - HPLC-UV, external standard Report No.: M-688742-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: No unpublished	No	Bayer
KCP 5.1.1 / 02	Hoffmann, D.; Garcia Sanchez, M. T.	2020	Validation of analytical method AM036120MF1 - Determination of flufenacet in the formulation flufenacet SC 508.8 (508.8 g/L) Report No.: FM0415(MVF00)G01, Edition Number: M-758632-01-1 Bayer AG, Crop Science Division, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 01 **... also filed:** **KCA 6.3.1.1 / 01**	Seym, M.	1996	Determination of residues of FOE 5043 & Diflufenican 60 WG in/on winter barley, winter rye and winter wheat following early post-emergence spray application in Germany Report No.: RA-2010/94, Edition Number: M-004451-01-2 Bayer AG, Leverkusen, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 02 **... also filed:** **KCA 6.3.1.1 / 02**	Hoffmann, M.	2002	Determination of residues of FOE 5043 in/on wheat and barley following spray application of FOE 5043 & Diflufenican (600 SC) to winter wheat and winter barley in the field in Northern and Southern France, Germany and Spain Report No.: RA-2144/00, Edition Number: M-058156-01-1 Bayer AG, Leverkusen, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.5 / 03	Rzepka, S.	2006	Supplement E004 of Method 00346 for the determination of residues of FOE 5043, FOE 5043 Oxalate, FOE 5043 Sulfonic Acid, and FOE 5043 Thioglycolate Sulfoxide in rice (grain) Report No.: 00346/E004, Edition Number: M-277805-01-1 Method Report No.: BAY-0610V Eurofins Analytik GmbH, Hamburg, Germany GLP/GEP: Yes unpublished	No	Bayer

Data Point	Author(s)	Year	**Title** **Company Report No.** **Source** **GLP or GEP status** **published or not**	**Vertebrate study Y/N**	**Owner**
KCP 5.1.2.5 / 04 **... also filed:** **KCA 6.6.2 / 01**	Melrose, I.; Erler, S.	2008	Determination of the residues of FOE 5043 in/on the rotational crops cereals after spraying of Artist (41.5 WG) and Liberator (500 SC) in the field in the United Kingdom, Germany and Northern France Report No.: RA-2020/06, Edition Number: M-306269-01-1 Bayer CropScience S.A., Lyon, France GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 01	Brumhard, B.	2009	Analytical method 01080 for the determination of residues of flufenacet (FOE 5043) in soil using LC-MS/MS Report No.: 01080, Edition Number: M-357296-01-1 Method Report No.: MR-07/352 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 02 **... also filed:** **KCP 10.4.1.2 / 01**	Leicher, T.	2008	Flufenacet SC 500: effect on the earthworm fauna of a grassland area within one year Report No.: LRT/RG-F-4/08, Edition Number: M-307211-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 03	Krebber, R.; Leppelt, L.	2009	Method 01169 for the determination of flufenacet-oxalate in test water by HPLC-MS/MS Report No.: 01169, Edition Number: M-357278-01-1 Method Report No.: MR-09/120 Bayer CropScience AG, Monheim, Germany GLP/GEP: No unpublished	No	Bayer
KCP 5.1.2.6 / 04 **... also filed:** **KCP 10.2.1 / 02**	Bruns, E.	2009	Pseudokirchneriella subcapitata growth inhibition test with flufenacet-oxalate Report No.: EBFOL137, Edition Number: M-358823-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 05 **... also filed:** **KCP 10.2.3 / 02**	Bruns, E.	2009	Lemna gibba G3 Growth inhibition test with flufenacet-oxalate under static conditions Report No.: EBFOL138, Edition Number: M-359515-02-1 Bayer CropScience AG, Monheim, Germany **... amended: 2009-12-08** GLP/GEP: Yes unpublished	No	Bayer

Data Point	Author(s)	Year	**Title** **Company Report No.** **Source** **GLP or GEP status** **published or not**	**Vertebrate study Y/N**	**Owner**
KCP 5.1.2.6 / 06 **... also filed:** **KCP 10.2.1 / 03**	Baetscher, R.	2001	Toxicity of flufenacet SC 500 to Pseudokirchneriella subcapitata (fornerly Selenastrum capricornutum in a 72-hour algal growth inhibition test Report No.: 796364, Edition Number: M-055471-01-1 RCC Ltd., Itingen, Switzerland GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 07 **... also filed:** **KCP 10.2.3 / 03**	Baetscher, R.	2001	Toxicity of flufenacet SC 500 to the aquatic higher plant Lemna gibba in a 7-day static growth inhibition test Report No.: 796342, Edition Number: M-055476-01-1 RCC Ltd., Itingen, Switzerland GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 08	Kling, A.	2014	Flufenacet (tech.) - Assessment of chronic effects to the honeybee, Apis mellifera L., in a 10 days continuous laboratory feeding limit test Report No.: S13-00145, Edition Number: M-477339-01-1 Eurofins-GAB GmbH, Niefern-Oeschelbronn, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 09 **... also filed:** **KCP 10.3.1.3 / 01**	Rathjen, K. A.	2018	Flufenacet: Honey bee (Apis mellifera L.) larval toxicity test, repeated exposure Report No.: 13798.6448, Edition Number: M-615473-01-1 Smithers Viscient, LLC, Snow Camp, NC, USA GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 10 **... also filed:** **KCP 10.6.2 / 01** **KCP 6.5.2 / 01**	Friedrich, S.	2005	Flufenacet SC 500: seedling emergence and seedling growth test on terrestrial non-target plants Report No.: 041048104, Edition Number: M-248250-01-1 BioChem agrar GmbH, Gerichshain, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.1.2.6 / 11 **... also filed:** **KCP 10.6.2 / 02** **KCP 6.5.2 / 02**	Friedrich, S.	2005	Flufenacet SC 500: vegetative vigour test on non-target terrestrial plants Report No.: 041048105, Edition Number: M-248251-01-1 BioChem agrar GmbH, Gerichshain, Germany GLP/GEP: Yes unpublished	No	Bayer

Data Point	Author(s)	Year	**Title** **Company Report No.** **Source** **GLP or GEP status** **published or not**	**Vertebrate study Y/N**	Owner
KCP 5.2.1 / 01	Class, Th.; Merdian, H.	2010	Validation of BCS analytical method no. 01179 for the determination of residues of flufenacet in/on plant materials by HPLC-MS/MS Report No.: 01179, Edition Number: M-362716-01-1 Method Report No.: B 1778 G PTRL Europe GmbH, Ulm, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.2.1 / 02	Stuke, S.	2018	Amendment no. 02 to final report: Analytical method 01100 for the determination of residues of flufenacet (FOE5043) and its metabolites in/on plant material Report No.: 01100, Edition Number: M-362575-03-1 Method Report No.: MR-08/060 Bayer AG, Crop Science Division, Monheim, Germany **... amended: 2018-09-20** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.2.1 / 03 **... also filed:** **KCA 6.1 / 01**	Stuke, S.	2018	Amendment no. 01 to final report: Modification M001 of the residue analytical method 01100 for the determination of residues of flufenacet (FOE5043) and its metabolites in/on cereals (straw, grain, and green material) at a LOQ of 0.01 mg/kg for grain and green material and at a LOQ of 0.05 mg/kg for straw by HPLC-MS/MS Report No.: 01100/M001, Edition Number: M-433720-02-1 Method Report No.: MR-11/011 Bayer AG, Crop Science Division, Monheim, Germany **... amended: 2018-09-20** GLP/GEP: Yes unpublished	No	Bayer
KCP 5.2.1 / 04	Meyer, M.	2011	Independent laboratory validation of the Bayer CropScience methods 01100 and 01179 for the determination of residues of Flufenacet (FOE5043) in/on plant materials Report No.: P612107502, Edition Number: M-405654-01-1 Method Report No.: IF-10/01717126 SGS Institut Fresenius GmbH, Taunusstein, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.2.1 / 05	Stuke, S.; Weile, M.	2011	Position paper: Subject: Flufenacet: Answer to CRD questions related to the authorization of the product Liberator SC 500 (flufenacet + diflufenican 400 g/L + 100 g/L) - Comparison of flufenacet residue analytical method nos. 00346 vs. 01179 Report No.: M-416013-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: n.a. unpublished	No	Bayer

Data Point	Author(s)	Year	**Title** **Company Report No.** **Source** **GLP or GEP status** **published or not**	**Vertebrate study Y/N**	**Owner**
KCP 5.2.1 / 06 **... also filed:** **KCA 6.2.1 / 01**	Krolski, M. E.; Bosnak, L. L.	1997	The metabolism of [Fluorophenyl-UL-14C] FOE 5043 in wheat after postemergent foliar spray application Report No.: 107399, Edition Number: M-002275-01-1 Bayer Corporation, Stilwell, KS, USA GLP/GEP: Yes unpublished	No	Bayer
KCP 5.2.2 / 01	xxx	2013	Validation of the Bayer methods 00418 (M-019605-01-1) and 00418/M001 (M-019614-01-1) for the determination of residues of flufenacet (FOE 5043) and its metabolites in animal tissues and animal products Report No.: S12-00052, Edition Number: M-461242-01-1 xxx GLP/GEP: Yes unpublished	No	Bayer
KCP 5.2.3 / 01	Kaussmann, M.	2016	Analytical method 01486 for the determination of various pesticides and selected pesticide metabolites in plasma by HPLC-MS/MS Report No.: 01486, Edition Number: M-556577-01-1 Method Report No.: P683166504 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.2.4 / 01	Brumhard, B.	2005	Modification M001 of method 00359 for the determination of the herbicide FOE 5043 and its metabolite FOE 5043-alcohol, FOE 5043-oxalate and FOE 5043-sulfonic acid in soil using HPLC-MS/MS Report No.: 00359/M001, Edition Number: M-248543-01-1 Method Report No.: MR-028/05 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.2.5 / 01	Krebber, R.; Braune, M.	2013	Analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS Report No.: MR-13/085, Edition Number: M-466732-01-1 Bayer CropScience AG, Monheim, Germany GLP/GEP: Yes unpublished	No	Bayer
KCP 5.2.5 / 02	Stanislowski, T.	2013	Independent laboratory validation of BCS analytical methods 01333 and 01387 for determination of various pesticides in surface water by Di-HPLC-MS/MS Report No.: P3117 G, Edition Number: M-470714-02-1 PTRL Europe GmbH, Ulm, Germany **... amended: 2013-12-13** GLP/GEP: Yes unpublished	No	Bayer

Data Point	Author(s)	Year	Title Company Report No. Source GLP or GEP status published or not	Vertebrate study Y/N	Owner
KCP 5.2.6 / 01	Hellpointner, E.	2000	Confirmatory method for the determination of FOE 5043 in air (confirmed method: 00410) Report No.: 00410C, Edition Number: M-048783-01-1 Method Report No.: MR-469/00 Bayer AG, Leverkusen, Germany GLP/GEP: Yes unpublished	No	Bayer

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

Please note that all data mentioned as part of DAR, RAR, or EFSA journals are considered as 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 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 Flufenacet

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

Method 00346 (EU peer reviewed) has been used to analyse the supplementary residue trials reported in section B7 A2.1.3.1.1 and A2.1.3.1.2 (Study 1 RA-2010/94, study 2 RA-2144/00). The extension E004 of this method (method 00346/E004) was performed to validate the sample material cereal grain at a lower LOQ of 0.01 mg/kg and was used for the analysis of cereal grain from the field rotational crop study (B7, A 2.1.6, study RA-2020/06).
The method extension 00396/E004 was evaluated on Member State level and also by France in the framework of previous submissions as well as in the DRAR with France acting as co-RMS (Poland, 2017).

A 2.1.1.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.1)

A 2.1.1.1.1 Analytical method 00346 (wheat, barley, rye))

A 2.1.1.1.1.1 Method validation in study report RA-2010/94

Comments of zRMS:	Method 00346 was EU peer reviewed. The analytical method has been validated to determine the residue of flufenacet and all metabolites containing the N-fluorophenyl-N-isopropyl-amine moiety in /on cereal matrices relevant to the residue study with an LOQ of 0.05 mg/kg for green plant material and grain and 0.1 mg/kg for straw. The method is acceptable.

Reference:	**KCP 5.1.2.5/01**
Title:	Determination of residues of FOE 5043 & Diflufenican 60 WG in/on winter barley, winter rye and winter wheat following early post-emergence spray application in Germany
Report:	Seym, M.; 1996; RA-2010/94; M-004451-01-2
Authority registration No:	
Guideline(s):	not specified
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods
Method 00346 is EU peer reviewed for the matrices relevant to this study (cereal grain, green plant material, straw). Flufenacet and all metabolites containing the N-fluorophenyl-N-isopropyl-amine moiety can be determined by this total residue method. Additional validation data are reported within residue study RA-2010/94 reported in B7.
The residues are oxidized with potassium permanganate for 5 min and hydrolysed to the common moiety N-fluorophenyl-N-isopropyl amine (fluoroaniline) by digesting the crop mixture with 47 % sulfuric acid for 24 hours. The fluoroaniline is separated from the crop matrix by steam distillation after making the crop digest basic with 50 % sodium hydroxide. The aniline is extracted from the steam distillate and derivatized with trifluoroacetic anhydride. A cleanup on a C-18 cartridge follows. The derivative, 2,2,2-trifluoro-N-(4-fluorophenyl)-N-isopropylacetamide, is measured by gas chromatography/mass spectroscopy (GC/MS). The residue is expressed as flufenacet equivalents.
GC/MS determination of derivative 2,2,2-trifluoro-N-(4-fluorophenyl)-N-isopropylacetamide was done monitoring the 207 m/z fragment ion (quantifier), and the ions 138 m/z and 249 m/z ions (qualifiers).
In the basic method a mix of flufenacet metabolites containing the N-fluorophenyl-N-isopropyl-amine moiety (FOE oxalate, FOE sulfonic acid, FOE thioglycolate sulfoxide) has also been validated for these

matrices.

Results and discussions

Table A 1: Recovery results from method validation of flufenacet using the analytical method 00346

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)	Comments
Flufenacet (determined as FOE5043 trifluoroacetamide)					
Barley, green material	0.05	3	87	4.4	Study report RA-2010/94
Barley grain	0.05	3	86	5.9	Study report RA-2010/94
Barley straw	0.1	3	87	7.4	Erroneous in report (0.05)
Wheat, green material	0.05	6	86	11.2	Study report RA-2010/94
Wheat grain	0.05	4	95	12.5	Study report RA-2010/94
Wheat straw	0.1	3	85	9.6	Study report RA-2010/94
Rye green material	0.05	12	81	6.1	Study report RA-2010/94
Rye green material	0.5	8	89	5.7	Study report RA-2010/94
Rye grain	0.05	12	90	9.2	Study report RA-2010/94
Rye grain	0.5	8	85	9.1	Study report RA-2010/94
Rye straw	0.1	12	87	5.7	Study report RA-2010/94
Rye straw	1.0	8	81	7.4	Study report RA-2010/94

Table A 2: Characteristics for the analytical method used for validation of flufenacet residues in small cereal grain, straw and green plant material

	Flufenacet and its metabolites determined as FOE 5043 trifluoro acetamide
Specificity	Residues in control samples were well below 30% of the respective LOQ level, 3 fragment ions determined: for quantification (m/z 207), ions m/z 138 and 249 for confirmation
Calibration (type, number of data points)	The linearity of the detector response was confirmed in the original method 00346. The 2,2,2-trifluoro-N-(4-fluorophenyl)-N-isopropylacetamide standard was injected 4times each at 7 concentrations. The correlation between the injected amount of substance and the detector response was linear for standard in solvent and matrix. The correlation coefficients (r2) were between 0.9997 and 1.0000.
Calibration range	All matrices: 0.025 to 2.5 µg/ml (as flufenacett equivalents) corresponding to 0.025-2.5 mg/kg (as flufenacet equivalents) for green plant material, cereal grain and 0.05-5.0 mg/kg (as flufenacet equivalents) for straw
Assessment of matrix effects is presented	Yes, in basic method 00346
Limit of determination/quantification	0.05 mg/kg green plant material, small cereal grain (wheat, barley, rye) 0.1 mg/kg straw (wheat, barley, rye)

Conclusion

The method meets all guideline criteria for data generation methods to determine the residues of flufenacet in /on cereal matrices relevant to the residue study with an LOQ of 0.05 mg/kg for green plant material and grain and 0.1 mg/kg for straw.

A 2.1.1.1.1.2 Method validation in study report RA-2144/00

Comments of zRMS:	Method 00346 was EU peer reviewed. The method is acceptable.

Reference:	**KCP 5.1.2.5/02**
Title:	Determination of residues of FOE 5043 in/on wheat and barley following spray application of FOE 5043 & Diflufenican (600 SC) to winter wheat and winter barley in the field in Northern and Southern France, Germany and Spain
Report:	Hoffmann, M.; 2002; RA-2144/00; M-058156-01-1
Authority registration No:	
Guideline(s):	EU-Ref: Council Directive 91/414/EEC Residues in or on treated products, food and feed; not stated fulfils EU 7029/VI/95 rev.5 dated 22 July 1997
Deviations:	none
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

Method 00346 is EU peer reviewed for the matrices relevant to this study (cereal grain, green plant material, straw). Additional validation data are reported within residue study RA-2144/00 reported in B7.

For method description please refer to A.2.1.1.1.1.1 above (study report RA-2010/94).

The analytical method was validated prior to analysis by running a set of recoveries at the LOQ by spiking control samples with flufenacet and all metabolites as well as a mixture of all substances. In addition, during analysis of the samples, concurrent recovery experiments at the LOQ and the ten-fold LOQ were performed by spiking control samples with the mixture of flufenacet and metabolites.

Results and discussions

Table A 3: Recovery results from method validation of flufenacet using the analytical method 00346

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)	Comments
Flufenacet (determined as FOE5043 trifluoroacetamide)					
Barley grain	0.05	11	84	15.6	Study report RA-2144/00
Barley grain	0.5	1	80	--	Study report RA-2144/00
Barley straw	0.1	11	84	8.3	Study report RA-2144/00
Barley straw	1.0	1	81	--	Study report RA-2144/00
Wheat grain	0.05	11	86	9.3	Study report RA-2144/00
Wheat grain	0.5	1	73	--	Study report RA-2144/00
Wheat straw	0.1	11	86	8.4	Study report RA-2144/00
Wheat straw	1.0	1	75	--	Study report RA-2144/00

Spiked substances: 1. flufenacet
2. FOE 5043 oxalate
3. FOE 5043 sulfonic acid
4. FOE 5043 thioglycolate sulfoxide
5. FOE 5043 mixture of equivalent amounts of 1-4
FL: Fortification Level, expressed in flufenacet equivalents
RSD: Relative Standard Deviation
Residues calculated as: flufenacet equivalents

Table A 4: Characteristics for the analytical method used for validation of flufenacet residues in small cereal grain, straw and green plant material

	Flufenacet and its metabolites determined as FOE 5043 trifluoro acetamide
Specificity	Residues in control samples were below 30% of the respective LOQ level based on peak area of the chromatograms, 3 fragment ions determined: for quantification (m/z 207), ion m/z 138 reported for confirmation
Calibration (type, number of data points)	The linearity of the detector response was confirmed in the original method 00346. The FOE 5043 trifluoroacetamide standard was injected 4times each at 7 concentrations. The correlation between the injected amount of substance and the detector response was linear for standard in solvent and matrix. The correlation coefficients (r2) were between 0.9997 and 1.0000. In study RA-2144/00: The linearity of the detector response was confirmed for the derivative FOE 5043 trifluoroacetamide single injection at least 9 concentrations .
Calibration range	In basic method: All matrices: 0.025 to 2.5 µg/ml (as flufenacet equivalents) corresponding to 0.025-2.5 mg/kg for green plant material, cereal grain and 0.05-5.0 mg/kg (as flufenacet equivalents) for straw Study RA-2044/00: 0.0173 – 3.32 µg/ml, expressed as FOE 5043 trifluoroacetamide; corresponding to 0.025 – 4.82 µg/ml flufenacet.) In mass units: 0.025 – 4.82 mg/kg flufenacet; R= 0.9999
Assessment of matrix effects is presented	Yes, in basic method 00346
Limit of determination/quantification	0.05 mg/kg small cereal grain (wheat, barley) 0.1 mg/kg straw (wheat, barley)

Conclusion
The method meets all guideline criteria for data generation methods to determine the residues of flufenacet in /on cereal matrices relevant to the residue study with an LOQ of 0.05 mg/kg for grain and 0.1 mg/kg for straw.

A 2.1.1.1.2 Analytical method 00346/E004

A 2.1.1.1.2.1 Method validation

Comments of zRMS:	The method 00346 and its supplement E004 was evaluated by RMS-Poland in RAR for Flufenacet (Vol. 3 – B.5, April 2022). The Bayer CropScience Method 00346 and its supplement E004 has been validated to determination of residues of FOE 5043, FOE 5043 oxalate, FOE 5043 Sulfonic Acid and FOE 5043 Thioglycolate Sulfoxide after derivatisation as FOE 5043 Trifluoro Acetamide with capillary gas chromatography coupled to a mass selective detector (MSB) in rice grain with a lower LOQ of 0.01 mg/kg. For FOE 5043, FOE5043 Oxalate, FOE 5043 Sulfonic Acid, and FOE 5043 Thioglycolate Sulfoxide in rice (grain) the limit of quantitation (LOQ) was 0.01 mg/kg with a limit of detection (LOD) of 0.003 mg/kg. The metabolites are expressed as parent equivalents. Mean recovery values obtained for rice (grain) for both fortification levels (LOQ and ten times LOQ) comply with the standard acceptance criteria of SANTE/2020/12830 rev 1, which demand that the mean recovery at each fortification level should be in the range of 70 -110% with RSD< 20%. The method meets all requirements for pre-registration methods (SANCO/3029/99 rev.4) and SANCO 825/00 rev 8.1 with exception of data on efficiency and precision of the derivatisation step. However, this step was demonstrated in other studies Gould, T.

	J.; 1995; M-041609-01, Beedle, E.C., Ying, S. L.; 2000; M-020428-01 and Krolski, M. E.; Bosnak, L. L.; 1997; M-002275-01. Therefore the method is accepted.

Reference:	**KCP 5.1.2.5/03**
Title:	Supplement E004 of Method 00346 for the determination of residues of FOE 5043, FOE 5043 Oxalate, FOE 5043 Sulfonic Acid, and FOE 5043 Thioglycolate Sulfoxide in rice (grain)
Report:	Rzepka, S.; 2006; 00346/E004; M-277805-01-1
Authority registration No:	
Guideline(s):	EU Directive 91/414/EEC as amended by 96/46/EC 4.2.1; SANCO/825/00 rev.7 of 17/03/04; BBA Guidline July 21, 1998
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

Supplement E004 to the analytical method 00346 (Seym, M.; 1995; M-018864-02-1) was validated for the determination of flufenacet (FOE 5043) and its metabolites (FOE 5043 oxalate hydrate, FOE 5043 sulfonic acid sodium salt, FOE 5043 thioglycolate sulfoxide) in/on rice grain with an LOQ of 0.01 mg/kg. The method was used for analysis of cereal grain from the field rotational crop studies (small cereal grain was validated in study RA-2020/06 reported in Section B7).

Flufenacet and all metabolites containing the *N*-fluorophenyl-*N*-isopropyl-amine moiety can be determined by this common moiety method. The residues of flufenacet, FOE 5043 oxalate hydrate, FOE 5043 sulfonic acid sodium salt, FOE 5043 thioglycolate sulfoxide are oxidized with potassium permanganate for 5 min and hydrolyzed to the common moiety 4-fluoro-N-methylethyl benzenamine (fluoroaniline) by digesting the crop mixture with 47% sulfuric acid for 24 hours. The fluoroaniline is separated from the crop matrix by steam distillation after making the crop digest basic with 50% sodium hydroxide. The aniline is extracted from the steam distillate and derivatized with trifluoroacetic anhydride. A cleanup on a C-18 SPE cartridge follows. The derivative 2,2,2-trifluoro-N-(4-fluorophenyl)-N-isopropylacetamide, (called FOE 5043 trifluoacetamide in the documentation) is measured by gas chromatography/mass spectroscopy (GC/MSD). The residue is expressed as flufenacet equivalents.

For quantitation molecular ion m/z 249 was used. For verification the fragment ions m/z 207 and m/z 138 were selected. The standard solutions were prepared either with methanol or with acetone. FOE 5043 trifluoro acetamide (analytical standard) was used for preparing the calibration standards with tert-butyl methyl ether.

Results and discussions

High precision was demonstrated by low relative standard deviations (RSD always below 20%).

Mean recoveries for each fortification level were within the range of 70 – 110%, which shows an acceptable accuracy of the method.

Stability of the derivative 2,2,2-trifluoro-N-(4-fluorophenyl)-N-isopropylacetamide in extracts was shown up to a storage interval of 8 weeks in the original method 00346 when stored in a refrigerator at about +4°C.

Table A 5: Recovery results from method validation of flufenacet, FOE 5043 oxalate hydrate, FOE 5043 sulfonic acid sodium salt and FOE 5043 thioglycolate sulfoxide using the analytical method 00346/E004

Matrix	Analyte*	Fortification level (mg/kg) expressed as flufenacet (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Rice grain	Flufenacet	0.01 (n = 5)	77	9.5	m/z 249
		0.10 (n = 5)	74	5.0	m/z 249
	FOE 5043 oxalate hydrate	0.01 (n = 5)	77	5.2	m/z 249
		0.10 (n = 5)	75	8.5	m/z 249
	FOE 5043 sulfonic acid sodium salt	0.01 (n = 5)	77	11	m/z 249
		0.10 (n = 5)	72	10	m/z 249
	FOE 5043 thioglycolate sulfoxide	0.01 (n = 5)	74	10	m/z 249
		0.10 (n = 5)	71	11	m/z 249
Rice grain	Flufenacet	0.01 (n = 5)	79	3.9	m/z 138
		0.10 (n = 5)	78	8.6	m/z 138
	FOE 5043 oxalate hydrate	0.01 (n = 5)	83	16	m/z 138
		0.10 (n = 5)	80	15	m/z 138
	FOE 5043 sulfonic acid sodium salt	0.01 (n = 5)	84	3.8	m/z 138
		0.10 (n = 5)	75	12	m/z 138
	FOE 5043 thioglycolate sulfoxide	0.01 (n = 5)	87	14	m/z 138
		0.10 (n = 5)	74	6.2	m/z 138
Rice grain	Flufenacet	0.01 (n = 5)	71	7.7	m/z 207
		0.10 (n = 5)	73	4.5	m/z 207
	FOE 5043 oxalate hydrate	0.01 (n = 5)	74	3.5	m/z 207
		0.10 (n = 5)	76	14	m/z 207
	FOE 5043 sulfonic acid sodium salt	0.01 (n = 5)	72	6.8	m/z 207
		0.10 (n = 5)	72	9.2	m/z 207
	FOE 5043 thioglycolate sulfoxide	0.01 (n = 5)	71	7.2	m/z 207
		0.10 (n = 5)	70	10	m/z 207

*All analytes determined as FOE5043 trifluoroacetamide calculated and expressed as flufenacet

Table A 6: Characteristics for the analytical method used for validation of flufenacet residues in rice grain

	Flufenacet and its metabolites determined as FOE 5043 trifluoro acetamide
Specificity	Blank value <30% LOQ. One fragment ion with a m/z ratio >100 was used for quantification and two fragment ions with a m/z ratio >100 were used for confirmation. Therefore, this GC/MSD method can be considered as highly specific. Mass spectrum is provided in the original report.
Calibration (type, number of data points)	Calibration with solvent standards of FOE 5043 trifluoroacetamide, 1/x weighted linear regression (calibration line equations are presented in the original report). Coefficient of correlation: m/z = 249: R^2 = 1.0000 m/z = 138: R^2 = 1.0000

	Flufenacet and its metabolites determined as FOE 5043 trifluoro acetamide
	m/z = 207: R^2 = 1.0000 Number of data points for all mass transitions: 6
Calibration range	0.002 – 2.00 µg/mL of FOE 5043 trifluoroacetamide (0.0029 – 2.91 µg/mL expressed as parent flufenacet), corresponding to 0.002 – 2.00 mg/kg of FOE 5043 trifluoroacetamide (0.0029 – 2.91 mg/kg expressed as parent flufenacet)
Assessment of matrix effects is presented	Matrix effects were assessed in the original method 00346. The presence of matrix does not affect the detector response.
Limit of determination/quantification	The limit of quantification for flufenacet (FOE 5043), FOE 5043 oxalate hydrate, FOE 5043 sulfonic acid sodium salt, and FOE 5043 thioglycolate sulfoxide was established and validated at 0.01 mg/kg in rice grain with a limit of detection of 0.003 mg/kg. The metabolites are expressed as parent equivalents.

Conclusion
All method validation results are in compliance with the European guideline requirements for data generation methods (SANCO/3029/99 rev. 4, 11/07/2000). Supplement E004 of method 00346 meets all necessary performance criteria to determine the total residue flufenacet in/on rice grain (representative for small cereal grain) with an LOQ of 0.01 mg/kg. The method is considered to comply with the quality criteria as set out in SANTE/2020/12830 rev 1.

A 2.1.1.1.2.2 Method validation of method 00346/E004 (grain) and method 00346 (green material, straw) in residue report RA-2020/06

Comments of zRMS:	The method was evaluated at EU level. The method is acceptable.

Reference:	**KCP 5.1.2.5/04**
Title:	Determination of the residues of FOE 5043 in/on the rotational crops cereals after spraying of Artist (41.5 WG) and Liberator (500 SC) in the field in the United Kingdom, Germany and Northern France
Report:	Melrose, I.; Erler, S.; 2008; RA-2020/06; M-306269-01-1
Authority registration No:	
Guideline(s):	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7524/VI/95 rev. 2 (1997-07-22) OECD Guideline for testing of Chemicals; Residues in rotational crops (limited field studies), No. 504, 8 Jan. 2007
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods
Method 00346 and its extension 00346/E004 was applied as described above with slight modifications.

Modifications to Analytical Method 00346/E004 in study RA-2020/06:
No SPE clean-up was performed for all the grain specimens. GC/MS determination of the TFA derivative was done in the MS/MS mode, isolating the 207 m/z fragment ion for collision induced dissociation (CID), monitoring the 138 m/z (quantifier), 110 m/z and 112 m/z daughter ions (qualifiers). The method extension E004 was validated for cereal (wheat) grain, additional validation data were generated for wheat green material and straw with method 00346.

Results and discussions

Table A 7: Recovery results from method validation of flufenacet, FOE 5043 oxalate hydrate, FOE 5043 sulfonic acid sodium salt and FOE 5043 thioglycolate sulfoxide using the analytical method 00346/E004 (grain) and 00346 (green material, straw)

Matrix	Analyte**	Fortification level (mg/kg) expressed as flufenacet (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Wheat grain	Flufenacet	0.01 (n = 5)	94	16	Pre-validation 0346/E004
	FOE 5043 oxalate hydrate	0.01 (n = 4)	75	16	Pre-validation 0346/E004
	FOE 5043 sulfonic acid sodium salt	0.01 (n = 4)	69	6	Pre-validation 0346/E004
	FOE 5043 thioglycolate sulfoxide	0.01 (n = 5)	73	5	Pre-validation 0346/E004
Wheat grain	FOE 5043 Mix*	0.01 (n=2)	89	--	Procedural recoveries 00346/E004
		0.4 (n=2)	83	--	Procedural recoveries 00346
Wheat/barley green material	FOE 5043 Mix*	0.05 (n=3)	94	13.3	Procedural recoveries 00346
Wheat/barley straw	FOE 5043 Mix*	0.1 (n=2)	113	--	Procedural recoveries 00346
		1.0 (n=2)	94	--	Procedural recoveries 00346

*FOE 5043 Mix : % of FOE 5043, % FOE 5043 Oxalate Hydrate, % of FOE 5043 Sulfonic Acid Sodium Salt, % of FOE 5043 Thioglycolate Sulfoxide
** all analytes determined as FOE5043 trifluoroacetamide calculated and expressed as flufenacet)

Table A 8: Characteristics for the analytical method used for validation in residue study RA-2020/06

	Flufenacet and its metabolites determined as FOE 5043 trifluoro acetamide
Specificity	Blank value <30% LOQ. Mass spectrum provided
Calibration (type, number of data points)	Calibration with solvent standards of FOE 5043 trifluoroacetamide, 1/x weighted linear regression (calibration line equations are presented in the original report). Coefficient of correlation: m/z = 138: $R^2 = 0.9951$ Number of data points: 5
Calibration range	0.001 – 0.1 ng/mL of FOE 5043 trifluoroacetamide
Assessment of matrix effects is presented	Matrix effects were assessed in the original method 00346. The presence of matrix does not affect the detector response.
Limit of determination/quantification	The limit of quantification for flufenacet (FOE 5043), FOE 5043 oxalate hydrate, FOE 5043 sulfonic acid sodium salt, and FOE 5043 thioglycolate sulfoxide was established and validated at 0.01 mg/kg in wheat grain with a limit of detection of 0.003 mg/kg. The metabolites are expressed as parent equivalents (method 00346/E004). Green material 0.05 mg/kg (basic method 00346) Straw 0.1 mg/kg (basic method 00346)

Conclusion

The methods referenced as 00346 and 00346/E004 was adequately validated for the determination of

residues of flufenacet determined as FOE 5043 trifluoro acetamide in cereal commodities (green plant material, grain and straw) within study RA-2020/06.

A 2.1.1.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.1)

No new or additional studies have been submitted.

A 2.1.1.3 Description of analytical methods for the determination of residues in support to environmental fate studies (KCP 5.1)

No new or additional studies have been submitted.

A 2.1.1.4 Description of analytical methods for the determination of residues in support to toxicological studies (KCP 5.1)

No new or additional studies have been submitted.

A 2.1.1.5 Description of analytical methods for the determination of residues in support of operator, worker, resident and bystander exposure studies (KCP 5.1)

No new or additional studies have been submitted.

A 2.1.1.6 Description of analytical methods for the determination of residues in of ecotoxicology studies (KCP 5.1)

A 2.1.1.6.1 Analytical method 01080

Comments of zRMS:	The analytical method 01080 has been validated for the determination of residues of flufenacet (FOE 5043) in soil by HPLC-ESI-MS/MS. The limit of quantitation (LOQ) for flufenacet is 4.0 µg/kg in soil. Mean recovery were within the 70 - 110% range for all matrices. Relative standard deviations were below 20% for all sample materials. The method meets all guideline criteria according to SANCO/825/00 rev.8.1 and SANCO/3029/99 rev. 4 and is suitable for the determination of residues of flufenacet (FOE 5043) in soil using LC-MS/MS.

Reference:	**KCP 5.1.2.6/01**
Title:	Analytical method 01080 for the determination of residues of flufenacet (FOE 5043) in soil using LC-MS/MS
Report:	Brumhard, B.; 2009; 01080; M-357296-01-1
Authority registration No:	
Guideline(s):	EU: 91/414/EEC amended by Commission Directive 96/68/EC European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99; SANCO/825/00 rev. 7, European Commission, Directorate General Health and Consumer Protection, 2004-03-17; US EPA: OPPTS 860.1340
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and Methods

The original method 01080 describes the determination of residues of flufenacet in soil by high performance liquid chromatography (HPLC) with mass-spectrometric (MS/MS) detection (ESI positive, MRM m/z: 364 → 152 used as qualifier and m/z: 364 → 194.1 used as quantifier).
Soil samples of 20 g were extracted in a microwave extractor with 40 mL of a mixture of acetonitrile/water (1/1, v/v). Then a subsample was centrifuged to remove fine particles of the soil. Possible Matrix effects of flufenacet were eliminated by using an internal standard solution of isotopically labelled reference item. This solution was added to the sample solutions after extraction. The method was validated using a silt soil (Höfchen) and a sandy loam soil (Laacher Hof).
Two different soils were used in order to assess a possible influence of different soil characteristics. The soil samples were classified according to USDA specifications.

Table A 9: Soil Characteristics

	Soil Höfchen	**Soil Laacher Hof**
Description	Plot 4011; 0-30 cm soil layer	Plot 712/718; 0-30 cm soil layer
pH (in $CaCl_2$ solution)	6.7	6.8
pH (in H_2O)	7.4	7.4
Organic Carbon [%]	0.92	1.20
Organic Matter [%] *	1.58	2.06
Cation Exchange Capacity [meq / 100 g dry soil]	12.4	9.8
max. Water Holding Capacity [g / 100 g dry soil]	39.5	37.9
Textural Description according to USDA [Fraction %]	Fraction [%]	Fraction [%]
Clay (<0.002 mm)	19.4	12.0
Silt (0.002-0.050 mm)	76.3	18.3
Sand (0.050-2.000 mm)	4.3	69.7
Soil type	Silt loam	Sandy loam

* Organic matter = Organic carbon x 1.72

Results and discussions

Mean recoveries for each fortification level and the overall mean recovery were within the 70 - 110% range and relative standard deviations were below 20% for all matrices. Measurements of the confirmatory mass transition provided similar results.

Table A 10: Recoveries for Flufenacet m/z 364 → 194.1. Quantitation Mass Transition.

Soil	**Fortification**	**Recoveries % (Single Values)**	**Recoveries**		**Overall**	
			Mean	**RSD**	**Mean**	**RSD**

	Level (FL) [µg/kg]						[%]	[%]	[%]	[%]
Höfchen	4	104	102	103	99	103	102	1.9	102	2.4
	40	99	101	104	107	103	103	3.0		
Laacher Hof	4	98	96	98	101	100	98	1.8	98	2.1
	40	96	99	95	99	95	97	2.2		
Mean and RSD over both soils	4	--	--	--	--	--	100	2.5	100	3.3
	40	--	--	--	--	--	100	4.1		

RSD = relative standard deviation

Table A 11: Recoveries for Flufenacet m/z 364 → 152. Confirmatory Mass Transition.

Soil	Fortification Level (FL) [µg/kg]	Recoveries % (Single Values)					Recoveries		Overall	
							Mean [%]	RSD [%]	Mean [%]	RSD [%]
Höfchen	4	102	104	104	103	111	105	3.2	104	3.1
	40	99	102	103	108	102	103	3.0		
Laacher Hof	4	98	90	95	100	96	96	4.1	96	3.0
	40	97	98	94	98	98	97	1.6		
Mean and RSD over both soils	4	--	--	--	--	--	100	5.9	100	4.8
	40	--	--	--	--	--	100	3.9		

RSD = relative standard deviation

The stability in final soil extracts was checked for the tested sample materials over a period of six days. Results of the initial analysis and after six days of storage in the refrigerator at <6 °C under dark conditions are presented. Flufenacet was found to be stable in final soil extracts for at least six days.

Table A 12: Storage Stability of Flufenacet in Soil Extracts Determined for the Quantitation Mass Transition.

Analyte	Mean Recovery for Soil Höfchen [%] *		Mean Recovery for Soil Laacher Hof [%] *	
	initial analysis	Six days re-analysis	initial analysis	Six days re-analysis
Flufenacet m/z 364 → 194.1	91	110	93	91

*: mean of four replicates fortified at 4 µg/kg

Table A 13: Characteristics for the analytical method 01080 used for validation of flufenacet residues in matrix standard solution

	flufenacet
Specificity	HPLC-MS/MS method is highly specific. Apparent residues and the blank values in all control samples were below 30% of the respective LOQ.
Calibration (type, number of data points)	individual calibration data is presented calibration line equations are presented (1/x weigthed): quantitation MRM: y = 1.20811x – 0.0066383, r = 0.9980 confirmatory MRM: y = 1.06309x – 0.00548626, r = 0.9975 number of data points: 8
Calibration range	1.0 – 150 µg/L (corresponding to about 2.0 to 300 µg/kg sample equivalents)
Assessment of matrix effects is presented	Matrix effects were eliminated by using an internal standard solution of isotopically labelled reference item.
Limit of determination/quantification	LOQ = 4 µg/kg (in soil) LOD = 1.0 µg/kg

Conclusion
The analytical method 01080 was successfully validated and complies with all guidance criteria according to SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4. The method is therefore suitable for the determination of residues of flufenacet in soil with a limit of quantitation of 4 µg/kg.

A 2.1.1.6.1.1 Concurrent validation of analytical method 01080 in support of study M-307211-01-1

Comments of zRMS:	The analytical method 01080 complies with all criteria according to SANCO/825/00 rev.8.1 and SANCO/3029/99 rev. 4 and is suitable for the determination of residues of flufenacet (FOE 5043) in soil using LC-MS/MS. The method fits the purpose with regard to the study Leicher, T.; 2008; M-307211-01-1.

Reference:	**KCP 5.1.2.6/02**
Title:	Flufenacet SC 500: effect on the earthworm fauna of a grassland area within one year
Report:	Leicher, T.; 2008; LRT/RG-F-4/08; M-307211-01-1
Authority registration No:	
Guideline(s):	BBA (Federal Biological Research Centre for Agriculture and Forstry, Germany): Guidelines for the Testing of Plant Protection Products within Registration, Part VI, 2 - 3 (January 1994): Effects of Plant Protection Products on Earthworms in the Field ISO (International Standard Organisation): Guideline CD 11268-3 (E), Soil Quality - Effects of pollutants on Earthworms, Part 3: Guidance on the determination of effects in field situations (1999)
Deviations:	none
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation
The purpose of the analytical phase of the study was the determination of the active ingredient Flufenacet of the test item Flufenacet SC 500 in soil samples of a tests on earthworm populations under field conditions. The samples were analysed according to Method 01080 which was developed and fully validated for the determination of flufenacet in soil samples by HPLC-MS/MS (Brumhard, B.; 2009; M-357296-01-1).

The determination was conducted using high performance liquid chromatography (HPLC) with mass-spectrometric (MS/MS) detection (ESI positive, MRM m/z: 364 → 194.1).

In the present study the method was validated concurrently with the sample analyses of the study by evaluation of the standard injections. Recovery experiments were performed to verify the integrity of the analysed residues. These concurrent recoveries were performed with control soil Höfchen (silt loam). The evaluation was done by comparison of the peak area ratio of the samples with the peak area ratio of the external standard solutions. Isotopically labelled internal standard was used to compensate for possible matrix effects in the MS/MS-detector. This solution is added to the sample solutions after extraction.

Table A 14: Concurrent Recovery Rates of Flufenacet during Analysis of Soil Samples

Matrix	Fortification Level [µg/kg]	Recovery [%]	Overall Mean Recovery [%]	Overall RSD [%]
Soil Höfchen	4	94 / 95	97	3.7
	40	99 / 102		

RSD: Relative Standard Deviation

Conclusion
The applicability of the HPLC-MS/MS method for the analysis of flufenacet in soil samples was assessed. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.1.1.6.2 Analytical method 01169

A 2.1.1.6.2.1 Method validation of method 01169

Comments of zRMS:	The method was evaluated by RMS-Poland in RAR for Flufenacet (Vol. 3 – B.5, April 2022). The analytical method 01169 has been validated for the determination of residues of flufenacet-oxalate in test water by HPLC-MS/MS. The limit of quantitation (LOQ) for flufenacet-oxalate is 0.9 µg/L. The method meets the majority guideline criteria of SANCO/3029/99 rev. 4 except GLP and is suitable for the determination of flufenacet-oxalate in test water by HPLC-MS/MS at the LOQ of 0.9 µg/L.

Reference:	**KCP 5.1.2.6/03**
Title:	Method 01169 for the determination of flufenacet-oxalate in test water by HPLC-MS/MS
Report:	Krebber, R.; Leppelt, L.; 2009; 01169; M-357278-01-1
Authority registration No:	
Guideline(s):	not applicable
Deviations:	not applicable
GLP/GEP:	no
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and Methods
The method 01169 describes the determination of flufenacet-oxalate in test water by HPLC-MS/MS and provides validation data for test water for Multiple Reaction Monitoring (MRM) transition using electrospray ionisation in the negative mode.

The water samples were adjusted to pH 3 with formic acid and analysed by direct injection into an HPLC-MS/MS instrument. For quantitation a MRM transition was monitored for flufenacet-oxalate (m/z 224 → m/z 152).

Results and discussions
For method validation test water samples were fortified with flufenacet-oxalate at 0.910 µg/L and at 9.10 µg/L. These test solutions were injected ten times each into the HPLC-MS/MS instrument. The peak areas and retention times for flufenacet-oxalate were determined.

The relative standard deviation for the peak areas of flufenacet-oxalate was 3.9% (0.91 µg/L) and 1.7% (9.1 µg/L). The relative standard deviation for the retention time was ≤ 0.33% for both fortification levels.

Table A 15: Recovery rates and precision results (repeatability) from method validation of Flufenacet-oxalate using analytical method 01169

Sample concentration [µg/L]	Peak area			Retention time	
	Single values	Mean value	RSD [%]	Mean [min]	RSD [%]
0.91	8540, 8272, 8901, 8295, 9058, 9532, 9000, 9010, 8732, 8902	8806	3.9	2.14	0.33

9.1	93243, 90938, 91532, 92031, 93265, 95721, 90329, 92280, 93461, 93879	92668	1.7	2.12	0.22

RSD: Relative Standard Deviation

Because of the direct measurement of fortified samples without separate extraction and clean-up steps it is not possible to determine recovery rates and an estimate of the accuracy of the analytical technique was made by an assessment of the linearity of calibration and by determination of the reproducibility of sample analysis.

However, for additional demonstration of the reliability of the method, the validation samples were evaluated like recovery rates.

Table A 16: Recovery rates and precision results (repeatability) of Flufenacet-oxalate

Sample concentration [µg/L]	Percentages found		
	Single values	Mean value	RSD [%]
0.91	104, 101, 107, 101, 109, 112, 108, 108, 106, 107	106	3.0
9.1	94, 92, 93, 93, 94, 97, 92, 93, 95, 95	94	1.5

RSD: Relative Standard Deviation

Table A 17: Characteristics for the analytical method 01169 used for validation of flufenacet-oxalate residues in test water

	Flufenacet-oxalate
Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30% of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted, quadratic): $y = 1.01 \cdot 10^4 x - 1.92 \cdot 10^3$, Correlation coefficient r: 0.9997, number of data points: 6
Calibration range	0.91 to 91 µg./L
Limit of determination/quantification	LOQ = 0.91 µg/L
Assessment of matrix effects is presented	No effects observed

Conclusion

The analytical method 01169 complies with all guideline criteria according to SANCO/3029/99 rev. 4. It was successfully validated and is suitable for the determination of flufenacet-oxalate in water via HPLC-MS/MS with an LOQ of 0.91 µg/L.

A 2.1.1.6.2.2 Concurrent validation of analytical method 01169 in support of study M-358823-01-1

Comments of zRMS:	The analytical method 01169 complies with all criteria according to SANCO/3029/99 rev. 4 and is suitable for the determination of flufenacet-oxalate in test water by HPLC-MS/MS. It fits the purpose with regard to the studies Bruns, E.; 2009; M-358823-01-1.

Reference:	**KCP 5.1.2.6/04**
Title:	Pseudokirchneriella subcapitata growth inhibition test with flufenacet-oxalate
Report:	Bruns, E.; 2009; EBFOL137; M-358823-01-1
Authority registration No:	
Guideline(s):	OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (March 23, 2006)
Deviations:	none
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation
The water samples were analysed according to Method 01169 which was developed for the determination of flufenacet-oxalate in test water by HPLC-MS/MS (Krebber, R.; Leppelt, L.; 2009; M-357278-01-1).

The water samples were adjusted to pH 3 with formic acid and analysed by direct injection into an HPLC-MS/MS instrument after appropriate dilution. Identification and quantitative determination are done by means of electrospray MS/MS-detection in the negative mode. The HPLC-MS/MS was operated in MRM-Mode (Multiple Reaction Monitoring) using the mass transition m/z 224 → 152.

In the method 01169 the linearity of the MS-detector was checked for flufenacet-oxalate in the concentration range from 0.91 µg/L to 91 µg/L by an injection volume of 100 µL. The correlation coefficient was 0.9997.

In the present study the method was validated concurrently with the sample analyses of the study by evaluation of the standard injections.

Because of the direct measurement of the samples recovery rates cannot be calculated. Thus, the presented precision data is based on 10 injections of a standard solution. The relative standard deviations for the peak areas was 3.3%.

Table A 18: Precision results from concurrent method validation for flufenacet-oxalate

Flufenacet-oxalate standard concentration [µg/L]	n	Peak area		Retention Time	
		Mean Value	RSD	Mean Value	RSD
		[area counts]	[%]	[min]	[%]
5.673	10	84748	3.3	2.05	0.3

RSD: Relative Standard Deviation

Conclusion
The applicability of the HPLC-MS/MS method for the analysis of flufenacet-oxalate in test water was assessed. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.1.1.6.2.3 Concurrent validation of analytical method 01169 in support of study M-359515-02-1

Comments of zRMS:	The analytical method 01169 complies with all criteria according to SANCO/3029/99 rev. 4 and is suitable for the determination of flufenacet-oxalate in test water by HPLC-MS/MS. It fits the purpose with regard to the studies Bruns, E.; 2009; M-359515-02-1.

Reference:	**KCP 5.1.2.6/05**
Title:	Lemna gibba G3 Growth inhibition test with flufenacet-oxalate under static conditions
Report:	Bruns, E.; 2009; EBFOL138; M-359515-02-1
Authority registration No:	
Guideline(s):	OECD Guideline 221 (March 23, 2006);
Deviations:	none
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Concurrent validation
The water samples were analysed according to Method 01169 which was developed for the determination of flufenacet-oxalate in test water by HPLC-MS/MS (Krebber, R.; Leppelt, L.; 2009; M-357278-01-1).
The water samples were adjusted to pH 3 with formic acid and analysed by direct injection into an HPLC-MS/MS instrument after appropriate dilution. Identification and quantitative determination are done by means of electrospray MS/MS-detection in the negative mode. The HPLC-MS/MS was operated in MRM-Mode (Multiple Reaction Monitoring) using the mass transition m/z 224 → 152.

In the method 01169 the linearity of the MS-detector was checked for flufenacet-oxalate in the concentration range from 0.91 µg/L to 91 µg/L by an injection volume of 100 µL. The correlation coefficient was 0.9997.

In the present study the method was validated concurrently with the sample analyses of the study by evaluation of the standard injections.
Because of a drift of peak area counts within the sequence a calculation of mean area counts and relative standard deviation over all injections was not appropriate. However, the relative standard deviation over the bracketing standards was <10% to the mean value for standard solutions of flufenacet-oxalate.

Conclusion
The applicability of the HPLC-MS/MS method for the analysis of flufenacet-oxalate in test water was assessed. Thus, this method can be regarded as fit for purpose with regard to the present study.

A 2.1.1.6.3 Analytical method in support of study M-055471-01-1

A 2.1.1.6.3.1 Method validation

Comments of zRMS:	The analytical method has been validated to determine residues of flufenacet in test medium samples with LOQ of 0.01 µg/L. The analytical method complies with the guidance criteria according to SANCO/3029/99 rev. 4 with the exception of the accuracy/precision data. Only two instead of five determinations per fortification level were performed. One additional fortification level is presented. The method fits the purpose with regard to the studies Baetscher, R.; 2001; 796364; M-055471-01-1.

Reference:	**KCP 5.1.2.6/06**
Title:	Toxicity of flufenacet SC 500 to Pseudokirchneriella subcapitata (fornerly Selenastrum capricornutum in a 72-hour algal growth inhibition test
Report:	Baetscher, R.; 2001; 796364; M-055471-01-1
Authority registration No:	
Guideline(s):	--
Deviations:	--
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The purpose of the analytical phase of this study was to determine the concentrations of Flufenacet in test medium samples. The quantification of the test item was performed by HPLC analysis with MS-detection (Scan mode: SIM; masses for data evaluation: 152, 194, 364) based on analysis of the active ingredient.

Lower-level treatment samples, lower-level spiked samples and control samples were mixed with 3 g of sodium chloride and extracted two times with 30 ml of dichloromethane, each. The combined organic phases were evaporated to dryness using a rotary evaporator. The residues were diluted. Higher-level treatment samples and higher-level spiked samples were analysed directly. Injected samples were quantified by peak areas with reference to the respective calibration curve.

Results and discussions

Concurrent with the sample analysis, recoveries of spiked test water samples in the relevant concentrations (0.244, 1.95 and 61.0 µg/l of the test item) were performed in duplicate. The average concentrations were found to be 97%, 94% and 97% of the spiked values, with an overall mean of 96% (n = 6). Therefore, no correction for possible losses during the analytical procedure is necessary.

Table A 19: Recovery rates and precision results (repeatability) of flufenacet

Sample ID	Nominal concentration of Flufenacet SC 500 [µg/L]	Flufenacet SC 500 measured (based on analysis of active ingredient)			
		[µg/L]	[% of nominal]	Average [µg/L]	Average [% of nominal]
Analytical blank	0	<0.01	n.a	n.a	n.a
Spiked test water	0.244	0.240	98	0.237	97
		0.234	96		
	1.95	1.845	95	1.84	94
		1.837	94		
	61.0	59.6	98	59.2	97
		58.8	96		
			Mean		**96**

n.a. = not applicable

The limit of quantification (LOQ) for the test item in test medium was derived from the lowest standard solution, which fits into calibration curve: the value is 0.00214 mg/l. Taking a factor 0.003 into calculation and considering the content of the active ingredient in the test item, a LOQ of about 0.01 µg/L is obtained.

Table A 20: Characteristics for the analytical method used for validation of flufenacet

	Flufenacet
Specificity	HPLC-MS/MS method is highly specific.

	Blank values of all analytes were below 30% of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 3765762x, Correlation coefficient r: 0.9995, number of data points: 7
Calibration range	0.00212 – 2.36 mg/L
Limit of determination/quantification	LOQ = 0.01 µg/L
Assessment of matrix effects is presented	No effects observed.

Conclusion
The analytical method complies with the guidance criteria according to SANCO/3029/99 rev. 4 with the exception of the accuracy/precision data. Only two instead of five determinations per fortification level were performed. However, this deviation can be regarded as acceptable due to the fact that one additional fortification level is presented. An average recovery over all determinations of 96% was determined and the recoveries were performed in a concentration range which is appropriate for the studies and showed good results. Therefore, the method can be regarded as fit for purpose with regard to the present study.

A 2.1.1.6.4 Analytical method in support of study M-055476-01-1

A 2.1.1.6.4.1 Method validation

Comments of zRMS:	The analytical method has been validated to determine residues of flufenacet in test medium samples with LOQ of 0.4 µg/L. The analytical method complies with the guidance criteria according to SANCO/3029/99 rev. 4 with the exception of the accuracy/precision data. Only two instead of five determinations per fortification level were performed. The method fits the purpose with regard to the studies Baetscher, R.; 2001; 796342; M-055476-01-1.

Reference:	**KCP 5.1.2.6/07**
Title:	Toxicity of flufenacet SC 500 to the aquatic higher plant Lemna gibba in a 7-day static growth inhibition test
Report:	Baetscher, R.; 2001; 796342; M-055476-01-1
Authority registration No:	
Guideline(s):	--
Deviations:	--
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods
The purpose of the analytical phase of this study was to determine the concentrations of Flufenacet in test medium samples. The quantification of the test item was performed by HPLC analysis with MS-detection (Scan mode: SIM; masses for data evaluation: 152, 194, 364) based on analysis of the active ingredient.
The samples were mixed with approx. 3 g of sodium chloride and extracted two times with 30 ml of dichloromethane, each. After drying with sodium sulfate, the combined organic phases were evaporated to a final volume of approx. 2 ml, using a rotary evaporator and finally to dryness with a gentle stream of nitrogen. The residues were dissolved in a mixture of acetonitrile/purified water (1:1; v:v). Injected samples were quantified by peak areas with reference to the respective calibration curve.
Results and discussions
Concurrent with the sample analysis, recoveries of spiked test water samples in the relevant

concentrations (0.891 and 178 µg/l of the test item) were performed in duplicate. The average concentrations were found to be 88% and 107% of the spiked values, with an overall mean of 98% (n = 4). Therefore, no correction for possible losses during the analytical procedure is necessary.

Table A 21: Recovery rates and precision results (repeatability) of flufenacet

Sample ID	Nominal concentration of Flufenacet SC 500 [µg/L]	Flufenacet SC 500 measured (based on analysis of active ingredient)			
		[µg/L]	[% of nominal]	Average [µg/L]	Average [% of nominal]
Analytical blank	0	<0.04	n.a	n.a	n.a
Spiked test water	0.891	0.8107	91.0	0.786	88
		0.7604	85.3		
	178	183.3	102.8	191.5	107
		199.8	112.1		
			Mean		**98**

n.a. = not applicable

The limit of quantification (LOQ) for the test item in test medium was derived from the lowest standard solution which fits into the calibration curve: the value is 31.7 µg/l. Taking a factor of 0.005 into calculation and considering the content of the active ingredient in the test item, a LOQ of about 0.4 µg/l is obtained.

Table A 22: Characteristics for the analytical method used for validation of flufenacet

	Flufenacet
Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30% of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 6000 x + 133975, Correlation coefficient r: 0.9996, number of data points: 5
Calibration range	31.7 – 2641 µg/L
Limit of determination/quantification	LOQ = 0.4 µg/l
Assessment of matrix effects is presented	No effects observed

Conclusion

The analytical method complies with the guidance criteria according to SANCO/3029/99 rev. 4 with the exception of the accuracy/precision data. Only two instead of five determinations per fortification level were performed. An average recovery over all determinations of 98% was determined and the recoveries were performed in a concentration range which is appropriate for the studies and showed good results. Therefore, the method can nevertheless be regarded as fit for purpose with regard to the present study.

A 2.1.1.6.5 Analytical method 01080 in support of study M-477339-01-1

A 2.1.1.6.5.1 Method validation

Comments of zRMS:	The analytical method 01080 complies with all criteria according to SANCO/825/00 rev.8.1 and SANCO/3029/99 rev. 4 and is suitable for the determination of residues of flufenacet (FOE 5043) in soil using LC-MS/MS. This method fits the purpose with regard to the study Kling, A.; 2014; M-477339-01-1 to determine residues of flufenacet in feeding solution. The limit of quantitation (LOQ) was 4 µg/kg for aqueous sugar solution.

	The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%.

Reference:	**KCP 5.1.2.6/08**
Title:	Flufenacet (tech.) - Assessment of chronic effects to the honeybee, Apis mellifera L., in a 10 days continuous laboratory feeding limit test
Report:	Kling, A.; 2014; S13-00145; M-477339-01-1
Authority registration No:	
Guideline(s):	US EPA OCSPP Guideline 850.SUPP
Deviations:	not applicable
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and Methods

The concentration of flufenacet was determined in the feeding solution employed to determine the chronic effects of the test item flufenacet on the honeybee, *Apis mellifera* L, in a 10 days continuous feeding test in the laboratory.

The analytical method 01080 (Brumhard, B.; 2009; M-357296-01-1) was developed for the determination of flufenacet in soil by HPLC-MS/MS. An analytical method summary of the original method validation is not provided as this validation is superseded by the following method validation.

In deviation to the original method 01080, only parts of the analytical method like chromatography conditions, mass transitions etc. were necessary to determine the concentration of flufenacet in the employed feeding solutions. According to the original method, flufenacet was extracted from samples of soil origin with a mixture of acetonitrile/water using a microwave oven. After centrifugation, an aliquot of the raw extract was subjected to liquid chromatography.

Due to the fact that the concentration in the feeding solutions of the present study were at a very high level, it was only necessary to dilute the samples with acetonitrile/water (1/1, v/v). Thereafter, aliquots of the diluted samples were subjected to reversed phase High Performance Liquid Chromatography (HPLC) coupled with electrospray and mass spectrometry (MS/MS) detection without a further clean-up step (ESI positive; 1st MRM Flufenacet m/z: 364 → 194; 2nd MRM Flufenacet m/z: 364 → 152; MRM Flufenacet-ISTD m/z: 371 → 201). Possible matrix effects of flufenacet are eliminated by using an internal standard solution of isotopically labelled reference item.

The limit of quantitation (LOQ) for flufenacet is 0.004 mg/kg (= 4 µg/kg) for the sample material aqueous sugar solution, corresponding to the lowest fortification level of successfully conducted recovery experiments.

Results and discussions

Recovery rates were determined at fortification levels of 0.004 mg/kg, 0.04 mg/kg and 200 mg/kg.

The individual recovery values for flufenacet ranged from 95 to 102 %, with an overall recovery of 98% and a relative standard deviation (RSD) of 0.7 % (n = 12).

Table A 23: Recovery rates and precision results (repeatability) of flufenacet

Analyte	Crop/Sample Material	FL [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
Flufenacet	Aqueous sugar solution	0.004	98, 100, 100, 99, 102	100	1.5	0.004
		0.04	95, 97, 98, 97, 96	97	1.2	
		200	97, 96	98	-	
			Overall recovery (n = 12)	**98**	**2.0**	

FL = Fortification Level, RSD = Relative Standard Deviation, LOQ = Practical Limit of Quantitation

Table A 24: Characteristics for the analytical method 01080 used for validation of flufenacet in feeding solution (aqueous sugar solution)

	flufenacet
Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30% of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 0.928618 x + 0.00308273, Correlation coefficient r: 0.9997, number of data points: 7
Calibration range	0.01 µg/L - 5 µg/L (corresponds to 0.01 mg/kg – 5 mg/kg)
Limit of determination/quantification	LOQ = 0.004 mg/kg
Assessment of matrix effects is presented	Matrix effects not monitored, as the internal standard procedure using stable isotopically labelled internal standards compensates for matrix effects.

Conclusion
The analytical method complies with all guideline criteria according to SANCO/3029/99 rev. 4 and is suitable for the determination of flufenacet in feeding solution via HPLC-MS/MS.

A 2.1.1.6.6 Analytical method in support of study M-615473-01-1

A 2.1.1.6.6.1 Method validation

Comments of zRMS:	The analytical method has been validated to determine of the residue of flufenacet in royal jelly diet. Recoveries for this method validation averaged 103 ± 2.77% with a limit of quantification (LOQ) of 1.00 µg/g, the lowest fortification level. The method is acceptable.

Reference:	**KCP 5.1.2.6/09**
Title:	Flufenacet: Honey bee (Apis mellifera L.) larval toxicity test, repeated exposure
Report:	Rathjen, K. A.; 2018; 13798.6448; M-615473-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.SUPP OECD Guidance Document No 239, Honey Bee (Apis mellifera) Larval Toxicity Test, Repeated Exposure
Deviations:	none
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and Methods
The analytical method was validated to quantify the amount of flufenacet present in recovery samples prepared in royal jelly diet.

Recovery samples were diluted with 50/50 acetonitrile/purified reagent water (v/v) and were analyzed using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS; ESI positive, MRM flufenacet m/z: 364.0 → 152.2).

Results and discussions
This method was validated in royal jelly diet by dosing with flufenacet at concentrations of 1.00 and 3000 µg/g. Recoveries averaged 103%. Relative standard deviations were below 20% for both

fortification levels.

Table A 25: Recovery rates and precision results (repeatability) of flufenacet

Sample ID	Fortified concentration (µg/g]	Retention time (minutes)	Dilution factor	Analytical result (µg/g)	Percent of fortified
Control A	0.00	NA	1000	<0.5	NA
Control B	0.00	NA	1000	<0.5	NA
Control C	0.00	NA	1000	<0.5	NA
Control D	0.00	NA	1000	<0.5	NA
Control E	0.00	NA	1000	<0.5	NA
LOQ A	1.00	3.47	1000	1.04	104
LOQ B	1.00	3.47	1000	1.00	100
LOQ C	1.00	3.47	1000	1.02	102
LOQ D	1.00	3.46	1000	1.02	102
LOQ E	1.00	3.46	1000	1.03	103
	Mean	**3.47**		**1.02**	**102**
	RSD	**0.158**		**1.44**	**1.44**
High A	3000	3.47	1000000	2990	99.7
High B	3000	3.47	1000000	3270	109
High C	3000	3.47	1000000	3100	103
High D	3000	3.47	1000000	3150	105
High E	3000	3.47	1000000	3010	100
	Mean	**3.47**		**3110**	**104**
	RSD	**0.258**		**3.71**	**3.71**
Overall mean (n = 10)					**103**
Overall RSD (%) (n = 10)					**2.77**

NA = not applicable, RSD = Relative Standard Deviation

Table A 26: Characteristics for the analytical method used for validation of flufenacet

	flufenacet
Specificity	HPLC-MS/MS method is highly specific. Blank values of all analytes were below 30% of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (1/x weighted): y = 3427.862 x – 90.9114, Correlation coefficient r: 0.998, number of data points: 6
Calibration range	0.5 µg/L – 5.0 µg/L (corresponds to 0.5 – 5.0 µg/g)
Limit of determination/quantification	LOQ = 1.0 µg/g
Assessment of matrix effects is presented	No effects observed

Conclusion

The analytical method complies with all guideline criteria according to SANCO/3029/99 rev. 4 and is suitable for the determination of flufenacet in royal jelly diet via HPLC-MS/MS.

A 2.1.1.6.7 Analytical method 00372 in support of studies M-248250-01-1 and M-248251-

01-1

A 2.1.1.6.7.1 Method validation

Comments of zRMS:	The analytical method has been validated to determine of the active ingredient Flufenacet of the test item Flufenacet SC 500 in spray solution applied to non-target plants. The determination of the Limit of Quantification (LOQ) was 0.038 µg/mL. Mean recoveries per fortification level were within the acceptable range of 70 - 110% and RSD values were below 20%. The system and method validity tests show that the analytical system are suitable for the determination of Flufenacet. The method is acceptable.

Reference:	**KCP 5.1.2.6/10**
Title:	Flufenacet SC 500: seedling emergence and seedling growth test on terrestrial non-target plants
Report:	Friedrich, S.; 2005; 041048104; M-248250-01-1
Authority registration No:	
Guideline(s):	OECD 208 A (2000, draft): seedling emergence and seedling growth test US EPA OCSPP 850.4225
Deviations:	none
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Reference:	**KCP 5.1.2.6/11**
Title:	Flufenacet SC 500: vegetative vigour test on non-target terrestrial plants
Report:	Friedrich, S.; 2005; 041048105; M-248251-01-1
Authority registration No:	
Guideline(s):	OECD 208 B (Draft 2000) US EPA OCSPP 850.4250
Deviations:	--
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and Methods

The purpose of the analytical phase of the two studies was the determination of the active ingredient Flufenacet of the test item Flufenacet SC 500 in spray solution applied to non-target plants. The determination was conducted by a method using high performance liquid chromatography (HPLC) with UV-Vis detection.

The analytical method was based on Bayer method 00372 (König, T.; 1994; M-019173-02-1), an analytical method summary of the original method validation is not provided as this validation is superseded by the following method validation. The validation data is used for both studies.

Samples were analysed via HPLC-UV (detection at 233 nm) after appropriate dilution with acetonitrile / 0.01% trifluoroacetic acid 50/50 (v/v). For validation sample preparation, the test item was weighed into 1000 ml volumetric flask and diluted with ultrapure water to 1000 ml. These solutions were diluted with acetonitrile / 0.01% trifluoroacetic acid 50/50 (v/v). The identification of the peak has been confirmed by comparison with the retention time of Flufenacet in the control standard solutions.

Two calibration standard lines were made: the reference calibration line was made from test item Flufenacet SC 500 to determine the suitability of the system and the analytical method (linearity, working range, limit of quantification (LOQ), repeatability). The control standard line was made from

the certified control standard. This calibration line was used for quantification of the analytical results.

Results and discussions

To determine the precision of the analytical determination method, from both calibration functions two calibration points at the lower and upper level was measured with 7 repetitions each.

The precision (relative standard deviation) of all investigated concentration ranges is smaller than 1%.

Table A 27: Precision results (repeatability) of Flufenacet

Calibration line	Reference calibration		Control calibration	
Standard solution	**Standard 1 Area**	**Standard 7 Area**	**Standard 1 Area**	**Standard 7 Area**
Nominal concentration µg/mL	**0.0846**	**0.59**	**7.92**	**55.44**
Repetition no. n				
1	6098	41600	493464	3536369
2	6243	41631	495575	3536024
3	6213	41587	494178	3535150
4	6188	41600	493811	3536102
5	6243	41711	492494	3534071
6	6178	41676	494899	3534070
7	6217	41604	492264	3536458
Mean	6197.14	41629.86	493812.14	3535463.43
Relative standard deviation RSD (%)	0.81	0.11	0.24	0.03

In addition, recovery determinations were performed for spray solutions at the 75 g/ha level, 150 g/ha level and 600 g/ha level. Mean recoveries per fortification level were within the acceptable range of 70 - 110% and RSD values were below 20%.

Table A 28: Recovery and precision results (repeatability) of Flufenacet at 75 g/ha*

Repetition	Weight test item	Concentration Flufenacet	Nominal concentration	Measured result	Result	Recovery rate
	g/100 mL	**mg ai/mL**	**µg ai/mL**	**µg/mL**	**µg/mL**	**%**
1	0.1054	0.445842	445.84	45.722	449.93	100.9
2				45.834	451.03	101.2
3				45.754	450.24	101.0
4				45.922	451.89	101.4
5				46.000	452.66	101.5
Mean						**101.2**
Relative standard deviation RSD (%)						**0.25**

* Dilution factor = 10

Table A 29: Recovery and precision results (repeatability) of Flufenacet at 150 g/ha*

Repetition	Weight test item	Concentration Flufenacet	Nominal concentration	Measured result	Result	Recovery rate
	g/100 mL	**mg ai/mL**	**µg ai/mL**	**µg/mL**	**µg/mL**	**%**
1	0.2196	0.928908	928.91	48.334	951.26	102.4
2				47.850	941.73	101.4
3				48.407	952.69	102.6
4				48.366	951.89	102.5
5				49.334	970.94	104.5

Mean	**102.7**
Relative standard deviation RSD (%)	**1.11**

* Dilution factor = 20

Table A 30: Recovery and precision results (repeatability) of Flufenacet at 600 g/ha*

Repetition	Weight test item	Concentration Flufenacet	Nominal concentration	Measured result	Result	Recovery rate
	g/100 mL	mg ai/mL	µg ai/mL	µg/mL	µg/mL	%
1	0.8466	3.581118	3581.12	37.076	3648.45	101.9
2				36.703	3611.75	100.9
3				36.809	3622.18	101.1
4				36.328	3574.84	99.8
5				36.004	3542.96	98.9
Mean						**100.5**
Relative standard deviation RSD (%)						**1.15**

* Dilution factor = 100

In the present study, the limit of detection (LOD) is defined as the value of the lowest calibration standard chromatographed that gave a peak height to baseline noise ratio ≥3. The signal to noise ratio at the lowest reference standard concentration (reference standard 1: 0.0846 µg/ml) is approximately 10 to 1, so that the LOD would be estimated by 0.008 µg/ml. The limit of quantification (LOQ) can be calculated by the calibration method. The determination of the Limit of Quantification (LOQ) on base of the reference calibration function resulted in a value of 0.038 µg/ml.

Table A 31: Characteristics for the analytical method used for validation of Flufenacet

	Flufenacet
Specificity	HPLC-UV/Vis method is regarded as highly specific. Blank values were below 30% of the respective LOQ.
Calibration (type, number of data points)	Individual calibration data is presented, calibration equation (linear) of the reference standard: y = 70447.062 x – 83.571, Correlation coefficient R: 0.9999, number of data points: 7 Individual calibration data is presented, calibration equation (linear) of the control standard: y = 63625.610 x – 3630.067, Correlation coefficient R: 1.0000, number of data points: 6
Calibration range	0.08461 – 0.5922 µg/mL (reference standard) 7.92 – 47.52 µg/mL (control standard)
Limit of determination/quantification	LOQ = 0.038 µg/mL (calculated)
Assessment of matrix effects is presented	No effects observed.

Conclusion

The analytical method complies with all guidance criteria according to SANCO/3029/99 rev. 4. It is suitable for the determination of Flufenacet in spray solution and can be regarded as fit for purpose with regard to the present studies.

A 2.1.1.7 Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1)

No specific method developed for this purpose.

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

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

A 2.1.2.1.1 Analytical method 01179

The analytical method 01179 was developed in order to determine the total residue of flufenacet (flufenacet and its metabolites containing the N-fluorophenyl-N-isopropyl amine moiety) in/on cereal matrices (green material, straw, grain) by HPLC-MS/MS using matrix matched standards. The matrices to be analyzed are considered to be representative for the matrix groups of high starch content and high water content. In addition, straw was validated as a representative for dry matrices.
Green material, grain and straw were fortified with flufenacet or with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid Na/flufenacet thioglycolate sulfoxide; 1/1/1).
The method may be used as a data collection method as well as for enforcement purposes.

A 2.1.2.1.1.1 Method validation

Comments of zRMS:	The method 01179 was evaluated by RMS-Poland in RAR for Flufenacet (Vol. 3 – B.5, April 2022). The analytical method 01179 was validated to determine the total residue of flufenacet (flufenacet and its metabolites containing the N-fluorophenyl-N-isopropyl amine moiety) in/on cereal matrices (green material, straw, grain) by LC-MS/MS using matrix matched standards with an LOQ of 0.01 mg/kg for cereal grain and green material and 0.05 mg/kg for straw. The method is acceptable.

Reference:	**KCP 5.2.1/01**
Title:	Validation of BCS analytical method no. 01179 for the determination of residues of flufenacet in/on plant materials by HPLC-MS/MS
Report:	Class, Th.; Merdian, H.; 2010; 01179; M-362716-01-1
Authority registration No:	
Guideline(s):	91/414/EEC, SANCO/3029/99 rev. 4, 11/07/00, and SANCO/825/00 rev. 7 17/03/04; OECD Guidance,(ENV/JM/Mono (2007) 17, 2007-08-13)
Deviations:	none
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods
The sample material (5 g of grain and green material; 2.5 g or 5 g of straw) in water was oxidized with potassium permanganate in the presence of sulfuric acid. After the oxidation, the mixture was hydrolyzed with concentrated sulfuric acid for 21 h in order to cleave the amide bonding to achieve the common moiety compound 4-fluoro-N-isopropylaniline. After making the solution basic water steam distillation out of alkaline medium into an acidified receiver followed. To remove the acid content from the distillation receiver flask the obtained solution was 3 times liquid/liquid distributed with dichloromethane. The total residue of flufenacet was determined as 4-fluoro-N-isopropylaniline by HPLC-MS/MS.
Two MRMs were used, m/z 154 → 112 m/z for quantitation and m/z 154 → 95 m/z for quantitative

confirmation of 4-fluoro-N-isopropylaniline.
Stock solutions of flufenacet and the metabolites were prepared by dissolving the substances in methanol. From these, fortification solutions (expressed as flufenacet) were prepared by diluting with methanol.
For quantification external calibration with matrix-matched standard solutions was applied. From the flufenacet-4-fluoro-N-isopropylaniline stock solution, an intermediate calibration solution (expressed as flufenacet) was prepared by volumetric dilution into methanol/water (1/1, v/v). The intermediate calibration solution was used to prepare calibration solutions in methanol/water (4/1, v/v), expressed as flufenacet.

Results and discussions
Precision: For the matrices investigated and for both mass transitions monitored, overall relative standard deviations (RSD) were < 20%, except for transition 154 m/z → 95 m/z , where the overall relative standard deviation for green material fortified with flufenacet metabolites was 21%. The RSD for green material at LOQ level (metabolite mix) slightly exceeded 20% (up to 23%), however, the RSD was <20% for flufenacet which is determined by conversion to the same analytical target.
Accuracy: Green material, grain and straw were fortified with flufenacet or with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid sodium salt/flufenacet thioglycolate sulfoxide; 1/1/1) and detected as 4-fluoro-N-isopropylaniline. Mean recoveries at the LOQ level and for higher levels determined with the two mass transitions were within the range of 70-110%.
Stability: Acceptable recovery results of total flufenacet residues show that the extracts are stable up to 22 days when stored refrigerated after reprocessing. Standard solutions in matrix are stable during the injection series up to seven hours at ambient temperature. Standard solutions in solvent, stored refrigerated are stable for about two months.
The use of HPLC-MS/MS with two mass transitions for quantitation and quantitative confirmation (154 m/z → 112 m/z and 154 m/z → 95 m/z) ensures a high level of specificity. A confirmatory method is not required.

Table A 32: Recovery results from method validation of flufenacet or flufenacet metabolites (mix) using the analytical method 01179

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Green material	Flufenacet (quantification) m/z 154 → 112	0.01 (n = 5)	73	15	
		0.10 (n = 3)	78	5	
		0.25 (n = 1)	70	-	
		1.0 (n = 1)	57	-	
		2.0 (n = 1)	70	-	
		5.0 (n = 2)	57	-	
		12 (n = 1)	66	-	
		20 (n = 1)	75	-	
		30 (n = 3)	88	7	
	Overall	*n=18*	*73*	*16*	
	Flufenacet Metabolite mix* (quantification) m/z 154 → 112	0.01 (n = 4)	89	23	The 5th recovery was 230%, which was not included in calculations (Dixon outlier). Most likely contaminated in the laboratory
		0.10 (n = 2)	85	-	
		0.90 (n = 1)	60	-	

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
		2.40 (n = 1)	90	-	
		18 (n = 2)	73	-	
		27 (n = 1)	66	-	
		30 (n = 2)	66	-	
	Overall	*n=13*	*78*	*20*	
	Flufenacet (confirmation) m/z 154 → 95	0.01 (n = 5)	71	11	
		0.10 (n = 3)	78	6	
		0.25 (n = 1)	77	-	
		1.0 (n = 1)	58	-	
		2.0 (n = 1)	71	-	
		5.0 (n = 2)	57	-	
		12 (n = 1)	66	-	
		20 (n = 1)	75	-	
		30 (n = 3)	88	7	
	Overall	*n=18*	*73*	*15*	
	Flufenacet Metabolite mix* (confirmation) m/z 154 → 95	0.01 (n = 4)	91	22	The 5th recovery was 223%, which was not included in calculations (Dixon outlier). Most likely contaminated in the laboratory
		0.10 (n = 2)	83	-	
		0.90 (n = 1)	58	-	
		2.40 (n = 1)	95	-	
		18 (n = 2)	73	-	
		27 (n = 1)	63	-	
		30 (n = 2)	65	-	
	Overall	*n=13*	*78*	*21*	
Grain	Flufenacet (quantification) m/z 154 → 112	0.01 (n = 7)	86	17	
		0.10 (n = 7)	83	19	
	Overall	*n=15*	*84*	*17*	
	Flufenacet Metabolite mix* (quantification) m/z 154 → 112	0.01 (n = 6)	85	16	
		0.10 (n = 6)	77	11	
	Overall	*n=12*	*81*	*14*	
	Flufenacet (confirmation) m/z 154 → 95	0.01 (n = 7)	87	15	
		0.10 (n = 7)	83	19	
	Overall	*n=15*	*85*	*17*	
	Flufenacet Metabolite mix* (confirmation) m/z 154 → 95	0.01 (n = 6)	84	15	
		0.10 (n = 6)	77	12	

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
	Overall	*n=12*	*80*	*14*	
Straw	Flufenacet (quantification) m/z 154 → 112	0.05 (n = 11)	84	12	
		0.10 (n = 2)	110	-	
		0.20 (n = 1)	98	-	
		0.50 (n = 5)	74	16	
		1.0 (n = 1)	73	-	
	Overall	*n=20*	*84*	*17*	
	Flufenacet Metabolite mix* (quantification) m/z 154 → 112	0.05 (n = 6)	74	14	
		0.60 (n = 5)	76	16	
	Overall	*n=11*	*75*	*14*	
	Flufenacet (confirmation) m/z 154 → 95	0.05 (n = 11)	83	12	
		0.10 (n = 2)	109	-	
		0.20 (n = 1)	98	-	
		0.50 (n = 5)	73	14	
		1.0 (n = 1)	73	-	
	Overall	*n=20*	*83*	*17*	
	Flufenacet Metabolite mix* (confirmation) m/z 154 → 95	0.05 (n = 6)	73	14	
		0.60 (n = 5)	75	15	
	Overall	*n=11*	*74*	*14*	

* Fortified with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid sodium salt/flufenacet thioglycolate sulfoxide; 1/1/1), detected as 4-fluoro-N-isopropylaniline and calculated as flufenacet.

Table A 33: Characteristics for the analytical method used for validation of flufenacet residues in cereal green material, grain and straw

	Flufenacet and metabolites determined as 4-fluoro-N-isopropylaniline
Specificity	Blank value <30% LOQ. The use of HPLC-MS/MS with two mass transitions for quantitation and quantitative confirmation (154 m/z → 112 m/z and 154 m/z → 95 m/z) ensures a high level of specificity. The control chromatograms generally show no peaks above the chromatographic background within the designated retention time of interest. The spiked sample chromatograms contain only the analyte peak of interest at the corresponding peak retention time. Peaks were well defined and symmetrical.
Calibration (type, number of data points)	Calibration with matrix-matched standards, 1/x weighted linear regression (calibration line equations are presented in the original report). Coefficient of correlation: MRM for quantification (154 m/z → 112 m/z): Green material:r = 0.9991 – 1.0000 Grain:r = 0.9992-0.9999 Straw: r = 0.9947-1.0000 MRM for confirmation (154 m/z → 95 m/z): Green material: r = 0.9955 - 1.0000 Grain: r = 0.9989 – 1.000 Straw:r = 0.9947 – 1.0000 Number of data points for both mass transitions and all matrices: 6
Calibration range	In all plant matrices: 0.0002 – 0.025 µg/mL expressed as flufenacet parent,

	Flufenacet and metabolites determined as 4-fluoro-N-isopropylaniline
	corresponding to 0.002 – 0.25 mg/kg straw (exceptional cases): corresponding to 0.004 – 0.5 mg/kg
Assessment of matrix effects is presented	Significant matrix effects were observed for both mass transitions and all commodities. For quantification external calibration with matrix-matched standard solutions was applied to compensate matrix effects.
Limit of determination/quantification	The limit of quantification (LOQ) is 0.01 mg/kg in green material and grain, and 0.05 mg/kg in straw (expressed as flufenacet equivalents). The limit of detection (LOD) is estimated to be 0.002 mg/kg (green material and grain) respectively 0.01 mg/kg (straw).

Conclusion
Method 01179 was validated for the determination of flufenacet residues (flufenacet and its metabolites containing the N-fluorophenyl-N-isopropyl amine moiety) in cereal materials, exemplified by green material and grain with an LOQ of 0.01 mg/kg and 0.05 mg/kg for straw. The method was successfully validated and thus was demonstrated to be applicable for data gathering and monitoring purposes. The analytical method fulfils the requirements of EC Guidance documents on residue analytical methods (SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1) and in OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13).
The method is considered to comply with the quality criteria as set out in SANTE/2020/12830 rev 1.

A 2.1.2.1.2 Analytical method 01100

A 2.1.2.1.2.1 Method validation

Comments of zRMS:	The analytical method 01100 has been validated for the determination of flufenacet residues in/on orange (fruit), dry bean seed and rapeseed with LOQ 0.01 mg/kg each in all matrices. Mean recoveries for each fortification level were within the range of 70 - 110% with RSD≤20%. The method is acceptable.

Reference:	**KCP 5.2.1/02**
Title:	Amendment no. 02 to final report: Analytical method 01100 for the determination of residues of flufenacet (FOE5043) and its metabolites in/on plant material
Report:	Stuke, S.; 2018; 01100; M-362575-03-1
Authority registration No:	
Guideline(s):	EU Council Directive 91/414/EEC amended by Commission Directive 96/68/EC Guidance document on residue analytical methods; SANCO/825/00 rev. 7, European Commission, Directorate General Health and Consumer Protection OECD guidance document ENV/JM/Mono(2007)17, 2007-08-13, Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

The analytical method 01100 was validated for the determination of flufenacet residues in/on orange (fruit), dry bean seed and rape seed representative for the matrix groups of high acid content, high protein content and high fat content.

The method may be used as a data collection method as well as for enforcement purposes.
Sample materials were fortified with flufenacet or with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid Na/flufenacet thioglycolate sulfoxide; 1/1/1).

Materials and methods
The sample material (5 g) was oxidized with potassium permanganate in the presence of sulfuric acid. After the oxidation, the mixture was hydrolyzed with concentrated sulfuric acid. After oxidation and hydrolysis, residues of flufenacet and metabolites were cleaned-up by distillation followed by a liquid-liquid partition with dichloromethane. Finally, the total residue of flufenacet was determined as 4-fluoro-N-isopropylaniline by HPLC-MS/MS.
The following MRM transitions were used for quantification and confirmation:
4-fluoro-N-isopropylaniline: m/z = 154.1 → m/z = 112.0 (quantification),
m/z = 154.1 → m/z = 92.0 (confirmation).
Stock solutions with concentrations expressed as flufenacet were prepared dissolving the substances in methanol. From these, secondary standard solutions were prepared by diluting with methanol or for fluoro-N-isopropylaniline with ACN/water (1/4, v/v) + 0.1 mL/L acetic acid.
4-Fluoro-N-isopropylaniline residues were quantified using matrix-matched standards. The extracts of blank control samples were diluted with standard solutions containing flufenacet-4-fluoro-N-isopropylaniline.

Results and discussions
Precision: For the matrices investigated and for both mass transitions monitored, relative standard deviations (RSD) per fortification level and overall were < 20% except for transition 154 m/z → 92 m/z where the relative standard deviation for dry bean seed fortified with flufenacet metabolite mix at the LOQ level was 20.9%.
Accuracy: Orange fruit, dry bean seed and rape seed were fortified with flufenacet or with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid Na/flufenacet thioglycolate sulfoxide; 1/1/1) and detected as 4-fluoro-N-isopropylaniline. Mean recoveries for each fortification level were within the range of 70 - 110%.
Stability: The stability of the analytes in extract was checked after approx. four weeks for 0.10 mg/kg rape seed recoveries. Final extracts were stable for at least four weeks under refrigerated conditions. Stability of standard solutions in solvent up to 2 months is known from previous experiments and was not monitored again.
The use of HPLC-MS/MS with two mass transitions for quantitation and quantitative confirmation (154 m/z → 112 m/z and 154 m/z → 92 m/z) ensures a high level of specificity. A confirmatory method is not required.

Table A 34: Recovery results from method validation of flufenacet or flufenacet metabolites (mix) using the analytical method 01100

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Orange fruit	Flufenacet (quantification) m/z 154 → 112	0.01 (n = 5)	103	11.6	
		1.0 (n = 5)	94	7.4	
	Flufenacet (confirmation) m/z 154 → 92	0.01 (n = 5)	101	10.2	
		1.0 (n = 5)	90	5.6	
	Flufenacet Metabolite mix* (quantification) m/z 154 → 112	0.01 (n = 5)	72	3.8	
		1.0 (n = 4)	80	6.4	
	Flufenacet Metabolite mix* (confirmation)	0.01 (n = 5)	71	3.2	
		1.0 (n = 4)	80	5.4	

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
	m/z 154 → 92				
Dry bean seed	Flufenacet (quantification) m/z 154 → 112	0.01 (n = 5)	75	10.9	
		0.10 (n = 5)	103	11.0	
	Flufenacet (confirmation) m/z 154 → 92	0.01 (n = 5)	83	11.8	
		0.10 (n = 5)	107	3.0	
	Flufenacet Metabolite mix* (quantification) m/z 154 → 112	0.01 (n = 5)	91	17.2	
		0.10 (n = 4)	77	16.7	
	Flufenacet Metabolite mix* (confirmation) m/z 154 → 92	0.01 (n = 5)	88	20.9	
		0.10 (n = 4)	77	17.4	
Rape seed	Flufenacet (quantification) m/z 154 → 112	0.01 (n = 5)	102	11.1	
		0.10 (n = 5)	98	11.4	
	Flufenacet (confirmation) m/z 154 → 92	0.01 (n = 5)	99	14.8	
		0.10 (n = 5)	99	10.2	
	Flufenacet Metabolite mix* (quantification) m/z 154 → 112	0.01 (n = 5)	76	10.8	
		0.10 (n = 5)	80	10.3	
	Flufenacet Metabolite mix* (confirmation) m/z 154 → 92	0.01 (n = 5)	73	8.8	
		0.10 (n = 5)	83	10.2	

* Fortified with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid sodium salt/flufenacet thioglycolate sulfoxide; 1/1/1), detected as 4-fluoro-N-isopropylaniline and calculated as flufenacet.

Table A 35: Characteristics for the analytical method used for validation of flufenacet residues in orange fruit, dry bean seed and rape seed

	Flufenacet and metabolites determined as 4-fluoro-N-isopropylaniline
Specificity	Blank value < 30% LOQ. The use of HPLC-MS/MS with two mass transitions for quantitation and quantitative confirmation (154 m/z → 112 m/z and 154 m/z → 92 m/z) ensures a high level of specificity. Mass spectrum is provided in the original report.
Calibration (type, number of data points)	Calibration with matrix-matched standards, 1/x weighted linear regression (calibration line equations are presented in the original report). Coefficient of correlation: <u>MRM for quantification:</u> Orange fruit: r = 0.9957 Dry bean seed: r = 0.9990 Rape seed: r = 0.9972 <u>MRM for confirmation:</u> Orange fruit: r = 0.9986 Dry bean seed: r = 0.9975 Rape seed: r = 0.9993 Number of data points for both mass transitions and all matrices: 6
Calibration range	In all plant matrices: 0.1 –50 µg/L (corresponding to 0.002 – 1 mg/kg).
Assessment of matrix effects is	Matrix-matched standards used for quantification compensate for matrix effects.

	Flufenacet and metabolites determined as 4-fluoro-N-isopropylaniline
presented	
Limit of determination/quantification	The limit of quantification (LOQ) is 0.01 mg/kg for all matrices (expressed as flufenacet equivalents).

Conclusion
Method 01100 was validated for the determination of flufenacet residues (flufenacet and its metabolites containing the N-fluorophenyl-N-isopropyl amine moiety) in orange fruit, dry bean seed and rape seed with an LOQ of 0.01 mg/kg. The method was validated successfully and thus was demonstrated to be applicable for data gathering and monitoring purposes. The analytical method fulfils the requirements detailed in the EC Guidance documents on residue analytical methods (SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1) and in OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13). The method is considered to comply with the quality criteria set out in SANTE/2020/12830 rev1.

A 2.1.2.1.2.2 Independent laboratory validation

The independent validation is applicable to method 01179, 01100 and 01100/M001 and reported below under A 2.1.2.1.3.2 (Meyer, M.; 2011; M-405654-01).

A 2.1.2.1.2.3 Confirmatory method

The use of HPLC-MS/MS with two mass transitions for quantitation and quantitative confirmation (154 m/z → 112 m/z and 154 m/z → 92 m/z) ensures a high level of specificity. Thus, an additional confirmatory method is not necessary.

A 2.1.2.1.2.4 Extraction efficiency

The extraction efficiency of the method has been investigated and is described in detail under point A 2.1.2.1.3.5.

A 2.1.2.1.3 Analytical method 01100/M001

A 2.1.2.1.3.1 Method validation

Comments of zRMS:	The analytical method 01100/M001 has been validated for the determination of residues of flufenacet and three main metabolites (FOE 5043-thioglycolate sulfoxide, FOE 5043-oxalate, FOE 5043-sulfonic acid) as the common moiety compound 4-Fluoro-Nisopropylaniline (FOE 5043-aniline) in/on samples of plant origins: wheat green material, wheat grain, wheat straw with LOQ of 0.01 mg/kg in wheat grain and green material and LOQ of 0.05 mg/kg in straw by LC-MS/MS. Mean recoveries for each fortification level were within the range of 70 - 110% with RSD≤20%. The method is acceptable.

Reference:	**KCP 5.2.1/03**
Title:	Amendment no. 01 to final report: Modification M001 of the residue analytical method 01100 for the determination of residues of flufenacet (FOE5043) and its metabolites in/on cereals (straw, grain, and green material) at a LOQ of 0.01 mg/kg for grain and green material and at a LOQ of 0.05 mg/kg for straw by HPLC-MS/MS
Report:	Stuke, S.; 2018; 01100/M001; M-433720-02-1
Authority registration No:	
Guideline(s):	EC Guidance Document SANCO/825/00 rev. 8.1 of November 16, 2010 EC Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration Data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of Directive 91/414, SANCO 3029/99 Commission Regulation (EU) No 544/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances OECD Guideline, ENV/JM/MONO(2007)17, August 13, 2007 U.S. EPA Guideline, OPPTS 850.7100 Data Reporting for Environmental Chemistry Methods of April 1996 U.S. EPA Guideline, OPPTS 860.1340 Residue Analytical Method of August 1996
Deviations:	None
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

The analytical method 01100/M001 was validated for the determination of flufenacet residues in/on cereal grain, straw and green material by HPLC-MS/MS using matrix matched standards. The method provides validation data on cereal matrices in addition to method 01179 with only minor adaptations justified by different laboratory equipment and procedures. All extraction and work-up steps are the same for both methods. The method may be used as a data collection method as well as for enforcement purposes. Sample materials were fortified with flufenacet or with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid Na/flufenacet thioglycolate sulfoxide; 1/1/1).

Materials and methods

The analytical method 01100/M001 was validated for the determination of residues of flufenacet and three main metabolites (FOE-thioglycolate sulfoxide, FOE-oxalate, FOE-sulfonic acid) as the common moiety compound 4-fluoro-N-isopropylaniline (FOE 5043 aniline) in/on samples of plant origin – here represented by wheat green material, wheat grain, wheat straw – by HPLC-MS/MS. The analytical work is based on method 01100. Residues are expressed as parent equivalents.

5 g of the plant sample was treated with potassium permanganate for oxidation and refluxed under sulfuric acidic conditions for hydrolysis purposes. Residues were purified by water steam distillation of the formed common moiety compound 4-fluoro-N-isopropylaniline followed by a liquid-liquid partition with dichloromethane. Finally, residues were dissolved and subjected to HPLC-MS/MS. Residues of flufenacet and metabolites (all determined as 4-fluoro-N-isopropylaniline) were quantified using matrix-matched standards.

The following MRM transitions were used for quantification and confirmation:

4-fluoro-N-isopropylaniline: m/z = 154.1 → m/z = 112.0 (quantification),
m/z = 154.1 → m/z = 92.0 (confirmation).

Stock solutions with concentrations expressed as flufenacet were prepared dissolving the substances in methanol. From these, secondary standard solutions were prepared by diluting with methanol or for fluoro-N-isopropylaniline with ACN/water (1/4, v/v) + 0.1 mL/L acetic acid. 4-Fluoro-N-isopropylaniline residues were quantified using matrix-matched standards. The extracts of blank control samples were diluted with standard solutions containing flufenacet-4-fluoro-N-isopropylaniline.

Results and discussions

Precision: For the matrices investigated and for both mass transitions monitored, relative standard deviations (RSD) per fortification level were <20% except for transition 154 m/z → 92 m/z where the RSD for wheat green material fortified with flufenacet metabolite mix at the LOQ level was 21.2%. The individual recovery causing the elevated RSD was identified as an outlier according to a statistical approach (Nalimov outlier). Not considering the respective recovery value, mean RSD resulted in 12.1%.

Accuracy: Recovery rates were determined after fortification with flufenacet or after fortification with a mixture of flufenacet metabolites (flufenacet oxalate hydrate/flufenacet sulfonic acid Na/flufenacet thioglycolate sulfoxide; 1/1/1) at fortification levels of 0.01 mg/kg and 0.1 mg/kg (wheat grain and wheat green material) and 0.05 mg/kg and 0.5 mg/kg (wheat straw). Each analyte is expressed as flufenacet equivalents.

Mean recovery rates per fortification level were within the range of 70-110% for both fortification levels and for both mass transitions monitored.

Stability: Residues of the formed common moiety compound 4-fluoro-N-isopropylaniline were found to be stable in final plant extracts for at least 14 days when stored in a refrigerator at < 6°C.

The use of HPLC-MS/MS with two mass transitions for quantitation and quantitative confirmation (154 m/z → 112 m/z and 154 m/z → 92 m/z) ensures a high level of specificity. Validation data for a 2nd mass transition are reported in the present report for straw and in method report Class, Th.; Merdian, H.; 2010, (M-362716-01-1) for all cereal commodities. A confirmatory method is not required.

The method may be used as a data collection method and for enforcement purposes.

Table A 36: Recovery results from method validation of flufenacet or flufenacet metabolites (mix) using the analytical method 01100/M001

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Wheat grain	Flufenacet (quantification) m/z 154 → 112	0.01 (n = 5)	85	13.9	
		0.10 (n = 5)	78	5.4	
	Flufenacet Metabolite mix* (quantification) m/z 154 → 112	0.01 (n = 5)	77	5.4	
		0.10 (n = 5)	76	3.1	
Wheat green material	Flufenacet (quantification) m/z 154 → 112	0.01 (n = 5)	77	10.3	
		0.10 (n = 5)	78	12.0	
	Flufenacet Metabolite mix* (quantification) m/z 154 → 112	0.01 (n = 4) (n=5 [a])	78 (85[a])	12.1 (21.2[a])	[a] one value (114%) was identified as Nalimov outlier, result including the outlier value
		0.10 (n = 5)	76	6.0	
Wheat straw	Flufenacet (quantification) m/z 154 → 112	0.05 (n = 5)	78	8.2	
		0.50 (n = 5)	82	4.5	
	Flufenacet Metabolite mix* (quantification) m/z 154 → 112	0.05 (n = 5)	72	2.9	
		0.50 (n = 5)	72	5.8	
	Flufenacet Metabolite mix* (confirmation) m/z 154 → 92	0.05 (n = 5)	71	9.4	
		0.50 (n = 5)	70	5.4	

* Fortified with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid sodium salt/flufenacet thioglycolate sulfoxide; 1/1/1), detected as 4-fluoro-N-isopropylaniline and calculated as flufenacet.

Table A 37: Characteristics for the analytical method used for validation of flufenacet residues in wheat green material, grain and straw

	Flufenacet and its metabolites determined as 4-fluoro-N-isopropylaniline
Specificity	Blank value 30% LOQ. The use of LC-MS/MS with two mass transitions for quantitation and quantitative confirmation (154 m/z → 112 m/z and 154 m/z → 92 m/z) ensures a high level of specificity. A 2nd MRM transition was monitored for the analyte 4-fluoro-N-isopropylaniline using the representative sample material wheat straw. The individual recoveries for this 2nd MRM (mz 154→92) confirm the results of the quantitation MRM very well. A complete set of recoveries for cereal grain, straw and green material using a confirmatory mass transition was performed within validation of method 01179 reported above (Class, Th.; Merdian, H.; 2010; M-362716-01). Mass spectrum is provided in the original report.
Calibration (type, number of data points)	Calibration with matrix-matched standards, 1/x weighted linear regression (calibration line equations are presented in the original report). Coefficient of correlation: MRM for quantification: Grain: r = 0.9986 Number of data points: 5 Green material: r = 0.9960 Number of data points: 5 Straw: r = 0.9930 Number of data points: 5 MRM for confirmation: Straw: r = 0.9990 Number of data points: 3
Calibration range	In green material and grain: 0.0001 – 0.010 µg/mL (corresponding to 0.002 – 0.2 mg/kg expressed as parent flufenacet) In straw: 0.0005 – 0.050 µg/mL (corresponding to 0.01 – 1.0 mg/kg expressed as parent flufenacet).
Assessment of matrix effects is presented	The calibration standard procedure using matrix-matches standard solutions compensates possible matrix effects. Matrix effects were assessed in method 01100 and 01179.
Limit of determination/quantification	The LOQ was set at a level of 0.01 mg/kg in the matrices wheat grain and wheat green material. In the matrix wheat straw the LOQ was set at 0.05 mg/kg (expressed as flufenacet).

Conclusion

Method 01100/M001 was validated for the determination of flufenacet residues in wheat grain and wheat green material with an LOQ of 0.01 mg/kg and for wheat straw with an LOQ of 0.05 mg/kg. The method was successfully validated and thus was demonstrated to be applicable for data gathering and monitoring purposes. The analytical method fulfils the requirements of the EC Guidance documents on residue analytical methods (SANCO/3029/99 rev. 4, 11/07/00, SANCO/825/00 rev. 8.1, 16/11/2010) and in OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13).

The method is considered to comply with the quality criteria set out in SANTE/2020/12830 rev1.

A 2.1.2.1.3.2 Independent laboratory validation

The ILV is relevant to methods 01179 and 01100 including extension M001 described above.

Comments of zRMS:	The study of Meyer, M.; 2011; P612107502; M-405654-01-1 was submitted in the ongoing AIR dossier for renewal of flufenacet and was evaluated by RMS-Poland in the frame of the re-approval (RAR for Flufenacet; Vol. 3 – B.5, April 2022). Therefore, the study is only reported but not evaluated in this dossier. Analytical methods 01179 and 01100 were successfully validated by an independent

	laboratory validation (ILV) in wheat green plant, rape seed, orange fruit and dry bean seed with an LOQ of 0.01 mg/kg. The independent laboratory validation is considered to be applicable to extension M001 which uses the same methodology.

Reference:	**KCP 5.2.1/04**
Title:	Independent laboratory validation of the Bayer CropScience methods 01100 and 01179 for the determination of residues of Flufenacet (FOE5043) in/on plant materials
Report:	Meyer, M.; 2011; P612107502; M-405654-01-1
Authority registration No:	
Guideline(s):	EU-guidance document on residue analytical methods. SANCO/825/00-rev 7, 17/03/04; OECD document ENV/JM/MONO(2007)17, 13-Aug-2007
Deviations:	none
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The ILV was performed at SGS Institut Fresenius, Taunusstein, Germany.
Flufenacet and its metabolites FOE5043-sulfonic acid sodium-salt, FOE5043-thioglycolate sulfoxide and FOE5043-oxalate hydrate were determined in fortified specimens of wheat green plant (high water content), oilseed rape seed (high fat content), orange fruit (high acid content) and bean seed (dry and high protein content). The metabolites were fortified as a mixture (1/1/1 as molar equivalents).
The Bayer methods 01100 (Billian, P.; 2010; amended Stuke, S.; 2018; M-362575-03-1) and 01179 (Class, Th.; Merdian, H.; 2010; M-362716-01-1) were independently validated for these matrices. Since with the extension M001 to method 01100 the same matrices were validated as for method 01179 the ILV is considered to be applicable. All methods differ only in minor details justified by different laboratory equipment and adaptations. Therefore, it is justified to consider them identical for validation purpose.
After oxidation and hydrolysis, residues of flufenacet and metabolites were cleaned-up by distillation followed by a liquid/liquid partition with dichloromethane. Finally, residues were dissolved and subjected to HPLC-MS/MS. Flufenacet and its metabolites FOE5043-sulfonic acid sodium-salt, FOE5043-thioglycolate sulfoxide and FOE5043-oxalate hydrate were determined as the common moiety 4-fluoro-N-isopropylaniline.

The following MRM transitions were used for quantification and confirmation:
4-fluoro-N-isopropylaniline: m/z = 154 → m/z = 112 (quantification)
m/z = 154 → m/z = 92 (confirmation)

Results and discussions

High precision was demonstrated by low relative standard deviations (RSD always below 20%) in all matrices at both mass transitions.
Mean recoveries for each fortification level were within the range of 70 - 110%, which shows an acceptable accuracy of the method.
The use of HPLC-MS/MS with two mass transitions for quantitation and quantitative confirmation (154 m/z → 112 m/z and 154 m/z → 92 m/z) ensures a high level of specificity. An additional confirmatory method is not required.

The standard solutions of the reference item were found to be stable for at least 2 months, covering their experimental use. The reference item fluoroaniline proved to be stable in final extracts in 2 different matrices for at least 3 weeks.

Table A 38: Recovery results from independent laboratory validation of flufenacet or flufenacet metabolites (mix) using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Wheat green plant	Flufenacet (quantification) m/z 154 → 112	0.01 (n = 5)	99	2.3	
		0.10 (n = 5)	84	3.5	
	Flufenacet (confirmation) m/z 154 → 92	0.01 (n = 5)	99	2.6	
		0.10 (n = 5)	85	3.7	
	Flufenacet Metabolite mix* (quantification) m/z 154 → 112	0.01 (n = 5)	85	6.9	
		0.10 (n = 5)	76	2.5	
	Flufenacet Metabolite mix* (confirmation) m/z 154 → 92	0.01 (n = 5)	88	8.0	
		0.10 (n = 5)	77	2.5	
Rape seed	Flufenacet (quantification) m/z 154 → 112	0.01 (n = 5)	88	5.3	
		0.10 (n = 5)	75	9.2	
	Flufenacet (confirmation) m/z 154 → 92	0.01 (n = 5)	87	3.7	
		0.10 (n = 5)	76	11	
	Flufenacet Metabolite mix* (quantification) m/z 154 → 112	0.01 (n = 5)	78	6.8	
		0.10 (n = 5)	79	5.2	
	Flufenacet Metabolite mix* (confirmation) m/z 154 → 92	0.01 (n = 5)	77	3.7	
		0.10 (n = 5)	78	6.0	
Orange fruit	Flufenacet (quantification) m/z 154 → 112	0.01 (n = 5)	80	9.2	
		0.10 (n = 5)	72	1.8	
	Flufenacet (confirmation) m/z 154 → 92	0.01 (n = 5)	87	8.1	
		0.10 (n = 5)	75	1.7	
	Flufenacet Metabolite mix* (quantification) m/z 154 → 112	0.01 (n = 5)	79	8.8	
		0.10 (n = 5)	73	7.4	
	Flufenacet Metabolite mix* (confirmation) m/z 154 → 92	0.01 (n = 5)	80	8.5	
		0.10 (n = 5)	71	8.0	
Bean seed (dry)	Flufenacet (quantification) m/z 154 → 112	0.01 (n = 5)	88	4.8	
		0.10 (n = 5)	97	7.2	
	Flufenacet (confirmation) m/z 154 → 92	0.01 (n = 5)	96	11.2	
		0.10 (n = 5)	96	4.0	
	Flufenacet Metabolite mix* (quantification) m/z 154 → 112	0.01 (n = 5)	76	3.8	
		0.10 (n = 5)	80	3.8	

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
	Flufenacet Metabolite mix* (confirmation) m/z 154 → 92	0.01 (n = 5)	82	6.0	
		0.10 (n = 5)	84	4.1	

* Fortified with flufenacet metabolites mix (flufenacet oxalate hydrate/flufenacet sulfonic acid sodium salt/flufenacet thioglycolate sulfoxide; 1/1/1), detected as 4-fluoro-N-isopropylaniline and calculated as flufenacet.

Table A 39: Characteristics for the analytical method used for independent laboratory validation of flufenacet residues in wheat green plant, rape seed, orange fruit and bean seed (dry)

	Flufenacet and its metabolites detected as 4-fluoro-N-isopropylaniline
Specificity	Blank value < 30% LOQ. The use of LC-MS/MS with two mass transitions for quantitation and quantitative confirmation (154 m/z → 112 m/z and 154 m/z → 92 m/z) ensures a high level of specificity. Mass spectrum is provided in the original report.
Calibration (type, number of data points)	Calibration with matrix-matched standards, 1/x weighted linear regression (calibration line equations are presented in the original report). Coefficient of correlation: MRM for quantification: (m/z 154→ m/z 112) Wheat green plant: r = 0.9998 Rape seed: r = 0.9998 Orange fruit:r = 0.9998 Bean seed (dry): r = 0.9999 MRM for confirmation: (m/z 154→ m/z 92) Wheat green plant: r = 0.9999 Rape seed: r = 0.9998 Orange fruit: r = 0.9998 Bean seed (dry): r = 0.9999 Number of data points for both mass transitions and all matrices: ≥7
Calibration range	Wheat green plant (1st and 2nd MRM): 0.05 -2.5 ng/ml (0.001-0.05 mg/kg expressed as 4-fluoro-N-isopropylaniline, corresponding to 0.002 – 0.12 mg/kg expressed as parent flufenacet). Rape seed (1st MRM): 0.1 -2.5 ng/ml (0.002-0.05 mg/kg expressed as 4-fluoro-N-isopropylaniline, corresponding to 0.005 – 0.12 mg/kg expressed as parent flufenacet) Rape seed (2nd MRM): 0.05 -2.5 ng/ml (0.001-0.05 mg/kg expressed as 4-fluoro-N-isopropylaniline, corresponding to 0.002 – 0.12 mg/kg expressed as parent flufenacet) Orange fruit (1st and 2nd MRM): 0.1 -2.5 ng/ml (0.002-0.05 mg/kg expressed as 4-fluoro-N-isopropylaniline, corresponding to 0.005 – 0.12 mg/kg expressed as parent flufenacet) Bean seed (dry) (1st MRM): 0.05 -2.0 ng/ml (0.001-0.04 mg/kg expressed as 4-fluoro-N-isopropylaniline, corresponding to 0.002 – 0.1 mg/kg expressed as parent flufenacet). Bean seed (dry) (2nd MRM): 0.05 -2.5 ng/ml (0.001-0.05 mg/kg expressed as 4-fluoro-N-isopropylaniline, corresponding to 0.002 – 0.12 mg/kg expressed as parent flufenacet).
Assessment of matrix effects is presented	For quantification external calibration with matrix-matched standard solutions was applied.
Limit of determination/quantification	The ILV confirmed the LOQ of 0.01 mg/kg (expressed as flufenacet) for flufenacet and its metabolites in all matrices.

Conclusion

Analytical methods 01179 and 01100 were successfully validated by an independent laboratory (ILV) in wheat green plant, rape seed, orange fruit and dry bean seed with an LOQ of 0.01 mg/kg. The independent laboratory validation is considered to be applicable to extension M001 which uses the same methodology. The ILV fulfils the requirements detailed in the EC "Guidance document on residue analytical methods" (SANCO/825/00 rev. 8.1) and in OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13).

A 2.1.2.1.3.3 Confirmatory method

The use of HPLC-MS/MS with two mass transitions for quantitation and quantitative confirmation (154 m/z → 112 m/z and 154 m/z → 92 m/z) ensures a high level of specificity. Validation data for a 2nd mass transition are reported in the present report for straw (method 01100/M001) and in method report Class, Th.; Merdian, H.; 2010, (M-362716-01-1) for all cereal commodities (cf. A 1.1.1.3). A confirmatory method is not required.

In addition, the multi-residue QuEChERS method in combination with HPLC-MS/MS, described in the European Standard EN 15662:2008 (CEN, 2008b), is available for the determination of flufenacet in high acid, dry, high sugar and high water commodities. However, this method does not include other metabolites containing the N-fluorophenyl-N-isopropyl moiety; it is therefore not suitable for enforcement of this substance according to its complete residue definition (EFSA Journal 2012;10(4):2689).

A 2.1.2.1.3.4 Extraction efficiency

The extraction efficiency of the method has been investigated and is described in detail under point A 2.1.2.1.3.5.

A 2.1.2.1.3.5 Expert statement: Comparison of extraction conditions of method 00346 (EU peer reviewed) and methods 01179, 01100 and 01100/M001

Comments of zRMS:	The position paper has been presented in RAR for Flufenacet (Vol. 3, B-5, RMS-PL, April 2022). RMS-PL conclusion: Methods 00346 and 01179 (as well as method 01100 and its extensions M001 and M002) are suitable for the determination of flufenacet residues in protected crops and the both methods were validated successfully and thus were demonstrated to be applicable for data gathering and monitoring purposes. The analytical methods fulfils the requirements of the Regulation (EC) 1107/2009, detailed in the EC Guidance documents on residue analytical methods (SANCO/3029/99 rev. 4, 11/07/2000, SANCO/825/00 rev. 8.1, 16/11/2010). The method (01179 and 01100) using an HPLC-MS/MS technique are more convenient because they do not include the step of derivatization.

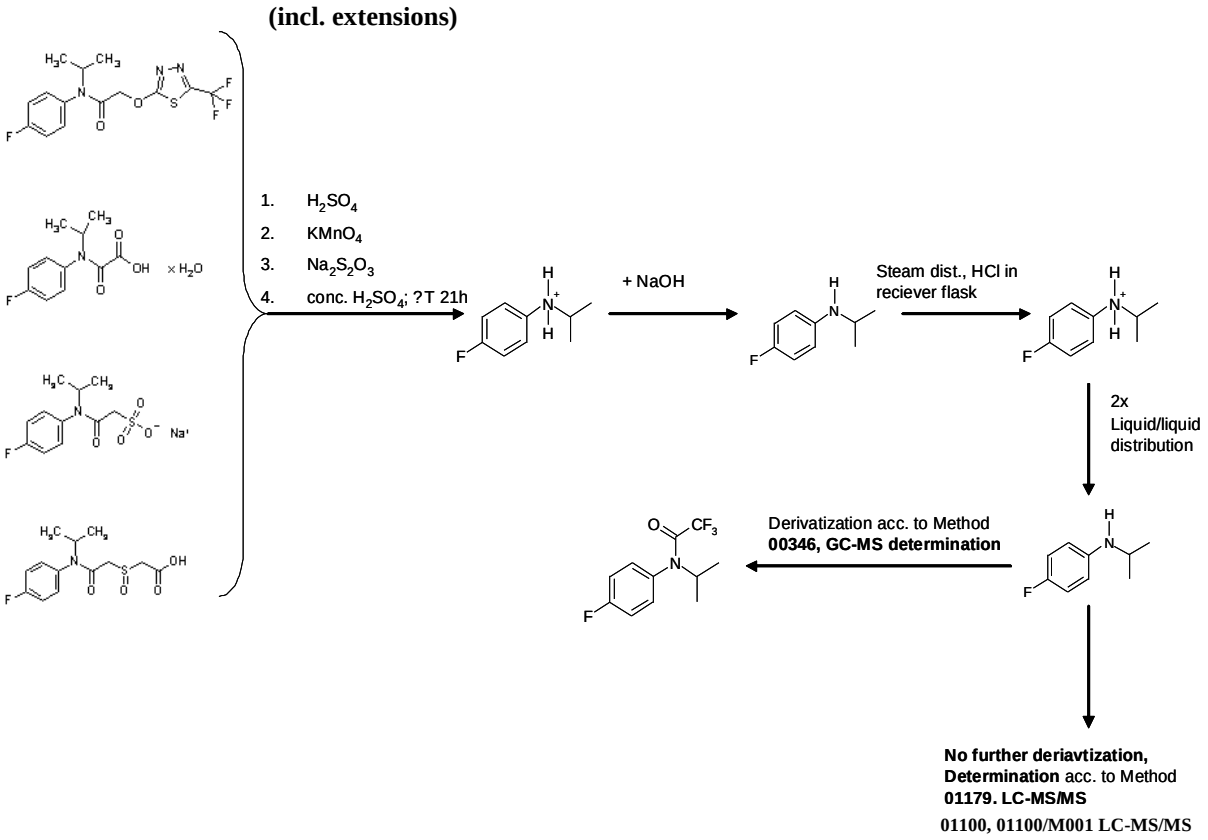
Reference:	**KCP 5.2.1/05**
Title:	Position paper: Subject: Flufenacet: Answer to CRD questions related to the authorization of the product Liberator SC 500 (flufenacet + diflufenican 400 g/L + 100 g/L) - Comparison of flufenacet residue analytical method nos. 00346 vs. 01179
Report:	Stuke, S.; Weile, M.; 2011; M-416013-01-1
Authority registration No:	
Guideline(s):	not specified
Deviations:	not specified
GLP/GEP:	not applicable
Acceptability:	yes
Duplication (if vertebrate study):	

Linked to the authorization of a flufenacet containing product (flufenacet 400 g/L + diflufenican 100 g/L) in/on cereals in the United Kingdom residue trials were submitted where the field samples were analyzed using method 01179. CRD requested clarification on the comparability of method 00346 evaluated in the EU review process and method 01179.
In the Monograph (France 1997) and the List of Endpoints (BBA 1999), method 00346 has been evaluated as the basic method for data generation and enforcement.
The residues are oxidized and hydrolyzed to the common moiety 4-fluoro-N-isopropylaniline. The 4-fluoro-N-isopropylaniline is separated from the crop matrix by steam distillation after making the crop digest basic. The 4-fluoro-N-isopropylaniline is extracted from the steam distillate and derivatized with trifluoroacetic anhydride. The derivative, 2,2,2-trifluoro-N-(4-fluorophenyl)-N-isopropylacetamide (trifluoroacetamide), is measured by gas chromatography/mass spectroscopy (GC/MS). Due to the physico-chemical properties of flufenacet and its metabolites (e.g. decomposition at higher

temperatures) the derivatisation step was necessary because these substances were not suitable for the determination as single substances themselves with GC (i.e. without prior derivatisation).
This method shows both very good repeatability and reproducibility as demonstrated in the method report of method 00346. During the validation of the method 00346 each compound of interest (i.e. flufenacet, FOE-oxalate, FOE sulfonic acid, FOE thioglycolate sulfoxide) was fortified separately or as a mixture of these showing sufficient recoveries at the end of the entire sample work-up.
Since HPLC determination has become more and more available and has become acceptable also for enforcement methods, method 00346 was modified by omission of the derivatisation step to form trifluoroacetamide. Instead, with method 01179 and 01100 (including its extensions, cf. A 2.1.2)) the resulting 4-fluoro-N-isopropylaniline is directly determined by HPLC-MS/MS in order to facilitate the analytical work.
CRD's questions center around the methodology used for formation and extraction of the common moiety in the new method 01179 and whether both methods can be considered comparable relative to the efficiency to form the common moiety.
In the position paper the individual steps of the sample work-up for method 00346 and 01179 are compared and a justification is provided that the metabolites containing the common fluorophenyl-isopropyl amine moiety can be fortified as a mixture because they are all converted to the 4-fluoro-N-isopropylaniline which forms the analytical target.
It is confirmed that all steps related to the work-up of the samples remain unchanged compared to method 00346. In method 00346 it has been demonstrated with fortifications of individual metabolites that each metabolite shows appropriate behavior to form the common moiety. Since the preparation steps in method 01179 (and 01100 including extensions) are identical it is considered appropriate to fortify the metabolites as mixture. Recovery rates and repeatability were acceptable. The same arguments as for method 01179 apply to method 01100 and its extension 01100/M001 which involve unchanged extraction of the residues and formation of the 4-fluoro-N-isopropylaniline which can be detected directly by HPLC-MS/MS without further derivatisation.
Method 00346 and 001179 (and method 01100 as well) are characterized by the following work-up steps:

- Extraction step is identical containing oxidation reagent in aqueous acidic environment.
- In following step the refluxing under strong acidic conditions cleaves the amide bonding to achieve the common moiety compound 4-fluoro-N-isopropylaniline. This step is identical in all described methods.
- This compound is protonated in acid medium and has to be deprotonated with alkaline to be distillable. This step is included in all described methods.
- Water steam distillation out of alkaline medium into an acidified distillation receiver flask (protonates the aniline again) avoids the loss of the target compound for all methods.
- To remove the acid content, the obtained solution is liquid/liquid distributed with dichloromethane. This step is included in methods 00346, 01179, 01100 and modification M001.
- In method 00346, the 4-fluoro-N-isopropylaniline is finally derivatized with trifluoroacetic anhydride to 2,2,2-trifluoro-N-(4-fluorophenyl)-N-isopropylacetamide (called 4-fluoro-*N*-methylethyl benzenamine trifluoroacetamide or FOE 5043 trifluoroacetamide in the method reports) to be amenable to GC-MS determination. In method 01179 (and 01100 including its extensions) this step is not necessary. The 4-fluoro-N-isopropylaniline can be determined by LC-MS/MS without further chemical modification.

Figure A1: Chemistry work-flow within residue analytical methods 00346 and 01179, 01100



Investigation of extraction efficiency was performed using samples/commodities from the metabolism studies. In the following paragraphs extraction efficiency of the residue analytical methods is compared to relative amount extracted from the metabolism studies.
Since the extraction conditions are the same in data generation methods and methods for post registration control the findings reported below are valid for enforcement and data generation methods.

A 2.1.2.1.3.6 Study report 107399 - extraction efficiency in cereal grain and straw

Comments of zRMS:	The extraction efficiency of the residue analytical method (Gould, T. J.; Lemke, V. J.; 1995) was submitted in the ongoing AIR dossier for renewal of flufenacet and was evaluated by RMS-Poland in the frame of the re-approval (RAR for Flufenacet; Vol. 3 – B.5, April 2022). Therefore, the study is only reported but not evaluated in this dossier.

Reference:	**KCP 5.2.1/06**
Title:	The metabolism of [Fluorophenyl-UL-14C] FOE 5043 in wheat after postemergent foliar spray application
Report:	Krolski, M. E.; Bosnak, L. L.; 1997; 107399; M-002275-01-1
Authority registration No:	
Guideline(s):	EPA Ref: 860.1300, Nature of the Residue - Plants
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Extraction efficiency of the residue analytical method (Gould, T. J.; Lemke, V. J.; 1995) was investigated in the course of the wheat metabolism study evaluated in the EU peer review (France 1997 and EFSA 2012) and the DRAR (Poland 2017). The method was evaluated on EU level (Monograph,

France 1997). Extraction conditions are identical to those in methods 00346 (including extensions and modifications) and methods 01179 and 01100 (including modifications), please cf. Stuke, S.; Weile, M., 2011, M-416013-01-1 above. The extraction efficiency was examined using wheat grain and straw samples with incurred residues after application of radiolabeled flufenacet.

In the metabolism study homogenized straw and grain were extracted separately with methanol/water (4/1, 1x) following steeping at room temperature for half an hour. Extraction was continued with pure methanol (2 times) at ambient temperature and under reflux, with hydrochloric acid and sodium hydroxide. The aqueous phases were neutralized and partitioned against chloroform. Between acid/basic hydrolysis at room temperature and under reflux an additional extraction step with methanol/water (3/1) under ultrasonication was inserted. All fractions/phases were radioassayed.

TRR levels of grain and straw samples used for this test amounted to 0.55 and 1.96 mg equ/kg. These levels were slightly lower than the initial levels, probably due to hydration of the dried grain and straw during freezer storage.

Applying the procedures of the residue analytical method, following oxidation, hydrolysis and steam distillation of formed common moiety N-fluorophenyl-N-isopropyl amine from wheat grain the distillate contained 97% of TRR in the original grain sample. 84% of TRR partitioned into the organic phase after addition of sodium hydroxide. Subsequent derivatisation revealed the analytical target N-4-fluorophenyl-N-isopropyl-trifluoroacetamide representing 81% of TRR in the original grain sample. Compared to the total extractability with methanol determined in the metabolism experiment (80% of TRR extractable at room temperature and under reflux conditions, with 66% of TRR identified as metabolites containing the common moiety) this figure represented a complete extraction of those residue components that contain the respective N-fluorophenyl-N-isopropyl amine moiety.

Applying the same method to a straw sample resulted in 86% of TRR in the distillate with 76% of TRR in the organic phase prior to derivatisation. The derivatized sample contained 70% of TRR in the original straw sample, which was identified as N-4-fluorophenyl-N-isopropyl-trifluoroacetamide. Compared to the total extractability with methanol determined in the metabolism experiment (86% of TRR extractable at room temperature and under reflux conditions, with 74% of TRR identified as metabolites with the common moiety) this figure represented also a complete extraction of those residue components that contain the respective N-fluorophenyl-N-isopropyl amine moiety.

Conclusion
Comparative extraction of the residues using methanol in the metabolism study and determination of the residues using the residue analytical method (oxidative acid hydrolysis and quantification of the hereby formed N-fluorophenyl-N-isopropyl amine) showed a good agreement of amount of residue compounds containing the common moiety. The analytical residue method adequately converts the residues in wheat straw and grain to the analyte 2,2,2-trifluoro-N-(4-fluorophenyl)-N-isopropylacetamide.

Table A 40: **Efficiency of the residue analytical method for extracting identified flufenacet residues from wheat grain and straw after application with [fluorophenyl-UL-^{14}C]flufenacet**

Metabolism study		Residue analytical method		Extraction efficiency[3]
Total extractablity of metabolites	**Identified metabolites[1]**	**Radioactivity in final CH_2Cl_2 extract**	**Analytical target[2]**	% analytical target referred to identified metabolites
[%TRR]	[%TRR]	[% TRR]	[%TRR]	
TRR = 0.62 ppm		**Wheat grain; TRR = 0.55 ppm**		
69	66	84	81	123
TRR = 2.04 ppm		**Wheat straw, TRR = 1.96 ppm**		
78	74	76	70	95

[1] Metabolites identified in metabolism study: FOE oxalate (FOEOX, M1), FOE sulfinyl lactic acid glucoside I (FAMSOL-Glu I+II, M37), FOE thioglycolate sulfoxide (FAMSOC, M4) FOE sulfinyl lactic acid I +II (FAMSOL I, M33), FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M41).
[2] Percentage of radioactivity in the final extract identified as analytical target 2,2,2-trifluoro-N-(4-fluorophenyl)-N-isopropylacetamide by radio-HPLC
[3] Extraction efficiency of residue method = ratio between analytical target [%TRR] / identified metabolites in the metabolism study [%TRR]. All identified metabolites contain the common moiety (= analytical target).

Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)
Bayer method 00418 (xxx, 1995), the extension for eggs 00418/M001 (xxx, 1995) and the independent validation (xxx, 1995)were evaluated during the EU peer review (Monograph France 1997 and Report of ECCO 73, Annex 2, Complete List of Endpoints (BBA 1999)).

Since the initial ILV was validated for bovine liver only a complete set of validation data was generated for all relevant animal tissues, milk and eggs.
The new ILV to methods 00418 and 00418/M001 is evaluated in the DRAR (Poland 2017) and summarised below.

A 2.1.2.1.4 Analytical methods 00418 and 00418/M001

A 2.1.2.1.4.1 Method validation

The method 00418 and its modification 00418/M001 have been evaluated in the EU peer-review. They are not reported again in the present dossier.

A 2.1.2.1.4.2 Independent laboratory validation

Comments of zRMS:	The validation of the Bayer methods 00418 (M-019605-01-1) and 00418/M001 (M-019614-01-1) for the determination of residues of flufenacet (FOE 5043) and its metabolites in animal tissues and animal products was submitted in the ongoing AIR dossier for renewal of flufenacet and was evaluated by RMS-Poland in the frame of the re-approval (RAR for Flufenacet; Vol. 3 – B.5, April 2022). Therefore, the study is only reported but not evaluated in this dossier. This method is acceptable.

Reference:	**KCP 5.2.2/01**
Title:	Validation of the Bayer methods 00418 (M-019605-01-1) and 00418/M001 (M-019614-01-1) for the determination of residues of flufenacet (FOE 5043) and its metabolites in animal tissues and animal products
Report:	xxx 2013; S12-00052; M-461242-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method
Deviations:	none
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods
The validation work was performed by Eurofins AgroScience Service, Hamburg, Germany.

The method follows the same methodology as the plant method 00346. The residues of flufenacet (FOE 5043), FOE 5043 oxalate hydrate, FOE 5043 sulfonic acid sodium salt, and FOE 5043 thioglycolate sulfoxide were extracted from the matrices under acidic and oxidative conditions. After steam distillation and clean-up by liquid / liquid partition an aliquot was derivatized with trifluoroacetic anhydride. The derivate (FOE 5043 trifluoroacetamide) was cleaned up on a C-18 SPE cartridge and subjected to GC-MSD.
For quantitation molecular ion m/z 249 was used. For verification the fragment ions m/z 207 and m/z 138 were selected.
The stock solutions were prepared with methanol (flufenacet and its metabolites) or MTBE (FOE 5043 trifluoroacetamide). Standard solutions (secondary standards of FOE 5043 trifluoroacetamide) were prepared from the stock solutions by dilution with MTBE. Calibration standards of FOE 5043 trifluoroacetamide for linearity investigations and for quantification of residues were prepared by dilution with MTBE and by dilution with matrix extract. These standards were used to check for matrix effects. Fortification solutions of flufenacet and its metabolites were prepared in methanol.

Results and discussions
Accuracy: Recovery rates were determined at fortification levels of 0.01 mg/kg (=LOQ level) and at 0.10 mg/kg for milk, 0.02 mg/kg (=LOQ level) and at 0.20 mg/kg for liver and 0.05 mg/kg (=LOQ level) and at 0.50 mg/kg for meat, fat and egg. Recovery experiments were conducted by separate fortification of untreated control samples with defined amounts of Flufenacet (FOE5043), FOE5043 oxalate hydrate, FOE5043 sulfonic acid sodium salt and FOE5043 thioglycolate sulfoxide prior to analysis.
Since all analytes are converted to the common moiety FOE5043 trifluoroacetamide recovery data from the parent compound and the individual metabolites are combined to form a full data set for calculation of the overall mean recovery per fortification level and commodity and the relative standard deviation (RSD). Recovery rates per fortification level and matrix were in the range of 70-110% for all fortification levels and for the three fragment ions investigated.
Precision: Fortified specimens were analyzed with n=8 - 12 for each fortification level. Relative standard deviations for n = 8-12 were <20% for fortification levels ≤0.1 mg/kg and <15% for fortifications above that concentration.
The stability in final extracts was checked for the tested sample material over a period of at least ten days. FOE5043 trifluoroacetamide was found to be stable in final extracts for at least 10 days in eggs, 18 days in liver, 28 days in fat and 34 days in milk and meat when stored at 3-8°C in the dark. FOE

5043 trifluoroacetamide in solvent standards was found to be stable up to 6 months.

Table A 41: Recovery results from independent laboratory validation of flufenacet, FOE 5043 oxalate hydrate, FOE 5043 sulfonic acid sodium salt and FOE 5043 thioglycolate sulfoxide

Matrix	Analyte	Fortification level (mg/kg)* (n = x)	Mean recovery (%)	RSD (%)	Comments
Fat	**m/z 249 (quantification)**				
	Flufenacet	0.05 (n = 2)	91	-	
		0.50 (n = 2)	87	-	
	FOE 5043 oxalate hydrate	0.05 (n = 2)	113	-	
		0.50 (n = 2)	96	-	
	FOE 5043 sulfonic acid sodium salt	0.05 (n = 2)	82	-	
		0.50 (n = 2)	84	-	
	FOE 5043 thioglycolate sulfoxide	0.05 (n = 2)	81	-	
		0.50 (n = 2)	66	-	
	Overall 0.05 mg/kg (n = 8)		*92*	*18*	
	Overall 0.50 mg/kg (n = 8)		*83*	*15*	
	m/z 207 (confirmation)				
	Flufenacet	0.05 (n = 2)	95	-	
		0.50 (n = 2)	88	-	
	FOE 5043 oxalate hydrate	0.05 (n = 2)	116	-	
		0.50 (n = 2)	94	-	
	FOE 5043 sulfonic acid sodium salt	0.05 (n = 2)	85	-	
		0.50 (n = 2)	84	-	
	FOE 5043 thioglycolate sulfoxide	0.05 (n = 2)	75	-	
		0.50 (n = 2)	67	-	
	Overall 0.05 mg/kg (n = 8)		*93*	*20*	
	Overall 0.50 mg/kg (n = 8)		*83*	*13*	
	m/z 138 (confirmation)				
	Flufenacet	0.05 (n = 2)	92	-	
		0.50 (n = 2)	88	-	
	FOE 5043 oxalate hydrate	0.05 (n = 2)	115	-	
		0.50 (n = 2)	96	-	
	FOE 5043 sulfonic acid sodium salt	0.05 (n = 2)	85	-	
		0.50 (n = 2)	84	-	
	FOE 5043 thioglycolate sulfoxide	0.05 (n = 2)	77	-	
		0.50 (n = 2)	66	-	
	Overall 0.05 mg/kg (n = 8)		*92*	*19*	
	Overall 0.50 mg/kg (n = 8)		*83*	*15*	
Egg	**m/z 249 (quantification)**				
	Flufenacet	0.05 (n = 2)	79	-	

Matrix	Analyte	Fortification level (mg/kg)* (n = x)	Mean recovery (%)	RSD (%)	Comments
		0.50 (n = 2)	94	-	
	FOE 5043 oxalate hydrate	0.05 (n = 2)	86	-	
		0.50 (n = 2)	74	-	
	FOE 5043 sulfonic acid sodium salt	0.05 (n = 2)	60	-	
		0.50 (n = 2)	95	-	
	FOE 5043 thioglycolate sulfoxide	0.05 (n = 2)	64	-	
		0.50 (n = 2)	73	-	
	Overall 0.05 mg/kg (n = 8)		*72*	*17*	
	Overall 0.50 mg/kg (n = 8)		*84*	*15*	
	m/z 207 (confirmation)				
	Flufenacet	0.05 (n = 2)	79	-	
		0.50 (n = 2)	96	-	
	FOE 5043 oxalate hydrate	0.05 (n = 2)	89	-	
		0.50 (n = 2)	74	-	
	FOE 5043 sulfonic acid sodium salt	0.05 (n = 2)	61	-	
		0.50 (n = 2)	94	-	
	FOE 5043 thioglycolate sulfoxide	0.05 (n = 2)	64	-	
		0.50 (n = 2)	73	-	
	Overall 0.05 mg/kg (n = 8)		*73*	*17*	
	Overall 0.50 mg/kg (n = 8)		*84*	*15*	
	m/z 138 (confirmation)				
	Flufenacet	0.05 (n = 2)	81	-	
		0.50 (n = 2)	95	-	
	FOE 5043 oxalate hydrate	0.05 (n = 2)	88	-	
		0.50 (n = 2)	74	-	
	FOE 5043 sulfonic acid sodium salt	0.05 (n = 2)	61	-	
		0.50 (n = 2)	95	-	
	FOE 5043 thioglycolate sulfoxide	0.05 (n = 2)	69	-	
		0.50 (n = 2)	72	-	
	Overall 0.05 mg/kg (n = 8)		*74*	*16*	
	Overall 0.50 mg/kg (n = 8)		*84*	*15*	
Meat	**m/z 249 (quantification)**				
	Flufenacet	0.05 (n = 2)	78	-	
		0.50 (n = 2)	73	-	
	FOE 5043 oxalate hydrate	0.05 (n = 2)	90	-	
		0.50 (n = 2)	83	-	
	FOE 5043 sulfonic acid	0.05 (n = 2)	82	-	
		0.50 (n = 2)	61	-	

Matrix	Analyte	Fortification level (mg/kg)* (n = x)	Mean recovery (%)	RSD (%)	Comments
	sodium salt				
	FOE 5043 thioglycolate sulfoxide	0.05 (n = 2)	70	-	
		0.50 (n = 2)	71	-	
	Overall 0.05 mg/kg (n = 8)		*80*	*11*	
	Overall 0.50 mg/kg (n = 8)		*72*	*14*	
	m/z 207 (confirmation)				
	Flufenacet	0.05 (n = 2)	78	-	
		0.50 (n = 2)	73	-	
	FOE 5043 oxalate hydrate	0.05 (n = 2)	91	-	
		0.50 (n = 2)	82	-	
	FOE 5043 sulfonic acid sodium salt	0.05 (n = 2)	84	-	
		0.50 (n = 2)	62	-	
	FOE 5043 thioglycolate sulfoxide	0.05 (n = 2)	71	-	
		0.50 (n = 2)	72	-	
	Overall 0.05 mg/kg (n = 8)		*81*	*10*	
	Overall 0.50 mg/kg (n = 8)		*72*	*13*	
	m/z 138 (confirmation)				
	Flufenacet	0.05 (n = 2)	84	-	
		0.50 (n = 2)	74	-	
	FOE 5043 oxalate hydrate	0.05 (n = 2)	92	-	
		0.50 (n = 2)	82	-	
	FOE 5043 sulfonic acid sodium salt	0.05 (n = 2)	82	-	
		0.50 (n = 2)	61	-	
	FOE 5043 thioglycolate sulfoxide	0.05 (n = 2)	70	-	
		0.50 (n = 2)	72	-	
	Overall 0.05 mg/kg (n = 8)		*82*	*11*	
	Overall 0.50 mg/kg (n = 8)		*72*	*14*	
Liver	**m/z 249 (quantification)**				
	Flufenacet	0.02 (n = 3)	82	6.3	
		0.20 (n = 3)	88	13	
	FOE 5043 oxalate hydrate	0.02 (n = 3)	88	1.7	
		0.20 (n = 3)	69	2.5	
	FOE 5043 sulfonic acid sodium salt	0.02 (n = 3)	85	1.2	
		0.20 (n = 3)	91	5.7	
	FOE 5043 thioglycolate sulfoxide	0.02 (n = 3)	82	6.3	
		0.20 (n = 3)	73	7.2	
	Overall 0.02 mg/kg (n = 12)		*84*	*5.1*	

Matrix	Analyte	Fortification level (mg/kg)* (n = x)	Mean recovery (%)	RSD (%)	Comments
	Overall 0.20 mg/kg (n = 12)		*80*	*14*	
	m/z 207 (confirmation)				
	Flufenacet	0.02 (n = 3)	81	5.7	
		0.20 (n = 3)	88	14	
	FOE 5043 oxalate hydrate	0.02 (n = 3)	88	0.65	
		0.20 (n = 3)	68	1.7	
	FOE 5043 sulfonic acid sodium salt	0.02 (n = 3)	82	2.4	
		0.20 (n = 3)	91	6.6	
	FOE 5043 thioglycolate sulfoxide	0.02 (n = 3)	92	10	
		0.20 (n = 3)	78	6.4	
	Overall 0.02 mg/kg (n = 12)		*86*	*7.7*	
	Overall 0.20 mg/kg (n = 12)		*81*	*14*	
	m/z 138 (confirmation)				
	Flufenacet	0.02 (n = 3)	90	11	
		0.20 (n = 3)	88	14	
	FOE 5043 oxalate hydrate	0.02 (n = 3)	110	2.9	
		0.20 (n = 3)	72	1.4	
	FOE 5043 sulfonic acid sodium salt	0.02 (n = 3)	96	4.4	
		0.20 (n = 3)	95	6.5	
	FOE 5043 thioglycolate sulfoxide	0.02 (n = 3)	92	7.6	
		0.20 (n = 3)	70	1.4	
	Overall 0.02 mg/kg (n = 12)		*97*	*10*	
	Overall 0.20 mg/kg (n = 12)		*81*	*15*	
Milk	**m/z 249 (quantification)**				
	Flufenacet	0.01 (n = 2)	89	-	
		0.10 (n = 2)	80	-	
	FOE 5043 oxalate hydrate	0.01 (n = 2)	93	-	
		0.10 (n = 2)	105	-	
	FOE 5043 sulfonic acid sodium salt	0.01 (n = 2)	92	-	
		0.10 (n = 2)	73	-	
	FOE 5043 thioglycolate sulfoxide	0.01 (n = 2)	95	-	
		0.10 (n = 2)	99	-	
	Overall 0.01 mg/kg (n = 8)		*92*	*13*	
	Overall 0.10 mg/kg (n = 8)		*89*	*18*	
	m/z 207 (confirmation)				
	Flufenacet	0.01 (n = 2)	92	-	
		0.10 (n = 2)	81	-	
	FOE 5043	0.01 (n = 2)	94	-	

Matrix	Analyte	Fortification level (mg/kg)* (n = x)	Mean recovery (%)	RSD (%)	Comments
	oxalate hydrate	0.10 (n = 2)	105	-	
	FOE 5043 sulfonic acid sodium salt	0.01 (n = 2)	98	-	
		0.10 (n = 2)	74	-	
	FOE 5043 thioglycolate sulfoxide	0.01 (n = 2)	97	-	
		0.10 (n = 2)	100	-	
	Overall 0.01 mg/kg (n = 8)		*95*	*12*	
	Overall 0.10 mg/kg (n = 8)		*90*	*17*	
	m/z 138 (confirmation)				
	Flufenacet	0.01 (n = 2)	105	-	
		0.10 (n = 2)	80	-	
	FOE 5043 oxalate hydrate	0.01 (n = 2)	97	-	
		0.10 (n = 2)	104	-	
	FOE 5043 sulfonic acid sodium salt	0.01 (n = 2)	90	-	
		0.10 (n = 2)	75	-	
	FOE 5043 thioglycolate sulfoxide	0.01 (n = 2)	97	-	
		0.10 (n = 2)	99	-	
	Overall 0.01 mg/kg (n = 8)		*97*	*13*	
	Overall 0.10 mg/kg (n = 8)		*89*	*17*	

* Fortification levels are expressed as Flufenacet equivalents
Determination as 2,2,2-trifluoro-N-(4-fluorophenyl)-N-isopropylacetamide (FOE5043 trifluoroacetamide), calculated and expressed as flufenacet

Table A 42: Characteristics for the analytical method used for independent laboratory validation of flufenacet residues in eggs, bovine fat, meat, liver and milk

	Flufenacet and its metabolites determined as FOE 5043 trifluoroacetamide
Specificity	Blank value <30% LOQ. Three fragments (m/z > 100) were evaluated for each analyte and sample. Therefore, the GC-MSD method is highly specific and an additional confirmatory method is not necessary. Mass spectrum is provided in the original report.
Calibration (type, number of data points)	Calibration with solvent standards of FOE 5043 trifluoroacetamide, 1/x weighted linear regression (calibration line equations are presented in the original report). Coefficient of correlation: m/z 249: r = 0.9997 m/z 207: r = 0.9997 m/z 138: r = 0.9997 Number of data points for all mass transitions: 11
Calibration range	0.0072 – 4.5 µg/mL of FOE 5043 trifluoroacetamide (expressed as flufenacet) which corresponds to 0.0072 – 4.5 mg/kg for egg, milk, fat and liver and 0.0029 – 1.8 mg/kg for meat.
Assessment of matrix effects is presented	Matrix effects were tested for each matrix for the three selected fragment ions by comparing the peak areas of matrix-matched standards with solvent standards. Matrix effects of <20% were measured for all matrices and considered to be not significant. Therefore solvent standards were used for calibration and quantification.
Limit of determination/quantification	The limit of quantification for flufenacet (FOE 5043), FOE 5043 oxalate, FOE 5043 sulfonic acid, and FOE 5043 thioglycolate sulfoxide was established and validated at 0.05 mg/kg in meat, egg and fat, 0.02 mg/kg in liver and 0.01 mg/kg in milk.

Conclusion
The method 00418 and its modification 00418/M001 are considered suitable for the determination of residues of flufenacet (FOE 5043) containing the N-fluorophenyl-N-isopropyl amine moiety in matrices of animal origin. Validation of the method was exemplified with flufenacet, FOE 5043 oxalate, FOE 5043 sulfonic acid, and FOE 5043 thioglycolate sulfoxide. The residue analytical method 00418 and the extension 00418/M001 were independently validated. The method fulfils the requirements of the Regulation (EC) 1107/2009, detailed in the EC "Guidance document on residue analytical methods" (SANCO/825/00 rev. 8.1) and in OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13). The method is considered to comply with the quality criteria set out in SANTE/2020/12830 rev 1.

A 2.1.2.1.4.3 Confirmatory method

The results of the method validation were confirmed using a second and a third fragment ion for confirmation. No additional confirmatory method is required.

A 2.1.2.1.4.4 Extraction efficiency

Not required, please refer to Point 5.3.2.3.

Since residues ≥LOQ are not anticipated in commodities of animal origin data on extraction efficiency in products of animal origin are not reported in the present submission which is in accordance with the requirements outlined in SANCO/825/00 rev. 8.1 and SANTE/2017/10632 rev 3 (Technical Guideline on the Evaluation of Extraction Efficiency). MRLs for animal commodities are established at the LOQ level.

A 2.1.2.2 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

A 2.1.2.2.1 Analytical method 01486

A 2.1.2.2.1.1 Method validation

Comments of zRMS:	The residue analytical method 01486 for the determination of residues of flufenacet and its metabolite flufenacet-thiadone in cattle plasma was submitted in the ongoing AIR dossier for renewal of flufenacet and was evaluated by RMS-Poland in the frame of the re-approval (RAR for Flufenacet; Vol. 3 – B.5, April 2022). Therefore, the study is only reported but not evaluated in this dossier. This method is accepted.

Reference:	**KCP 5.2.3/01**
Title:	Analytical method 01486 for the determination of various pesticides and selected pesticide metabolites in plasma by HPLC-MS/MS
Report:	Kaussmann, M.; 2016; 01486; M-556577-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1 of November 16, 2010 European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, July 11, 2000
Deviations:	none
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

The purpose of such analytical method for analysis of the active substance and relevant metabolites is the detection of intoxications in humans and animals or for biomonitoring purposes.
While with SANCO/825/00 rev. 8.1 a method was only required if the active substance or a relevant metabolite was classified as toxic or very toxic, or classification according to GHS as follows: Acute toxicity (Cat. 1-3), CMR (Cat. 1) or STOT (Cat. 1). Flufenacet or any of its metabolites is not classified according to those categories.
With SANTE/2020/12830 rev 1 repealing SANCO/825/00 rev 8.1 the requirement for an analytical method in body fluids is set out irrespective of the toxicological classification of the active substance.
No recommendation was provided during the EU peer review for analytes relevant for monitoring in body fluids.

The selection of the analytical targets is based on the assessment of rodent and livestock metabolism studies. For selection of the analytical targets in plasma – apart from the parent compound – flufenacet thiadone was considered to be most appropriate since it was observed in quantities of more than 80% of TRR in goat (and hen) liver, kidney and muscle.
Residues detected in the organs are transported via the blood into these organs and it is concluded that these residues are the same in blood and plasma. It was shown in the rat ADME studies that the thiadiazole moiety is quickly absorbed and reached a maximum concentration already about 2 - 4 hours after dosing.
Metabolites containing the common N-fluorophenyl-N-isopropyl moiety were not considered to be suitable markers since (i) the analytical method requires a separate hydrolysis step and is not compatible with a multi-residue method and (ii) summed quantities of those metabolites were lower than the portion of thiadone in livestock tissues even after high overdose experiments.

The method has been evaluated in the DRAR (Poland 2017).

Materials and methods
Plasma samples were deproteinized by mixing with a solution of acetonitrile/water (6/1, v/v) containing 56 mg/L ammonium acetate and 0.14 mL/L formic acid and subsequent centrifugation. An aliquot of the supernatant was analysed for flufenacet and flufenacet-thiadone using HPLCMS/MS (column Luna 5u C18(2) 100A, 150 mm length, 2.0 mm diameter,5.0 µm particle size) operating in the positive ion mode for flufenacet and in negative ion mode for flufenacet-thiadone.

Two MRM transitions were monitored for flufenacet m/z 364 → 152 for quantitation and m/z 364 → 112 for confirmation and for flufenacet- thiadone (m/z 169 → 113 for quantitation and m/z 169 → 109 for confirmation. Quantification was performed with matrix matched standards to compensate possible matrix effects.

Results and discussions

Apparent residues in control samples of flufenacet and flufenacet-thiadone were below 30% of the LOQ of 50 µg/L.

The correlation between the injected amount of flufenacet and flufenacet-thiadone and the detector response was linear for matrix-matched standards ranging from 1.5 µg/L to 75 µg/L corresponding to 15 µg/L to 750 µg/L in plasma (for flufenacet-thiadone expressed as parent equivalents).

The correlation coefficient r was 0.999 for both analytes and both MRM transitions.

Mean recoveries for each fortification level and the overall mean recovery were within the 70 - 110% range for plasma and relative standard deviations were below 20% for both MRM transitions.

Table A 43: Recovery results from method validation of flufenacet and flufenacet-thiadone using the analytical method 01486

Matrix	Fortification level (µg/L)	n	Mean recovery (%)	RSD (%)	Comment
Flufenacet (*m/z* 364 → 152) quantitation					
Plasma	50	5	101	7.0	-
	500	5	103	4.6	-
Aclonifen (*m/z* 364 → 124) confirmation					
Plasma	50	5	104	7.5	-
	500	5	103	6.0	-
Flufenacet-thiadone (*m/z* 169 → 113) quantitation					
Plasma	50	5	92	9.5	-
	500	5	99	5.1	-
Flufenacet-thiadone (*m/z* 169 → 109) confirmation					
Plasma	50	5	96	6.8	-
	500	5	99	9.0	-

Table A 44: Characteristics for the analytical method used for validation of flufenacet and flufenacet-thiadone residues in body fluids

	diflufenican
Specificity	MS is used : mass spectrum is provided blank value < 30 % LOQ
Calibration (type, number of data points)	individual calibration data in matrix matched standards are presented| calibration line equation are presented number of data points : >5 R > 0.999
Calibration range	1.5 to 75 µg/L (matrix-matched sdandards), equivalent to 15 to 750 µg/l in plasma
Assessment of matrix effects is presented	no
Limit of determination/quantification	50 µg/L

Conclusion

The analytical method 01486 was sufficiently validated for the determination of flufenacet and flufenacet-thaidone residues in plasma.

The method meets all guideline SANCO/825/00/rev.8.1 criteria to determine residues of flufenacet and flufenacet-thaidone in/on plasma at 50 µg/L. Allthough the method was validated at the higher LOQ of

0.05 mg/kg accoding to SANCO/825/00 re 8.1 the method is still considered fit for purpose.

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

A 2.1.2.3.1 Analytical method 00359/M001 for the determination of the herbicide FOE 5043 (flufenacet) in soil

A 2.1.2.3.1.1 Method validation

The study summarized below was submitted within the ongoing AIR dossier for renewal of flufenacet and will be evaluated in the frame of the re-approval. Therefore, the study is only reported and not evaluated in this dossier.

Comments of zRMS:	The residue analytical method 00359 for the determination of the herbicide FOE 5043 and its metabolite FOE 5043-alcohol, FOE 5043-oxalate and FOE 5043-sulfonic acid in soil using HPLC-MS/MS was submitted in the ongoing AIR dossier for renewal of flufenacet and was evaluated by RMS-Poland in the frame of the re-approval (RAR for Flufenacet; Vol. 3 – B.5, April 2022). Therefore, the study is only reported but not evaluated in this dossier. RMS-PL conclusion: Brumhard, B.; 2005; M-248543-01-1 is not fully applicable as analytical method for monitoring. No data on metabolites were provided by the applicant.

Reference:	**KCP 5.2.4/01**
Title:	Modification M001 of method 00359 for the determination of the herbicide FOE 5043 and its metabolite FOE 5043-alcohol, FOE 5043-oxalate and FOE 5043-sulfonic acid in soil using HPLC-MS/MS
Report:	Brumhard, B.; 2005; 00359/M001; M-248543-01-1
Authority registration No:	
Guideline(s):	Commission Directive 96/46/EC amending Council Directive 91/414/EEC European Commission Guidance Document SANCO/825/00 rev. 7 BBA Guideline: Residue Analytical Methods for Post-Registration Control Purposes
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	-
Duplication (if vertebrate study):	

Materials and methods

The original method 00359 describes the determination of flufenacet (and its metabolites, not further mentioned here) in soil by HPLC-MS/MS and provides validation data for one MRM transition. This modification M001 was prepared to provide additional validation data for flufenacet using a second MRM transition and a lower limit of quantitation (LOQ).

The MRM transition for quantitation is m/z 364 → 124 and the MRM transition for confirmation is m/z 364 → 152.

Soil samples of 30 g were extracted at ambient temperature using a shaker and 100 mL 0.1 N HCl/water 1/1 (v/v). After the soil had settled, the extract was filtered, and an aliquot of 40.0 mL was concentrated at 50°C to a volume of approx. 5 mL. The concentrated extract was quantitatively transferred to a 10 mL volumetric flask and made up to volume with 0.015% hydrochloric acid. Then an aliquot of this solution was centrifuged to remove fine particles of the soil.

Identification and quantitation of flufenacet was performed by HPLC using MS/MS detection in the multiple reaction monitoring (MRM) mode and external standards in matrix (matrix matched). The method was validated using a silt loam (Höfchen) and a sandy loam (Laacher Hof).

Results and discussions

Table A 45: Recovery results from method validation of flufenacet using the analytical method 00359/M001

Matrix	Analyte	Fortification level (µg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)
Höfchen	flufenacet m/z = 364 → 124 (quantitation)	4	84	2.6
Laacher Hof		4	65	3.1
		Overall	74	13.5
Höfchen	flufenacet m/z = 364 → 152 (confirmation)	4	87	3.4
Laacher Hof		4	70	3.8
		Overall	78	12.0

Table A 46: Characteristics for the analytical method used for validation of flufenacet residues in matrix standard solution

	flufenacet
Specificity	mass spectrum is provided blank value < 30% LOQ
Calibration (type, number of data points)	individual calibration data is presented calibration line equations are presented (1/x weigthed): m/z 124 (soil Höfchen): y = 31160.6 x, r = 0.9990 m/z 124 (soil Laacher Hof): y = 26186.4 x, r = 0.9990 m/z 152 (soil Höfchen): y = 76772.3 x, r = 0.9993 m/z 152 (soil Laacher Hof): y = 65550.2 x, r = 0.9989 number of data points: 5
Calibration range	2 – 100 µg/L (corresponding to 0.4 - 20 µg/kg sample equivalents)
Assessment of matrix effects is presented	Matrix effects were eliminated by using matrix matched standard solutions.
Limit of determination/quantification	LOQ = 4 µg/kg (in soil) LOD = 1.5 µg/kg

Conclusion

The analytical method 00359 was successfully modified to meet the current guideline requirements to determine residues of flufenacet in soil with a limit of quantitation of 4 µg/kg. The modification was named as analytical method 00359/M001.

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

A 2.1.2.4.1 Analytical method 01387 for the determination of various pesticides in drinking and surface water

A 2.1.2.4.1.1 Method validation

The study summarized below was submitted in the ongoing AIR dossier for renewal of flufenacet and will be evaluated in the frame of the re-approval. Therefore, the study is only reported but not evaluated in this dossier.

Comments of zRMS:	Analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS was submitted in the ongoing AIR dossier for renewal of flufenacet and was evaluated by RMS-Poland in the frame of the re-approval (RAR for Flufenacet; Vol. 3 – B.5, April 2022). Therefore, the study is not evaluated in this dossier. RMS-PL conclusion: The method meets all guideline criteria to determine residues of flufenacet in drinking and surface water at a limit of quantitation (LOQ) of 0.05 µg/L.

Reference:	**KCP 5.2.5/01**
Title:	Analytical method 01387 for the determination of various pesticides in drinking and surface water by HPLC-MS/MS
Report:	Krebber, R.; Braune, M.; 2013; MR-13/085; M-466732-01-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 European Commission Guidance Document SANCO/825/00 rev. 8.1 European Commission Guidance Document SANCO/3029/99 rev. 4
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods

The analytical method 01387 was developed for the determination of flufenacet in drinking and surface water by direct injection into the HPLC-MS/MS instrument without further clean-up. Identification and quantitation of flufenacet was performed by HPLC using MS/MS detection in the multiple reaction monitoring (MRM) mode and external matrix-matched standard solutions. A second MRM transition was used for confirmation. The method was validated using surface water from the river Rhine. A validation for drinking water was not necessary because the limit of quantitation for surface water is below the drinking water limit of 0.1 µg/L.

The following MRM transitions were used for quantitation and confirmation of flufenacet:

m/z 364 → *m/z* 194 (quantitation)

m/z 364 → *m/z* 152 (confirmation)

Results and discussions

Because of the direct measurement of samples, recovery rates cannot be calculated, and repeatability was calculated instead.

Table A 47: Recovery/ repeatability results from method validation of flufenacet using the analytical method 01387

Matrix	Analyte	Fortification level (µg/L) (*n* = 10)	Mean recovery (area counts)	RSD (%)
surface water	flufenacet *m/z* 364 → 194 (quantitation)	0.05	117775	3.0
		0.5	997493	1.1
	flufenacet *m/z* 364 → 152 (confirmation)	0.05	121521	2.0
		0.5	1025270	1.6

Table A 48: Characteristics for the analytical method used for validation of flufenacet residues in surface water

	flufenacet
Specificity	mass spectrum is provided blank value < 30% LOQ No signals/peaks interfering with the detection of the analyte were observed
Calibration (type, number of data points)	individual calibration data is presented calibration line equations are presented (1/x weighted): m/z 194: $y = 2.43*10^6 x + 2.06*10^4$, r = 1.00 m/z 152: $y = 2.49*10^6 x + 1.9*10^4$, r = 1.00 number of data points: 6
Calibration range	0.012 – 4.0 µg/L (corresponding to 0.015 - 5 µg/L in surface water)
Assessment of matrix effects is presented	yes The MS/MS detection of flufenacet was not affected by the matrix.
Limit of determination/quantification	LOQ = 0.05 µg/L (in drinking and surface water)

Conclusion
The method meets all guideline criteria (SANCO/825/00 rev. 8.1) to determine residues of flufenacet in drinking and surface water at a limit of quantitation (LOQ) of 0.05 µg/L and is therefore suitable as enforcement method.

A 2.1.2.4.1.2 Independent laboratory validation of method 01387

Comments of zRMS:	The analytical method 01387 for the determination of flufenacet in drinking and surface water was independly validated. The report of the study was submitted in the ongoing AIR dossier for renewal of flufenacet and was evaluated by RMS-Poland in the frame of the re-approval (RAR for Flufenacet; Vol. 3 – B.5, April 2022). Therefore, the study is not evaluated in this dossier. The method is acceptable.

Reference:	**KCP 5.2.5/02**
Title:	Independent laboratory validation of BCS analytical methods 01333 and 01387 for determination of various pesticides in surface water by Di-HPLC-MS/MS
Report:	Stanislowski, T.; 2013; P3117 G; M-470714-02-1
Authority registration No:	
Guideline(s):	Regulation (EC) No 1107/2009 Commission Regulation (EU) No 283/2013 European Commission Guidance Document SANCO/825/00 rev. 8.1 European Commission Guidance Document SANCO/3029/99 rev. 4
Deviations:	none
GLP/GEP:	yes
Acceptability:	yes
Duplication (if vertebrate study):	

Materials and methods
The analytical method 01387 was independently validated for the determination of flufenacet in drinking and surface water by direct injection into the (DI-)HPLC-MS/MS instrument without further clean-up. Identification and quantitation of flufenacet was performed by HPLC using MS/MS detection in the multiple reaction monitoring (MRM) mode and external matrix-matched standard solutions. A second MRM transition was used for confirmation. The method was validated using surface water from the river Danube.
The following MRM transitions were used for quantitation and confirmation of flufenacet:

m/z 364 → *m/z* 194 (quantitation)

m/z 364 → *m/z* 152 (confirmation)

Results and discussions

Because of the direct measurement of samples, recovery rates cannot be calculated, and repeatability was calculated instead.

Table A 49: Recovery/ repeatability results from independent laboratory validation of flufenacet using the analytical method 01387

Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean recovery (area counts)	RSD (%)
surface water	flufenacet *m/z* 364 → 194 (quantitation)	0.05	196394	1.7
		0.5	1913297	1.7
	flufenacet *m/z* 364 → 152 (confirmation)	0.05	205505	2.9
		0.5	2003163	2.6

Table A 50: Characteristics for the analytical method used for independent laboratory validation of flufenacet residues in surface water

	flufenacet
Specificity	blank value < 30% LOQ No signals/peaks interfering with the detection of the analyte were observed.
Calibration (type, number of data points)	individual calibration data is presented calibration line equations are presented (1/x weighted): m/z 194: $y = 5.11*10^6 x – 7.60*10^3$, r = 0.9998 m/z 152: $y = 5.35*10^6 x – 6.34*10^3$, r = 0.9998 number of data points: 5
Calibration range	0.012 – 0.8 µg/L (corresponding to 0.015 to 1.0 µg/L in surface water)
Assessment of matrix effects is presented	Matrix effects were eliminated by using matrix matched standard solutions.
Limit of determination/quantification	LOQ = 0.05 µg/L (in drinking and surface water)

Conclusion

The laboratory PTRL Europe performed the independent laboratory validation (ILV) of the analytical method 01387 for the determination of flufenacet in drinking and surface water as described in BCS Report M-466732-01-1. The method was shown to be selective and yield accurate and repeatable results and further fulfils the criteria for SANCO/825/00 rev. 8.1.

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

A 2.1.2.5.1 Analytical method 00410C for the determination of FOE 5043 (flufenacet) in air

A 2.1.2.5.1.1 Method validation

The study summarized below was submitted in the ongoing AIR dossier for renewal of flufenacet and will be evaluated in the frame of the re-approval. Therefore, the study is only reported and not evaluated in this dossier.

Comments of zRMS:	The analytical method 00410C for the determination of flufenacet in air was submitted in the ongoing AIR dossier for renewal of flufenacet and was evaluated by RMS-Poland in the frame of the re-approval (RAR for Flufenacet; Vol. 3 – B.5, April 2022). Therefore, the study is not evaluated in this dossier. RMS-PL conclusion: The analytical method can be considered as validated but not highly specific. This one can be accepted after providing following information: concentration levels used for the calibration, correlation coefficient and equation. The LOQ provided in analytical methods for residue in air complies with the concentration C calculated from the AOEL systemic. Proposed residue definition for air: Flufenacet, FOE Thiadone and Trifluoroacetic acid. Data on metabolites are missing.

Reference:	**KCP 5.2.6/01**
Title:	Confirmatory method for the determination of FOE 5043 in air (confirmed method: 00410)
Report:	Hellpointner, E.; 2000; 00410C; M-048783-01-1
Authority registration No:	
Guideline(s):	Commission Directive 96/46/EC
Deviations:	not specified
GLP/GEP:	yes
Acceptability:	-
Duplication (if vertebrate study):	

Materials and methods

The analytical method 00410C for the determination of flufenacet in air was developed as confirmatory method for method no. 00410. The modified method uses a cyanopropyl stationary phase for the HPLC-UV analysis of the extracts of the Tenax® adsorption tubes, instead of the reversed phase stationary phase described in the original method. No deviation from the Tenax® sampling and extraction technique described in method no. 00410 was necessary.
The method was validated using blank samples spiked with flufenacet.

Results and discussions

Individual recovery rates were already elaborated within the method validation of method 00410 (M-012833-01-2) and were already evaluated and accepted. Therefore, this information is not repeated here.

Table A 51: Characteristics for the analytical method used for validation of flufenacet residues in acetonitrile

	flufenacet
Specificity	The chromatograms of the blank sample did show a chromatographic signal at the retention time of FOE 5043 above the background noise, corresponding to about 18.5% of the signal at the LOQ.
Calibration (type, number of data points)	individual calibration data is presented calibration line equation: $y = 60.39 x – 0.6786$, $r^2 = 0.99997$ number of data points: 5
Calibration range	0.16 – 1-6 mg/L
Limit of determination/quantification	LOQ = 0.0022 mg/m^3 (in air)

Conclusion

The method 00410C is suitable as confirmatory method to confirm the results of method no. 00410 at a limit of quantitation (LOQ) of 0.0022 mg/m^3.

A 2.1.2.6 Other Studies/ Information

No new or additional studies have been submitted