FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods,

Detailed summary of the risk assessment

Product code: BAS 765 00 F

Product name(s): Daxur

Chemical active substance(s):

Mefentrifluconazole, 100 g/L
Kresoxim-methyl, 150 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: BASF

Submission date: May 2021

Finalisation date: 03/11/2021

Version history

When	What
12/2020	Initial dRR – BASF DocID 2020/2096191
02/2021	Dossier sent for evaluation to Merit Mark (PL)
05/2021	Update dRR – BASF DocID 2021/2018222
08/2021	zRMS finalised evaluation
11/2021	Evaluation after commenting period - RR

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Evaluator comments:
The text highlighted in grey was provided by the evaluator.

5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are not available for the active substance(s) and relevant impurities in the plant protection product.

~~Noticed data gaps are:~~

- ~~data gap 1~~
- ~~data gap 2~~
- ~~data gap 3~~

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions of both actives.
Noticed data gaps are: None
Mefentrifluconazole (BAS 750 F): the analytical methods developed in plant and animal matrices, soil and air, were already submitted and evaluated in the context of the Inclusion of mefentrifluconazole excepting of two new pre-authorization water methods, a new enforcement water method with its ILV (however, L0359/01 was accepted already within the evaluation of the another applicant product), and a new enforcement body fluids method (the previous data gap fulfilled), which are submitted with the current dossier. Regarding the triazole derivatives the validated pre-authorisation methods developed for 1,2,4 triazole (T; M750F001), triazole alanine (TA), triazole acetic acid (TAA) and triazole lactic acid (TLA) in plant, animal, soil and water matrices, are available as EU agreed methods.
Kresoxim-methyl (BAS 490 F): the analytical methods developed in plant, animal, soil, water and air matrices has been already evaluated and approved at EU level (RAR, revised 2010). Moreover, new residue studies (see section 7: BASF DocID 2020/2100869 and BASF DocID 2020/2093149) using HPLC-MS/MS method L0095/01 provide procedural recovery data and additional validation data for the determination of kresoxim-methyl, metabolite BF 490-2 and metabolite BF 490-9 in cereal plants, ears, and grain with an associated LOQ of 0.01 mg/kg and for cereal straw with an LOQ of 0.05 mg/kg.
Within the current dossier additional pre-authorization methods in water, new method/storage stability study of BF 490-1, BF 490-2, BF 490-9 metabolites in animal matrices as well as a new enforcement method (R0062/01) and its related ILV for the determination of BF 490-9 in matrices of animal origin, were submitted.

Commodity/crop	Mefentrifluconazole Supported/ Not supported	Kresoxim-methyl Supported/ Not supported
Plant matrices	Supported	Supported
Animal matrices	Supported	Supported
Soil	Supported	Supported
Water	Supported	Supported
Air	Supported	Supported
Body fluids	Supported	n/a

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)

The plant protection product was not a representative formulation. Analytical methods for determination of active substances, impurities and relevance of CIPAC methods in BAS 765 00 F were not evaluated as part of the EU review. Therefore, all relevant data are provided and are considered adequate.

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

This method AFL0999/01 is applicable for the determination of the content of Kresoxim-methyl (Reg.No.242009) and Mefentrifluconazole (Reg.No.5834378) in BAS 765 00 F SC-Formulation. This method is also applicable for the determination of the content of Kresoxim-methyl (Reg.No.242009) and Mefentrifluconazole (Reg.No.5834378) in suspensions of BAS 765 00 F SC-Formulation in CIPAC standard water D at use rates of 0.1% (v/v) to 10% (v/v).
The following analytical method for the determination of the active substances mefentrifluconazole and Kresoxim-methyl in the plant protection product performed on BAS 765 00 F has not previously been reviewed and is provided in support of this assessment.

Comments of zRMS:	This study contains description of an analytic method used for analysing active substances in BAS 765 00 F (SC formulation).

Reference:	CP 5.1.1/1
Report	Determination of the Active Ingredients Kresoximmethyl and Mefentrifluconazole in BAS 765 00 F and Aqueous Solutions of BAS 765 00 F by HPLC and UPLC Nemitz A. 2019 Report No. N/A BASF DocID 2019/2071131 Authority registration No.
Guideline(s):	None, no guidelines available
Deviations:	No
GLP:	No, not subject to GLP regulations
Acceptability:	Yes

Materials and methods

The samples are analyzed using liquid chromatographic procedure that employs UV detection and external calibration. The separation is achieved by reversed phase chromatography using gradient conditions with acetonitrile, formic acid and water on a HPLC and UPLC system.

U(H)PLC parameters

Column	Waters BEH Cl8 50 mm x 2.1 mm; 1.7pm or equivalent
Column temperature	40 °C
Mobile phase	A: 1000 mL water + 1mL formic acid cone. B: 1000 mL acetonitrile + 1 mL formic acid cone.
Gradient	Time [min] / A [%] / B [%] / Flow [mL/min] 0.00 / 45 / 55 / 0.9 1.00 / 45 / 55 / 0.9 1.05 / 1 / 99 / 0.9 2.00 / 1 / 99 / 0.9 2.05 / 45 / 55 / 0.9 3.00 / 45 / 55 / 0.9
Injection volume	1 µL
Detector wavelength	232 nm (Reg.No.242009 and Reg.No.5834378)
Total running time	3 min
Approximate retention times	Mefentrifluconazole (Reg. No. 5834378): 0.53 min Kresoximmethyl (Reg. No. 242009): 0.71 min

HPLC parameters

Column	Waters Xbridge Cl8 (100 mm x 4.6 mm; 3.5 pm or equivalent)
Column temperature	40 °C
Mobile phase	A: 1000 mL water + 1mL formic acid cone. B: 1000 mL acetonitrile + 1 mL formic acid cone.
Gradient	Time [min] / A [%] / B [%] / Flow [mL/min] 0.00 / 45 / 55 / 1.6 5.00 / 45 / 55 / 1.6 5.05 / 1 / 99 / 1.6 8.00 / 1 / 99 / 1.6 8.05 / 45 / 55 / 1.6 12.00 / 45 / 55 / 1.6
Injection volume	5 µL
Detector wavelength	232 nm (Reg.No.242009 and Reg.No.5834378)
Total running time	12 min
Approximate retention times	Mefentrifluconazole (Reg. No. 5834378): 2.5 min Kresoximmethyl (Reg. No. 242009): 3.5 min

Comments of zRMS:	This study presents information on validation parameters acquired from analytical method used for analysing active substances in the formulation and aqueous suspensions. All validation data meet the requirements. So, these methods are accepted for evaluation purpose in physicochemical section.

Reference:	CP 5.1.1/2
Report	Validation of the Analytical Method AFL0999/01: Determination of the Active Ingredients Kresoxim-methyl and Mefentrifluconazole in BAS 765 00 F and Aqueous Solutions of BAS 765 00 F by HPLC and UPLC Nemitz A. 2019 Report No. N/A BASF DocID 2019/2052210 Authority registration No.
Guideline(s):	OECD Principles of Good Laboratory Practice, GLP Principles of the German "Chemikaliengesetz" (Chemicals Act), Directive 2004/10/EC, EU Regulation 1107/2009, CIPAC Guidelines on method validation, SANCO/3030/99 rev.5, SANCO/3029/99, US EPA OPPTS Harmonized Test Guideline 830.1000, US EPA OPPTS Harmonized Test Guideline 830.1800, ABNT NBR 14029
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany),
Acceptability:	Yes

Validation - Results and discussions

Table 5.2- 1: Methods suitable for the determination of active substances mefentrifluconazole and kresoxim-methyl in plant protection product BAS 765 00 F

	Mefentrifluconazole (Reg. No. 5834378)		**Kresoximmethyl (Reg. No. 242009)**	
Author(s), year	Nemitz A., 2019		Nemitz A., 2019	
Principle of method	HPLC with DAD/UV-detector	U(H)PLC with DAD/UV-detector	**Principle of method**	HPLC with DAD/UV-detector
Linearity (Accuracy) (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as R) n=5	Range:45–184mg/L R: 1.0000 Slope: 0.1304 Intercept: 0.1240	Range:45–184mg/L R: 1.0000 Slope: 0.0430 Intercept: 0.0430	**Linearity (Accuracy) (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as R) n=5**	Range:45–184mg/L R: 1.0000 Slope: 0.1304 Intercept: 0.1240
Linearity (Suspensibility) (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as R) n=5	Range:36–204mg/L R: 1.0000 Slope: 0.1302 Intercept: 0.1186	Range:45–184mg/L R: 1.0000 Slope: 0.0433 Intercept: 0.0296	**Linearity (Suspensibility) (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as R) n=5**	Range:36–204mg/L R: 1.0000 Slope: 0.1302 Intercept: 0.1186
Accuracy n = 5 per each level (approx. 50%, 100%, and 150 % of the nominal concentration) (% Recovery)	99.5% 99.9% 99.3%	100.0% 100.5% 99.8%	**Accuracy n = 5 per each level (approx. 50%, 100%, and 150 % of the nominal concentration) (% Recovery)**	99.5% 99.9% 99.3%
Precision – Repeatability within one day n = 5 (%RSD)	Mean:9.26% w/w %RSD: 1.03 %RSDr[1]: 1.92 RSD Acceptable: Yes Horrat=0.54	Mean:9.30% w/w %RSD: 1.03 %RSDr[1]: 1.92 RSD Acceptable: Yes Horrat=0.54	**Precision – Repeatability within one day n = 5 (%RSD)**	Mean:9.26% w/w %RSD: 1.03 %RSDr[1]: 1.92 RSD Acceptable: Yes Horrat=0.54
Precision – Repeatability between different days n = 5 (%RSD)	Mean:9.35% w/w %RSD: 1.26 %RSDr[1]: 1.91 RSD Acceptable: Yes Horrat=0.66	Mean:9.37% w/w %RSD: 1.08 %RSDr[1]: 1.91 RSD Acceptable: Yes Horrat=0.56	**Precision – Repeatability between different days n = 5 (%RSD)**	Mean:9.35% w/w %RSD: 1.26 %RSDr[1]: 1.91 RSD Acceptable: Yes Horrat=0.66
LoQ mg/L	75	75	**LoQ mg/L**	75
Comment	Method suitable	Method suitable	**Comment**	Method suitable

[1] Acceptable spread of the applied modified Horwitz equation % RSD_r = % $2^{(1-0.5 \log C)}$ x 0.67

Table 5.2- 2: Methods suitable for the determination of active substances mefentrifluconazole and kresoxim-methyl in aqueous suspensions

	Mefentrifluconazole (Reg. No. 5834378)		**Kresoximmethyl (Reg. No. 242009)**	
Author(s), year	Nemitz A., 2019		Nemitz A., 2019	
Principle of method	HPLC with DAD/UV-detector	U(H)PLC with DAD/UV-detector	HPLC with DAD/UV-detector	U(H)PLC with DAD/UV-detector
Accuracy (% Recovery) n=5 per each level	Suspensibility 10%: 100.3% Suspensibility 0.1%: 98.2%	Suspensibility 10%: 99.7% Suspensibility 0.1%: 96.7%	Suspensibility 10%: 100.5% Suspensibility 0.1%: 98.2%	Suspensibility 10%: 99.8% Suspensibility 0.1%: 96.6%
Precision – Repeatability within one day n = 5 (%RSD)	Suspensibility 10% %RSD: 0.32 %RSD[1)] expected:≤20 RSD Acceptable: Yes Suspensibility 0.1% %RSD: 0.93 %RSD[1)] expected:≤20 RSD Acceptable: yes	Suspensibility 10% %RSD: 0.27 %RSD[1)] expected:≤20 RSD Acceptable: Yes Suspensibility 0.1% %RSD: 1.03 %RSD[1)] expected:≤20 RSD Acceptable: yes	Suspensibility 10% %RSD: 0.37 %RSD[1)] expected:≤20 RSD Acceptable: Yes Suspensibility 0.1% %RSD: 0.80 %RSD[1)] expected:≤20 RSD Acceptable: yes	Suspensibility 10% %RSD: 0.32 %RSD[1)] expected:≤20 RSD Acceptable: Yes Suspensibility 0.1% %RSD: 1.05 %RSD[1)] expected:≤20 RSD Acceptable: yes
Comment	Method suitable	Method suitable	Method suitable	Method suitable

[1)] SANCO/3029/99 rev. 4

Conclusion

With respect to the conditions described for the analytical method AFL0999/01 all validation parameters (identity, specificity, accuracy, precision, intermediate precision and stability) are acceptable for Kresoxim-methyl (Reg.No. 242009) and Mefentrifluconazole (Reg.No. 5834378). Therefore, the method is valid without restriction in the tested concentration range and is suitable for the determination of Kresoxim-methyl (Reg.No. 242009) and Mefentrifluconazole (Reg.No. 5834378) in BAS 765 00 F and in aqueous suspensions of BAS 765 00 F.

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

Determination of the relevant impurity DMF

Mefentrifluconazole contains ≤ 0.5 g/kg dimethylformamide (DMF) which is considered to be an impurity of toxicological concern (equivalent to 47.65 mg/kg DMF in the SC-formulation BAS 765 00 F).
The analytical method AFL1010/01 has been developed for the determination of dimethylformamide (Reg. No. 159267) in the SC-formulation BAS 765 00 F and has been validated.

Comments of zRMS:	Accepted. Description of the analytical method only.

Reference:	CP 5.1.1/3
Report	Determination of Dimethylformamide in Formulations containing Mefentrifluconazole (BAS 750 F) D. Frohn. 2020 Report No. N/A BASF DocID 2020/2028497 Authority registration No.
Guideline(s):	None, no guidelines available
Deviations:	No
GLP:	No, not subject to GLP regulations
Acceptability:	Yes

Materials and methods

This method is applicable to the determination of the content of Dimethylformamide (Reg.No. 159267) in Formulations containing Mefentrifluconazole (BAS 750 F).
The samples are analysed using a gas chromatographic procedure that employs external standard. The separation is achieved by using gradient conditions for detection and quantification. A RTX-200 column or equivalent is used. The analyses are detected using a MS detector and quantified by comparing the specific response ratio of the sample with those of the standard of known quality.

GC parameters

Column	Rtx-200, 30m x 0.32mm, 1.5 µm
Injector temperature	250 °C
Detector temperature	250 °C
Oven temperature	160 °C; hold for 5 min.; to 250 °C at 30 °C/min.; hold for 4 min.
Carrier gas	Helium
Detector	MSD
Split ratio	10:1
Column flow	1.5 mL/min. (constant flow)
Injection volume	1.5 □L
Source temperature	230 °C
Quad Temperature	150 °C
Analysis time	12 min.
Solvent delay	3 min.
MS off	After 5 min.

Detection: MS detector

Target compound	**Retention time [min]**	**m/z [quantifier]**	**m/z [quantifier]**
Reg.No. 159267	4.0	73	44

Comments of zRMS:	The method is validated and accepted for analysing Dimethylformamide in the PPP.

Reference:	CP 5.1.1/4
Report	Validation of the Analytical Methode AFL1010/01: "Determination of Dimethylformamide in Formulations containing Mefentrifluconazole (BAS 750 F)"
	D. Frohn, 2020
	Report No.
	BASF DocID 2020/2032727
	Authority registration No.
Guideline(s):	CIPAC Guidelines on method validation, SANCO/3030/99 rev. 5 (22 March 2019)
Deviations:	No
GLP:	Yes, certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbe-aufsicht, Mainz, Germany
Acceptability:	Yes

Validation - Results and discussions

Table 5.2- 3: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) BAS 765 00 F

	DMF (Reg. No. 159267) **max. 51.6 mg/L or 47.7 mg/kg in BAS 765 00 F**
Author(s), year	D. Frohn 2020
Principle of method	GC-MS
Accuracy **n=5 per each level**	Concentration 0.013% Recovery 110.8% Concentration 0.001% Recovery 90.8% Concentration 0.040% Recovery 93.8%
Precision **n=5**	The content of Dimethylformamide in BAS 765 00 F was smaller than the limit of quantification (LoQ). Therefore, the intraday precision (repeatability within one day) was determined by using the 0.001% accuracy samples and calculating the relative standard deviation of the recovery rates in %. The relative standard deviation (RSD) for the whole procedure of sample preparation and measurement of the precision in BAS 765 00 F was found to be 5.91% (limit: 7.69%). Hr = 0.77
Intermediate Precision **n=5**	The intermediate precisions (repeatability between different days) of the method was also determined by using the 0.001% accuracy level. Therefore, the results of the precision (repeatability) and five fresh prepared sample weights of the formulation, which were prepared 8 days after the precision, were compared. The %RSD of two different sets of the lowest accuracy level, which were prepared on different days, was calculated. The %RSD was found to be 7.08% (limit: 11.47%). This results to an Horrat value of Hr = 0.62.
Identity	A solution of the reference substance and a solution of the test item fortified with the reference substance were measured. The identity was confirmed.
Specificity	A solution of the reference substance, a solution of the test item, a solution of the blank formulation and a solution of the test item fortified with the reference substance were measured. No interferences were detected.
LoQ	The lowest accuracy level (0.001%) was used for the LoQ. The LoQ was defined as 0.24 mg/L.
Linearity **n=11**	Data evaluation confirmed a linear detector response at least between 0.06 mg/L and 12.89 mg/L. The correlation coefficient was found to be 0.9995.
Comment	Method suitable

Conclusion

The objective of this study was the validation of the analytical method AFL1010/01 to be used for the determination of Reg.No. 159267 in BAS 765 00 F by GC-MS in accordance to the guidelines cited above. The results of the validation report include data to confirm the linearity, the specificity, the identity, the precision, the accuracy, the intermediate precision, the limit of quantification (LoQ) and the stability of the

analytical method AFL1010/01.
These investigations have shown that the analytical conditions employed in the respective method (AFL1010/01) are suitable for these quantifications.

Determination of the relevant impurity 1,2,4-(1H)-triazole:

Mefentrifluconazole contains ≤ 1 g/kg 1,2,4-(1H)-triazole which is considered to be an impurity of toxicological concern (equivalent to 103.1 mg/L or 95.20 mg/kg 1,2,4-(1H)-triazole in the SC-formulation BAS 765 00 F).
The analytical method AFL0977/01 and AFL0977/02 have been developed for the determination of 1,2,4-(1H)-triazole (Reg. No. 87084) in the EC-formulation BAS 750 01 F. A new version of the method (AFL0977/03) was created and validated to add one new analytical procedure (Part B) and calculation for the determination of Triazole in BAS 763 00 F, and it is also validated for BAS 765 00 F as described in BASF DocID 2020/2034385. Therefore analytical method AFL0977/03 was used for the determination of Triazole in BAS 765 00 F.

Comments of zRMS:	The method is accepted.

Reference:	CP 5.1.1/5
Report	Validation of the Analytical Method AFL0977/01: Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole, Rilinger, D., 2018 Report No 869531_1 BASF DocID 2018/1144190 Authority registration No
Guideline(s):	CIPAC Guidelines on method validation, ABNT NBR 14029, EPA 830.1000, 2004/10/EC, SANCO/3030/99, EC 1107/2009, EPA 830.1800
Deviations:	No
GLP:	Yes, certified by Landesamt fuer Umwelt, Mainz, Germany
Acceptability:	Yes

Comments of zRMS:	The method is used for analyzing the impurity in EC-Formulation BAS 750 01 F.

Reference:	CP 5.1.1/6
Report	Analytical method AFL0977/02 - Determination of the impurity Reg.No. 87084 in formulations containing Mefentrifluconazole, Rilinger, D., 2019 Report No BASF DocID 2019/1009546 Authority registration No
Guideline(s):	no guidelines available
Deviations:	No
GLP:	No, not subject to GLP regulations
Acceptability:	Yes

Comments of zRMS:	This study contains data on HPLC-MS determination of Triazole (Reg.No. 87084) in BAS 763 00 F using standard addition for calculation.

Reference:	CP 5.1.1/7
Report	Additional Validation to the Analytical Method AFL0977/02: Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole, Frohn, D., 2020 Report No 885000_1 BASF DocID 2019/2044380 Authority registration No
Guideline(s):	ABNT NBR 14029, CIPAC Guidelines on method validation, US EPA OPPTS Harmonized Test Guideline 830.1000, US EPA OPPTS Harmonized Test Guideline 830.1800, SANCO/3030/99 rev. 5 (22 March 2019)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Mainz, Germany)
Acceptability:	Yes

Comments of zRMS:	Accepted LOQ is set at 2 ppm.

Reference:	CP 5.1.1/8
Report	Analytical Method AFL0977/03: Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole D. Frohn. 2020 Report No. N/A BASF DocID 2019/2070568 Authority registration No.
Guideline(s):	None, no guidelines available
Deviations:	No
GLP:	No, not subject to GLP regulations
Acceptability:	Yes

Materials and methods

This method is applicable to the determination of the impurity Reg.No. 87084 (1,2,4-(1H)-triazole) in Formulations containing Reg.No. 5834378 (Mefentrifluconazole or BAS 750 F).
The method consists of two parts for the analytical procedure with different calculation methods (part A and part B). All samples are analysed using a high performance liquid chromatograph with MS-detector.
A Synergi Polar-RP 4 µm, 150 x 4.6 mm column (or equivalent type) is used. The separation is achieved by using isocratic conditions with water, acetonitrile and formic acid for detection and quantification followed by a rinsing step with a high acetonitrile ratio and an equilibration step.
Part A: The analyte is quantified by comparing the specific response ratio of the sample with those of the standard of known quality.
Part B: The analyte is quantified by standard addition method.

HPLC parameters

Column	Synergi Polar-RP 4µm, 150 mm x 4.6 mm (or equivalent type)
Mobile phase A	1000 mL water + 1mL formic acid (100%)
Mobile phase B	1000 mL acetonitrile + 1mL formic acid (100%)
Gradient	

Time	A [%]	B [%]
0.0	95	5
5.0	95	5
5.1	1	99
10.0	1	99
10.1	95	5
15.0	95	5

Flow	1.0 mL/min (constant)
Column temperature	40 °C
Injection volume	10 □L
Detection	MS-Detection SIR-mode
MS Detection Signal	70 m/z (monoisotopic mass M + H+)
Retention time	Reg.No. 87084: approx. 2.2 minutes

Specific Waters SQD2 Tune parameters:

Cappilary (kV)	1.00
Cone (V)	50.00
RF (V)	2.50
Extractor (V)	3.00
Source Temperature (°C)	150
Desolvation Temperature (°C)	600
Cone Gas Flow (L/Hr)	50
Desolvation Gas Flow (L/Hr)	600

Comments of zRMS:	The method is validated and may be used for analysing the impurity in the PPP.

Reference:	CP 5.1.1/9
Report	Additional Validation of the Analytical Method AFL0977/03: "Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole", Frohn, D., 2020 Report No 887001_1 BASF DocID 2020/2034385 Authority registration No
Guideline(s):	CIPAC Guidelines on method validation, EPA 830.1000, EPA 830.1800, SANCO/3030/99 rev. 5 (22 March 2019)
Deviations:	No
GLP:	Yes, (certified by Landesamt fuer Umwelt, Mainz, Germany)
Acceptability:	Yes

Validation - Results and discussions

Table 5.2- 4: Methods suitable for the determination of the relevant impurity 1,2,4-(1H)-triazole in plant protection product BAS 765 00 F

	1,2,4-(1H)-triazole (Reg. No. 87084) max. 103.1 mg/L or 95.20 mg/kg in BAS 765 00 F
Author(s), year	D. Frohn, 2020;
Principle of method	HPLC-MS
Accuracy (% Recovery) n=6	The mean recovery for Reg.No. 87084 in the preparation was found to be 102.9% for the 0.0020% accuracy level, 105.0% for the 0.0050% accuracy level and 114.2% for the 0.0075.% accuracy level, calculate corresponding to the nominal weigh in concentration of the formulation (6.4 g/L).
Precision (%RSD) n=6	The precision was taken from the 0.0020% accuracy level. The relative standard deviation (RSD) for the whole procedure of sample preparation and measurement of the precision for Reg.No. 87084 in BAS 765 00 F was found to be 4.82% (limit: 6.83%). This results to an Horrat value of Hr = 0.71.
Specificity	The specificity of the method was demonstrated by analyzing typical chromatograms of the pure solvent water, the reference item, the test item, the test item fortified and the blank formulation. No interferences with the peak of the analytical substance were observed.
Linearity n=9	Data evaluation confirmed a linear detector response at least between 0.04 mg/L and 0.62 mg/L. The correlation coefficient was found to be 0.9981.
Comment	Method suitable

Conclusion

With respect to the conditions described for the analytical method AFL0977/03 all validation parameters (accuracy, precision, linearity and specificity) are acceptable. Therefore, the method is valid without restriction in the tested concentration range and is suitable for the determination of Reg.No. 87084 in BAS 765 00 F.

Determination of the relevant impurity toluene:

Mefentrifluconazole contains ≤ 1 g/kg Toluene which is considered to be an impurity of toxicological concern. Kresoximmethyl contains ≤ 1 g/kg Toluene as well. In total, BAS 765 00 F contains max. 267.9 mg/l or 247.4 mg/kg toluene as the impurity of toxicological concern.
The analytical method AFL0948/01 has been originally developed for quantitative determination of toluene (Reg. No. 4005250) in EC formulation BAS 751 05 F. Based on that the analytical method AFL0948/02 has been developed for the determination of toluene (Reg. No. 4005250) in the SC-formulation BAS 765 00 F and has been validated.

Comments of zRMS:	Accepted. The method's LOQ is set at 100 ppm.

Reference:	CP 5.1.1/10
Report	Analytical method AFL0948/01: Derterminatin of Toluene in Formulations containing Mefentrifluconazole (BAS 750 F) M. Marsch. 2017 Report No. N/A BASF DocID 2017/1077926 Authority registration No.
Guideline(s):	None, no guidelines available
Deviations:	No
GLP:	No, not subject to GLP regulations
Acceptability:	Yes

Materials and methods

This method AFL0948/01 is applicable to the determination of the content of Toluene (Reg.No. 4005250) in Formulations containing Mefentrifluconazole (BAS 750 F).
The samples are analyzed using a gas chromatographic procedure that employs external standard. The separation is achieved by using gradient conditions for detection and quantification. A RTX 200 column or equivalent is used. The analyses are detected using a MS detector and quantified by comparing the specific response ratio of the sample with those of the standard of known quality. The GC parameters described in AFL0948/01 had to be improved due to matrix effects of the test item. A bake out step was added at the end of the temperature gradient to create a new version of the method AFL0948/02 which is validated to be applicable for the determination of Reg.No. 4005250 in the test item BAS 765 00 F.

GC parameters in AFL0948/02

Column	Rtx 200, 60m x 0.32mm, 1.5 µm
Injector temperature	280 °C
Detector temperature	300 °C
Oven temperature	95 °C; hold for 3 minutes; to 280 °C at 15 °C/minute; hold for 10.7 minutes to 300 °C at 20 °C/minute; hold for 10 minutes
Carrier gas	Helium
Detector	MSD
Split ratio	10:1
Column flow	0.9 mL/minute (constant flow)
Injection volume	1.5 □L
Analysis time	37 min.
MS off	14 min.
MS Retention time	Approx.. 10.1 min.

Detection: MS detector

Target compound	**Retention time [min]**	**m/z [quantifier]**	**m/z [quantifier]**
Reg.No. 4005250	approx. 10.1	91	92

Comments of zRMS:	Accepted. This study contains validation data on method used for analyzing toluene in BAS 751 05 F (EC formulation)

Reference:	CP 5.1.1/11
Report	Validation of the analytical method AFL0948/01: Determination of Toluene in formualtions containing Mefentrifluconazole (BAS 750 F), Harsch, M., 2017 Report No 844268_1 BASF DocID 2017/1078235 Authority registration No
Guideline(s):	2004/10/EC, ABNT NBR 14029, CIPAC Guidelines on method validation, EC 1107/2009, EPA 830.1000, EPA 830.1800, SANCO/3030/99
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Mainz, Germany)
Acceptability:	Yes

Comments of zRMS:	Accepted. The method is validated and may be applied for analysing toluene in the PPP (BAS 765 00 F). Linearity was tested using 5 samples in range from 0.058 to 0.417 mg/l.

Reference:	CP 5.1.1/12
Report	Additional Validation to the Analytical Method AFL0948/01: Determination of Toluene in Formulations containing Mefentrifluconazole (BAS 750 F), Frohn, D., 2020 report No 884516_1 2019/2048576 Authority registration No
Guideline(s):	2004/10/EC, ABNT NBR 14029, CIPAC 3807, EC 1107/2009, EPA 830.1000, EPA 830.1800, SANCO/3030/99 rev. 5 (22 March 2019)
Deviations:	No
GLP:	Yes, (certified by Landesamt fuer Umwelt, Mainz, Germany),
Acceptability:	Yes

Validation - Results and discussions

Table 5.2- 5: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) BAS 765 00 F

	Toluene (Reg. No. 4005250) **max. 267.9 mg/l or 247.4 mg/kg in BAS 765 00 F**
Author(s), year	D. Frohn 2020
Principle of method	GC-MS
Accuracy **n=5**	The mean recovery rate at all concentration levels (0.010%, and 0.015%) was found to be in the range between 107.8% and 112.5%,
Precision **n=5**	The precision was determined by analyzing five individual sample weights of the formulation BAS 765 00 F and calculating the Horrat value. The relative standard deviation (RSD) was found to be 4.96%. The Horrat value was found to be 1.0.
Specificity	The specificity of the method was demonstrated by analyzing typical chromatograms of the pure solvent acetonitrile, the reference item, the test item, the blank formulation and the blank formulation fortified. No interferences with the peak of the analytical substance were observed.
Comment	Method suitable

Conclusion

The analytical method AFL0948/01 was developed and validated for the determination of Reg.No. 4005250 in Formulations containing Mefentrifluconazole.
With respect to the conditions described in the analytical method AFL0948/01 the validation parameters are acceptable with the addition of a bake out step at the end of the temperature gradient. Therefore, the method is valid. A new version of the method (AFL0948/02) was created.
This additional validation confirms that the analytical method AFL0948/02 is applicable for the determination of Reg.No. 4005250 in the test item BAS 765 00 F.

Determination of the relevant impurities Methanol and Methylchloride

Kresoximmethyl contains ≤ 5 g/kg methanol which is considered to be an impurity of toxicological concern (equivalent to max. 824.2 mg/L or 761.03 mg/kg methanol in the SC-formulation BAS 765 00 F).
Kresoximmethyl contains also ≤ 1 g/kg methylchloride which is considered to be an impurity of toxicological concern (equivalent to max. 164.8 mg/L or 152.17 mg/kg methylchloride in the SC-formulation BAS 765 00 F).

The analytical method AM/01445/01e (AFL1013/01) has been developed for the determination of methanol and methylchloride in the SC-formulation BAS 765 00 F and has been validated.

Comments of zRMS:	This method is validated and can be used for analysing methanol and methylchloride in the PPP

Reference:	CP 5.1.1/13
Report	Determination of Methanol and Methylchloride in Formulations of the Active Ingredient Kresoximmethyl Report No. 862259_1 BASF DocID 2020/2034265 Authority registration No.
Guideline(s):	• OECD Principles of Good Laboratory Practice • GLP Principles of the German "Chemikaliengesetz" (Chemicals Act) • EC Guideline SANCO/3030/99 rev.5
Deviations:	No
GLP:	Yes, (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Materials and methods

This newly developed method AM/01445/01e (AFL1013/01) is applicable to the determination of the content of methanol and methylchloride in formulations containing Kresoxim-methyl (BAS 490 F).
The samples are analysed using a headspace gas chromatography - mass spectrometry (HSGC-MS), quantified with standard addition.
Because both analytes, methanol and methylchloride, are dissolved in solvent N,N-Dimethylacetamide (DMAA), the system suitability has to be tested prior to the analysis of samples. The system suitability was tested prior to the analysis of the samples with DMAA containing approx. 4 mg/kg of methylchloride and approx. 24 mg/kg of methanol.
The system is suitable if methylchloride and methanol can be separated from each other (and form other components of the mixture like impurities) with R >= 1.5 and S/N >= 10 for both analytes.

HSGC-MS parameters in AM/01445/01e (AFL1013/01)

Headspace conditions		
Equilibration temperature		90 °C
Equilibration time		60 min
Tranfer line temperature		150 °C
Pressurization time		1.0 min
Initial pressure		115 kPa
Final headspace pressure		180 kPa
Injection time		6 s
GC conditions		
Column	Fused silica capillary	CP Sil 8 CB
	Length	50 m
	Internal diameter	0.32 mm
	Film thickness	5 µm
Carrier gas (Helium)	Column head pressure	0.65 bar
	Split	50 ml/min
	Septum purge	3 ml/min
Temperatures	Injector	150 °C
	Oven	40 °C, isothermal for 10 min 40 °C → 130 °C, 8 K/min 130 °C → 240 °C, 25 K/min 240 °C, isothermal for 10 min
	MS transfer line	240 °C
MS conditions		
Ionization mode		EI
Acquisition mode		SIM
Multiplier voltage		2644 V
Ion source temperature		230 °C
Quadrupol temperature		150 °C
Methanol ion in SIM		m/z 31, m/z 32, m/z 29
Methylchloride ion in SIM		m/z 50, m/z 52, m/z 35

Validation - Results and discussions

Table 5.2- 6: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) BAS 765 00 F

<table>
<tr><td></td><td>Methanol (max. 824.2 mg/L or 761.03 mg/kg in BAS 765 00 F)
and
Methylchloride (max. max. 164.8 mg/L or 152.17 mg/kg methylchloride in BAS 765 00 F)</td></tr>
<tr><td>Author(s), year</td><td>W. Stegmaier 2020</td></tr>
<tr><td>Principle of method</td><td>HSGC-MS</td></tr>
<tr><td>System suitability</td><td>System suitability is confirmed:
<table>
<tr><td></td><td>Experimentally determined</td><td>Required</td></tr>
<tr><td>R</td><td>2.1</td><td>≥ 1.5</td></tr>
<tr><td>S/N(Methylchloride)</td><td>248</td><td>≥ 10</td></tr>
<tr><td>S/N(Methanol)</td><td>129</td><td>≥ 10</td></tr>
</table></td></tr>
<tr><td>Selectivity and specificity</td><td>Selectivity and specificity result from the selective gas chromatgraphic separation of methylchloride and methanol from each other and from other components and the specific detection of the analytes by means of mass spectrometry based on specific molecular mass and fragmentations of the analytes.
Interferences, blank
The analytes cannot be detected in the solvent N,N-dimethylacetamide (DMAA). Hence, co-elution of interfering components in the solvent was not observed.
Confirmation of identity
<table>
<tr><td></td><td>Retention time [min]</td></tr>
<tr><td>Methylchloride</td><td>4.38</td></tr>
<tr><td>Methanol</td><td>4.74</td></tr>
</table>
Mass spectra of the analytes which were acquired corresponded to library spectra.</td></tr>
<tr><td>Linearity</td><td>Linearity was verified for both analytes without and with the influence of the sample matrix:
<table>
<tr><td></td><td colspan="2">Methanol</td><td colspan="2">Methylchloride</td></tr>
<tr><td></td><td>without matrix</td><td>with matrix</td><td>without matrix</td><td>with matrix</td></tr>
<tr><td>Number of concentration levels</td><td>13</td><td>11</td><td>12</td><td>10</td></tr>
<tr><td>Concentration range</td><td>4.0 mg/kg
to
478 mg/kg</td><td>3.65 mg/kg
to
436 mg/kg</td><td>0.41 mg/kg
to
205 mg/kg</td><td>0.37 mg/kg
to
186 mg/kg</td></tr>
</table></td></tr>
<tr><td>Limits of quantification</td><td>Peak areas of analytes of calibrations on the lowest concentration level were very large.
Therefore LOQs could only be roughly estimated:
LOQ(Methylchloride) << 1 mg/kg
LOQ(Methanol) << 4 mg/kg</td></tr>
</table>

	Methanol (max. 824.2 mg/L or 761.03 mg/kg in BAS 765 00 F) **and** **Methylchloride (max. max. 164.8 mg/L or 152.17 mg/kg methylchloride in BAS 765 00 F)**
Accuracy (Recovery) **n=6 for each fortifikation level**	Fortifications with mass fractions of methylchloride and methanol corresponding to about 80 %, 100 % and 120 % of the concentrations indicated in the specification were determined with mean recoveries in the range of 83.6 to 92.1 %.
Precision (Repeatability) **n=6**	Precicion was verified with Horwitz ratio values well below 1 determined for methylchloride (H_r=0.20) and methanol (H_r=0.82).
Comment	Method suitable

Conclusion

The analytical method AM/01445/01e (AFL1013/01) was developed and validated for the determination of methanol and methylchloride in Formulations containing Kresoxim-methyl (BAS 490 F).
With respect to the conditions described in the analytical method AM/01445/01e (AFL1013/01), this method is applicable for the determination of methanol and methylchloride in the test item BAS 765 00 F.

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

Under current EU legislation, analytical methods for the determination of formulants are not required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There is no CIPAC method available for the simultaneous determination of mefentrifluconazole and kresoxim-methyl in the plant protection product BAS 765 00 F.

There is no CIPAC method available for the analysis of mefentrifluconazole in technical or formulated material.

CIPAC method 568 is a RP-HPLC method for the determination of kresoxim-methyl and usable for suspension concentrates, water dispersible granules, and supo-emulsion formulations.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

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

Mefentrifluconazole

Table 5.2- 7: Validated methods for the generation of pre-authorization data for mefentrifluconazole in plant and animal matrices

Component of residue definition: plants/plant products: mefentrifluconazole **Animal/food of animal origin: mefentrifluconazole + M750F022 +fatty acid conjugates of M750F022**				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants, plant products				
Citrus- fruit Coffee - beans Dry beans Soya beans Tomato - fruit Wheat – grain, straw (Residues)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	HPLC-MS/MS UPLC-MS/MS *(parent only)*	Paula Jose W.F. de, 2015 BASF DocID 2015/3001681 Method L0076/09 EU agreed
Animal products, food of animal origin				
Cow – meat, kidney, liver, fat, milk, cream Hen – egg (Residues)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Devine C., 2015 BASF DocID 2015/1106707 Method L0272/01 EU agreed *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.2.3)*
Cow – muscle, kidney, liver, fat, milk Hen – egg (Residues)	Primary (VAL) Confirmatory method not necessary (three ions used for confirmation)	0.01 mg/kg	GC-MS *(M750F022 only)*	Heger N., Taraschewski I., 2016 BASF DocID 2015/1106706 Method L0309/01 EU agreed *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.2.3)*
Hen – egg, muscle, liver, fat (Residues)	Primary (VAL) Confirmatory method not necessary (three ions used for confirmation)	0.01 mg/kg	GC-MS *(fatty acid conjugates of M750F022 only)*	Guedez Orozco A.A.,Heger N., 2016 BASF DocID 2016/1001326 Method L0309/02 EU agreed

Table 5.2- 8: Validated methods for the generation of pre-authorization data for mefentrifluconazole in plant and animal matrices – Triazole derivative metabolites

Components on interests:	**1,2,4 triazole (T), triazole alanine (TA), triazole acetic acid (TAA) and triazole lactic acid (TLA)**			
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants, plant products				
Tomato - fruit Cucumber - fruit Lettuce - leaves Cereal – grain, straw, green plant Orange - fruit Melon – peel, fruit, pulp Sweet pepper - fruit Carrot – leaf, root Dry bean Oilseed rape Sunflower (Residues)	Primary (VAL)	0.01 mg/kg	LC-DMS/MS/MS	Class T., 2011 BASF DocID 2012/1294644 Method L0170/02 (01062) EU agreed
	Confirmatory	Different confirmatory method(s) are available. Monitoring a confirmatory mass transition (157→88 m/z, positive mode for triazolylalanine), a second LC (Hypercarb) column or monitoring in the negative ion modus (triazole acetic acid, triazole lactic acid), an additional stationary phase (e.g. Phenomenex Luna Synergi Polar-RP for 1,2,4-triazole) or multiple derivatisation with subsequent SPE clean-up were performed in former versions of the method (M001/M002 and M003). These versions were also submitted by the Triazole Derivative Metabolite Group to CRD. Therefore enough confirmatory methods are available.		
Animal products, food of animal origin				
Cow – whole milk, skimmed milk, cream, meat, liver, fat, kidney, Hen - whole egg, egg yolk, egg white (Residues)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	Billian P.,Druskus M., 2009 BASF DocID 2010/1230632 Method L0293/01 (01132) EU agreed

Table 5.2- 9: Validated methods for the generation of pre-authorization data for mefentrifluconazole in soil matrices

Component of residue definition:	**mefentrifluconazole and M750F001 (1,2,4-triazole)**			
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Soil (Environmental fate)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.002 mg/kg	LC-MS/MS *(also Reg.No 5924326)*	Studenroth S.,Lueer D, 2015 BASF DocID 2015/1039006 Report Amendment 1: 2016/1030227 Report Amendment 2: 2016/1215646 Method L0214/01 EU agreed

Table 5.2- 10: Validated methods for the generation of pre-authorization data for mefentrifluconazole and metabolites in surface water and sediment matrices

Component of residue definition: mefentrifluconazole + M750F001 (1,2,4-triazole) + M750F003 + M750F005 + M750F006 + M750F007 + M750F008				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Surface, drinking water (Environmental fate)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	30 ng/L	LC-(ESI)-MS/MS *(except 1,2,4-triazole)*	Malinsky D.S., 2016 BASF DocID 2015/7001125 Report Amendment DocID: 2016/7010048 Method D1506/01 EU agreed
Surface, drinking water (Environmental fate)	Primary (VAL) Confirmatory method not necessary (two columns used for confirmation)	0.05 ng/L	HPLC-MS/MS *(1,2,4-triazole only)*	Penning H. et al., 2013 BASF DocID 2012/1297158 Method L0199/01 EU agreed
M4-Medium, OECD-water and mixing water (Ecotoxicology)	Primary (VAL) Confirmatory method not necessary	0.001 mg/L	LC/MS (*BAS 750 F, M750F007)*	**New study** KCP 5.1/1, not peer-reviewed Ziegler G., 2017 BASF DocID 2017/1064882 Method APL0500/03
Test water (mixing water)	Primary (VAL) Confirmatory method not necessary	0.001 mg/L	HPLC-MS (BAS 750 F)	**New study** KCP 5.1/2, not peer-reviewed xxxxxxxxxxxx., 2016 BASF DocID 2016/1155889 Method APL0500/03
Tap water or M4-medium	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.1 µg/L	LC-MS/MS	**New study** KCP 5.1/3 not peer-reviewed Andre, M., 2017 BASF DocID 2017/1065621 Method L0631/01

Table 5.2- 11: Validated methods for the generation of pre-authorization data for mefentrifluconazole in air

Component of residue definition: mefentrifluconazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Air (Environmental fate)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 ng/L	LC-MS/MS	Obermann M.,Studenroth S., 2015 BASF DocID 2015/1111330 Method L0327/01 EU agreed *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.2.6)*

Kresoxim-methyl

Table 5.2- 12: Validated methods for the generation of pre-authorization data (kresoxim-methyl)

Component of residue definition: kresoxim-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants, plant products (residues, storage stability)				
Apple - fruit	Primary	0.05 mg/kg	GC-MS or GC-PND	Mackenroth C., Krotzky A.J., 1994 BASF DocID 1994/11097 Method no. 351/2 EU agreed (RAR, revised 2010) Validation of method 351/2 Mackenroth C., Krotzky A.J., 1994 BASF DocID 1994/10565 EU agreed (RAR, revised 2010) Additional data for the validation of method 351/2 Mackenroth C., Krotzky A.J., 1996 BASF DocID 1996/10794
Wheat – grain, green material and straw	Primary	0.05 mg/kg	GC-MS or GC-PND	Krotzky A.J., 1994 BASF DocID 1994/11176 Method no. 351/2 and 350/1 EU agreed (RAR, revised 2010) Validation of method 351/2 Mackenroth C., Krotzky A.J., 1994 BASF DocID 1994/10565 EU agreed (RAR, revised 2010) Additional data for the validation of method 351/2 Mackenroth C., Krotzky A.J., 1996 BASF DocID 1996/10794 Validation of method 350/1 Mackenroth C., Krotzky A.J., 1994 BASF DocID 1994/10566 EU agreed (RAR, revised 2010)

Component of residue definition: kresoxim-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Pecan	Primary	0.05 mg/kg	HPLC-UV	Thornton, J.B. 1998 BASF DocID 1997/5339 Method no. D9611 EU agreed (DAR, revised 2010) Validation of method D9611 Movassaghi, S., Thornton, J.B. 1997 BASF DocID 1997/5282
Apple – processed fractions	Primary	0.05 mg/kg	HPLC-UV	Wofford, J.T., Movassaghi, S., Riley, M.E. 1998 BASF DocID 1998/5021 Method no. 350/3 EU agreed (RAR, revised 2010) Validation of method 350/3 Rabe U., Mackenroth, C. 1996 BASF DocID 1996/10626 EU agreed (RAR, revised 2010)
Grapes Apple - fruit Apple – processed fractions	Primary	0.05 mg/kg	HPLC-UV	Movassaghi, S., Riley, M.E. 1998 BASF DocID 1998/5027 Method no. 350/3 EU agreed (RAR, revised 2010) Validation of method 350/3 Rabe U., Mackenroth, C. 1996 BASF DocID 1996/10626 EU agreed (RAR, revised 2010)
Cucumber	Primary	0.05 mg/kg	HPLC-UV	Abdel-Baky, S. Riley, M.E. 1998 BASF DocID 1998/5189 Method no. 350/3 EU agreed (RAR, revised 2010) Validation of method 350/3 Rabe U., Mackenroth, C. 1996 BASF DocID 1996/10626 EU agreed (RAR, revised 2010)

Component of residue definition: kresoxim-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Grapes Apple - fruit Apple – processed fractions	Primary	0.05 mg/kg	HPLC-UV	Jordan, J., Riley, M.E. 1999 BASF DocID 1999/5006 Method no. 350/3 EU agreed (RAR, revised 2010) Validation of method 350/3 Rabe U., Mackenroth, C. 1996 BASF DocID 1996/10626 EU agreed (RAR, revised 2010)
Wheat - grain	Primary & Confirmatory (2 mass transitions)	0.05 mg/kg	LC-MS/MS	Class, T. Senciuc, M. 2008 BASF DocID 2008/1014862 Method no. 445/0 EU agreed (RAR, revised 2010) Validation of method 445/0 Benz, A., Mackenroth, C. 2001 BASF DocID 2000/1012402 EU agreed (RAR, revised 2010)
Wheat - grain	Primary & Confirmatory (2 mass transitions)	0.05 mg/kg	LC-MS/MS	Class, T. Senciuc, M. 2009 BASF DocID 2009/1018683 Method no. 445/0 EU agreed (RAR, revised 2010) Validation of method 445/0 Benz, A., Mackenroth, C. 2001 BASF DocID 2000/1012402 EU agreed (RAR, revised 2010)
Wheat - grain Soybean Pea - dried	Primary & Confirmatory (2 mass transitions)	0.05 mg/kg	LC-MS/MS	Class, T. Senciuc, M. 2009 BASF DocID 2009/1120208 Method no. 445/0 EU agreed (RAR, revised 2010) Validation of method 445/0 Benz, A., Mackenroth, C. 2001 BASF DocID 2000/1012402 EU agreed (RAR, revised 2010

Component of residue definition: kresoxim-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Barley: Whole plant (no root) Ears Rest of plant (no roots) Grain Straw	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	Schroth, E. Martin, T. 2009 BASF DocID 2008/1090698 Method no. L0010/01 (also called method no. 445/0) EU agreed (RAR, revised 2010 Validation of method L0010/01 (445/0) Benz, A., Mackenroth, C. 2001 BASF DocID 2000/1012402 EU agreed (RAR, revised 2010
Wheat: Whole plant (no root) Ears Rest of plant (no roots) Grain Straw	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	Schroth, E. Martin, T. 2009 BASF DocID 2008/1090699 Method no. L0010/01 (also called method no. 445/0) EU agreed (RAR, revised 2010 Validation of method L0010/01 (445/0) Benz, A., Mackenroth, C. 2001 BASF DocID 2000/1012402 EU agreed (RAR, revised 2010
Wheat: Whole plant (no root) Ears Rest of plant (no roots) Grain Straw	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	Validation of method L0095/01 Class, T. 2007 BASF DocID 2007/1037728 EU agreed (RAR, revised 2010
Plants, plant products (residues, feeding study)				
Corn oil – feeding vehicle	Primary	2 mg/ml	HPLC-UV	Redgrave, V. 1994 BASF DocID 1994/10960 EU agreed (RAR, revised 2010)
Plants, plant products (stability study, stability of kresoxim-methyl in organic solvents)				
Various organic solvents	Primary	10 µg/ml	HPLC-UV	Funk, H., Mackenroth, C. 2001 BASF DocID 2000/1014856 EU agreed (RAR, revised 2010)

Component of residue definition: kresoxim-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Soil, sediment (Environmental fate)				
Soil	Primary & Confirmatory (2 mass transitions)	0.005 mg/kg	LC-MS/MS	Zangmeister W., 2008 BASF DocID 2007/1018914 Method no. L0084/01 EU agreed (RAR, revised 2010) *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.3.4)*
Soil	Primary	0.01 mg/kg	GC-ECD	Mackenroth C., Krotzky A.J., 1994 BASF DocID 1993/11572 Method no. 329 EU agreed (RAR, revised 2010)
Soil	Primary	0.01 mg/kg (sum of BAS 490F and BF 490-1 determined as BAS 490F)	GC method with ^{63}Ni-ECD (with pre-column derivatisation)	Krotzky A.J., 1994 BASF DocID 1994/10477 Method no. 325 EU agreed (RAR, revised 2010)
Sediment	Primary & Confirmatory (2 mass transitions)	0.005 mg/kg	LC-MS/MS	Zangmeister W., 2008 BASF DocID 2008/1015601 Method no. L0084/02 EU agreed (RAR, revised 2010)
Drinking water, groundwater, surface water	Primary & Confirmatory (2 mass transitions)	0.03 µg/L	LC-MS/MS	Zangmeister W., 2010 BASF DocID 2010/1007230 Method no. L0156/01 EU agreed (RAR, revised 2010) *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.3.5)*
Drinking water	Primary	0.05 µg/kg (sum of BAS 490 F and BF 490-1 determined as BAS 490 F)	GC method with ^{63}Ni-ECD (with pre-column derivatisation)	Krotzky A.J., 1994 BASF DocID 1994/10474 Method no. 323 EU agreed (RAR, revised 2010)
Drinking water, surface water	Confirmatory	0.05 µg/L	LC-MS	Staab G., 1997 BASF DocID 1997/11042 Method no. 417 EU agreed (RAR, revised 2010)

Component of residue definition: kresoxim-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Drinking water, surface water	Primary & Confirmatory (2 mass transitions)	0.5 µg/L	LC-MS/MS	Zangmeister W., 2008 BASF DocID 2008/1014887 Method no. L0112/01 EU agreed (RAR, revised 2010)
Water (Ecotoxicology)				
Onchorhynchus mykiss	Primary	0.05 ppm	HPLC-UV	Munk, R. 1992 BASF DocID 1992/10211 Method No. CP-No. 138 EU agreed (RAR, revised 2010) Amendment BASF DocID 1993/11444 Validation of method CP-No. 138 Petersen, M. 1992 BASF DocID 1992/10341
Onchorhynchus mykiss	Primary	0.5 µg/mL	GC-ECD	Graves, W.C., Swigert, J.P., Holmes, C.M., Patel, J.R. 1995 BASF DocID 1995/5167 Method no. D9209 EU agreed (RAR, revised 2010)
Lepomis macrochirus	Primary	1.44 mg/L	HPLC-UV	Munk, R. 1993 BASF DocID 1993/10483 Method No. CP-No. 138/1 EU agreed (RAR, revised 2010) Validation of method CP-No. 138/1 Petersen, M. 1993 BASF DocID 1993/10002
Lepomis macrochirus	Primary	0.5 µg/mL	GC-ECD	Graves, W.C., Swigert, J.P., Holmes, C.M., Patel, J.R. 1995 BASF DocID 1995/5168 Method no. D9209 EU agreed (RAR, revised 2010)
Cyprinus carpio	Primary	1.44 mg/L	HPLC-UV	Munk, R. 1993 BASF DocID 1993/10457 Method no. CP-No. 138/1 EU agreed (RAR, revised 2010)

Component of residue definition: kresoxim-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				Validation of method CP-No. 138/1 Petersen, M. 1993 BASF DocID 1993/10002
Cyprinodon variegatus	Primary	10 µg/L	GC-ECD	Graves, W.C., Swigert, J.P., Holmes, C.M. 1996 BASF DocID 1996/5153 Method no. D9209 EU agreed (RAR, revised 2010)
Onchorhynchus mykiss	Primary	1.44 mg/L	HPLC-UV	Munk, R. 1994 BASF DocID 1994/10921 Method no. CP-No. 138/1 EU agreed (RAR, revised 2010) Validation of method CP-No. 138/1 Petersen, M. 1993 BASF DocID 1993/10002
Pimephales promelas	Primary	10 µg/L	GC-ECD	Graves, W.C., Mank, M.A., Swigert, J.P., Holmes, C.M. 1996 BASF DocID 1996/5155 Method no. D9209 EU agreed (RAR, revised 2010)
Daphnia magna	Primary	1.44 mg/L	HPLC-UV	Jatzek, H. 1993 BASF DocID 1993/10497 Method no. CP-No. 138/1 EU agreed (RAR, revised 2010) Validation of method CP-No. 138/1 Petersen, M. 1993 BASF DocID 1993/10002
Daphnia magna	Primary	5.0 µg/L	GC-ECD	Graves, W.C., Swigert, J.P., Holmes, C.M., Patel, J.R. 1995 BASF DocID 1995/5169 Method no. D9209 EU agreed (RAR, revised 2010)
Daphnia similis	Primary	9.94 mg/L	HPLC-UV	Nozarka, T. 1991 BASF DocID 1991/10710 Method no. CF-A 405/1

Component of residue definition: kresoxim-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				EU agreed (RAR, revised 2010) Validation of method CF-A 405/1 Ziegler, H. 1991 BASF DocID 1991/11422 EU agreed (RAR, revised 2010)
Mysidopsis bahia	Primary	1.0 µg/L	GC-ECD	Graves, W.C., Swigert, J.P., Holmes, C.M. 1996 BASF DocID 1996/5151 Method no. D9209 EU agreed (RAR, revised 2010)
Crassostrea virginica	Primary	0.75 µg/L	GC-ECD	Graves, W.C., Swigert, J.P., Holmes, C.M. 1996 BASF DocID 1996/5152 Method no. D9209 EU agreed (RAR, revised 2010)
Daphnia magna	Primary	1.44 mg/L	HPLC-UV	Elendt-Schneider, B. 1993 BASF DocID 1993/10335 Method no. CP-No. 138/1 EU agreed (RAR, revised 2010) Validation of method CP-No. 138/1 Petersen, M. 1993 BASF DocID 1993/10002
Daphnia magna	Primary	0.75 µg/L	GC-ECD	Graves, W.C., Mank, M.A., Swigert, J.P., Holmes, C.M. 1996 BASF DocID 1996/5154 Method no. D9209 EU agreed (RAR, revised 2010)

Component of residue definition: kresoxim-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Selenastrum capricornutum (syn. P. subcapitata)	Primary	0.5 ppb	GC-ECD	Thompson, S.G., Swigert, J.P., Jackson, S.H. 1995 BASF DocID 1995/5051 Method no. D9209 (modified) EU agreed (RAR, revised 2010)
Navicula pelliculosa	Primary	0.5 ppb	GC-ECD	Thompson, S.G., Swigert, J.P., Jackson, S.H. 1995 BASF DocID 1995/5048 Method no. D9209 EU agreed (RAR, revised 2010)
Anabaena flos-aquae	Primary	0.5 ppb	GC-ECD	Thompson, S.G., Swigert, J.P., Jackson, S.H. 1995 BASF DocID 1995/5050 Method no. D9209 EU agreed (RAR, revised 2010)
Skeletonema costatum	Primary	0.5 ppb	GC-ECD	Thompson, S.G., Swigert, J.P., Jackson, S.H. 1995 BASF DocID 1995/5054 Method no. D9209 EU agreed (RAR, revised 2010)
Outdoor mesocosm (multiple spary application)	Method CP No. 213 Primary Method CP No. 233 Primary	1.002 µg/L 1 µg/kg sediment	HPLC-UV GC-ECD	Dohmen, G.P. 1995 BASF DocID 1995/11150 Method no. CP-No. 213 & CP-No. 232 EU agreed (RAR, revised 2010) Validation of method CP-No. 213 (water) Petersen-Thiery, M. 1995 BASF DocID 1995/10916 Validation of method CP-No. 232 (sediment) Petersen-Thiery, M. 1995 BASF DocID 1995/10917
Tap water, OECD- and M4-medium	Method L0631/03 Primary	0.1 µg/L	LC-MS/MS	**New study** KCP 5.1.2/1 and 5.1.2/2, not peer-reviewed Andre, M., 2019 BASF DocID 2019/1039564 Report Amendment DocID 2020/2090916
Diet (Ecotoxicology)				
Avian diet	Primary	50 mg/kg	HPLC-UV	Munk, R. 1994

Component of residue definition: kresoxim-methyl				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				BASF DocID 1994/10877 Method no. CP-No. 069/2 EU agreed (RAR, revised 2010) Validation of method CP-No. 069/2 Petersen, M. 1991 BASF DocID 1991/10874
Stock solution (Ecotoxicology)				
Apis mellifera (larvae)	Primary	8414 mg/L	HPLC-UV	**New Study** KCP 5.1.2/3 Kleebaum, K. 2015 BASF DocID 2014/1111118

Table 5.2- 13: Validated methods for the generation of pre-authorization data (kresoxim-methyl metabolite BF 490-1)

Component of residue definition: BF 490-1				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants, plant products (residues, storage stability)				
Wheat – grain, green material and straw	Primary	0.05 mg/kg	GC-MS or GC-PND	Krotzky A.J., 1994 BASF DocID 1994/11176 Method no. 351/2 and 350/1 EU agreed (RAR, revised 2010) Validation of method 351/2 Mackenroth C., Krotzky A.J., 1994 BASF DocID 1994/10565 EU agreed (RAR, revised 2010) Additional data for the validation of method 351/2 Mackenroth C., Krotzky A.J., 1996 BASF DocID 1996/10794 Validation of method 350/1 Mackenroth C., Krotzky A.J., 1994 BASF DocID 1994/10566 EU agreed (RAR, revised 2010)
Wheat: Whole plant (no root) Ears Rest of plant (no roots) Grain Straw	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	Validation of method L0095/01 Class, T. 2007 BASF DocID 2007/1037728 EU agreed (RAR, revised 2010
Animal products, food of animal origin (Residues, feeding studies) [1]				
Liver- bovine	Primary	10 ppb	HPLC-UV	Redgrave, V. 1995 BASF DocID 1995/10960 Method no. 354/2 EU agreed (RAR, revised 2010) Validation of method no. 354/2 Maxwell, J.G. 1994 BASF DocID 1994/10959 EU agreed (RAR, revised
Kidney - bovine				
Muscle - bovine				
Subcutaneous fat - bovine				
Peritoneal fat - bovine				

Component of residue definition: BF 490-1				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				2010)
Liver- bovine	Primary	10 ppb	HPLC-UV	Maxwell, G. 1996 BASF DocID 1996/10959 Method no. 354/2 EU agreed (RAR, revised 2010) Validation of method no. 354/2 Maxwell, J.G. 1994 BASF DocID 1994/10959 EU agreed (RAR, revised 2010)
Kidney - bovine				
Muscle - bovine				
Subcutaneous fat - bovine				
Peritoneal fat - bovine				
Animal products, food of animal origin (Residues, storage stability) [1]				
Liver -	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	**New study** KCP 5.1.2/4 xxxxxxxxx. 2016 BASF DocID 2016/1235729
Kidney -				
Muscle -				
Fat -				
Soil, sediment (Environmental fate)				
Soil	Primary & Confirmatory (2 mass transitions)	0.005 mg/kg	LC-MS/MS	Zangmeister W., 2008 BASF DocID 2007/1018914 Method no. L0084/01 EU agreed (RAR, revised 2010) *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.3.4)*
Soil	Primary	0.01 mg/kg (sum of BAS 490F and BF 490-1 determined as BAS 490F)	GC method with ^{63}Ni-ECD (with pre-column derivatisation)	Krotzky A.J., 1994 BASF DocID 1994/10477 Method no. 325 EU agreed (RAR, revised 2010)
Sediment	Primary & Confirmatory (2 mass transitions)	0.005 mg/kg	LC-MS/MS	Zangmeister W., 2008 BASF DocID 2008/1015601 Method no. L0084/02 EU agreed (RAR, revised 2010)

Component of residue definition: BF 490-1				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Water (Environmental fate)				
Drinking water, groundwater, surface water	Primary & Confirmatory (2 mass transitions)	0.03 µg/L	LC-MS/MS	Zangmeister W., 2010 BASF DocID 2010/1007230 Method no. L0156/01 EU agreed (RAR, revised 2010) *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.3.5)*
Drinking water	Primary	0.05 µg/kg (sum of BAS 490 F and BF 490-1 determined as BAS 490 F)	GC method with ^{63}Ni-ECD (with pre-column derivatisation)	Krotzky A.J., 1994 BASF DocID 1994/10474 Method no. 323 EU agreed (RAR, revised 2010)
Drinking water, surface water	Confirmatory	0.05 µg/L	LC-MS	Staab G., 1997 BASF DocID 1997/11042 Method no. 417 EU agreed (RAR, revised 2010)
Drinking water, surface water	Primary & Confirmatory (2 mass transitions)	0.05 µg/L	LC-MS/MS	Zangmeister W., 2008 BASF DocID 2008/1014887 Method no. L0112/01 EU agreed (RAR, revised 2010)
Water (Ecotoxicology)				
Selenastrum capricornutum (syn. P. subcapitata)	Primary	0.5 ppb	GC-ECD	Thompson, S.G., Swigert, J.P., Jackson, S.H. 1995 BASF DocID 1995/5051 Method no. D9209 (modified) EU agreed (RAR, revised 2010)
Navicula pelliculosa	Primary	0.5 ppb	GC-ECD	Thompson, S.G., Swigert, J.P., Jackson, S.H. 1995 BASF DocID 1995/5048 Method no. D9209 EU agreed (RAR, revised 2010)
Anabaena flos-aquae	Primary	0.5 ppb	GC-ECD	Thompson, S.G., Swigert, J.P., Jackson, S.H. BASF DocID 1995/5050 Method no. D9209 EU agreed (RAR, revised 2010)

Component of residue definition: BF 490-1				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Skeletonema costatum	Primary	0.5 ppb	GC-ECD	Thompson, S.G., Swigert, J.P., Jackson, S.H. 1995 BASF DocID 1995/5054 Method no. D9209 EU agreed (RAR, revised 2010)
Oncorhynchus mykiss	Primary	0.1 mg/L	HPLC-UV	Munk, R. BASF DocID 1994/10621 Method no. CP-No. 187 EU agreed (RAR, revised 2010) Validation of method CP-No. 187 Petersen-Thiery, M. 1993 BASF DocID 1993/11004
Daphnia magna	Primary	0.1 mg/L	HPLC-UV	Dohmen, G.P.1994 BASF DocID 1994/10622 Method no. CP-No. 186 EU agreed (RAR, revised 2010) Validation of method CP-No. 186 Petersen, M. 1992 BASF DocID 1992/11724
P. subcapitata	Primary	0.1 mg/L	HPLC-UV	Dohmen, G.P.1994 BASF DocID 1994/10616 Method no. CP-No. 186 EU agreed (RAR, revised 2010) Validation of method CP-No. 186 Petersen, M. 1992 BASF DocID 1992/11724

[1] The current residue definition for animal matrices does not include metabolite BF 490-1. Methods for the determination of BF 490-1 in animal matrices are therefore included to provide additional information only**.**

Table 5.2- 14: Validated methods for the generation of pre-authorization data (kresoxim-methyl metabolite BF 490-2)

Component of residue definition: BF 490-2				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants, plant products (residues, storage stability)				
Wheat – grain, green material and straw	Primary	0.05 mg/kg	GC-MS or GC-PND	BASF DocID 1994/11176 Method no. 351/2 and 350/1 Validation of method 350/1 Mackenroth C., Krotzky A.J., 1994 Validation of method 351/2 Mackenroth C., Krotzky A.J., 1994 BASF DocID 1994/10565 Additional data for the validation of method 351/2 Mackenroth C., Krotzky A.J., 1996 BASF DocID 1996/10794 Validation of method 350/1 Mackenroth C., Krotzky A.J., 1994 BASF DocID 1994/10566
Pecan	Primary	0.05 mg/kg	HPLC-UV	Thornton, J.B. 1998 BASF DocID 1997/5339 Method no. D9611 EU agreed (RAR, revised 2010) Validation of method D9611 Movassaghi, S., Thornton, J.B. 1997 BASF DocID 1997/5282
Apple – processed fractions	Primary	0.05 mg/kg	HPLC-UV	Wofford, J.T., Movassaghi, S., Riley, M.E. 1998 BASF DocID 1998/5021 Method no. 350/3 EU agreed (RAR, revised 2010) Validation of method 350/3 Rabe U., Mackenroth, C. 1996 BASF DocID 1996/10626
Grapes	Primary	0.05 mg/kg	HPLC-UV	Movassaghi, S., Riley, M.E. 1998
Apple - fruit				

Component of residue definition: BF 490-2				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Apple – processed fractions				BASF DocID 1998/5027 Method no. 350/3 EU agreed (RAR, revised 2010) Validation of method 350/3 Rabe U., Mackenroth, C. 1996 BASF DocID 1996/10626
Cucumber	Primary	0.05 mg/kg	HPLC-UV	Abdel-Baky, S. Riley, M.E. 1998 BASF DocID 1998/5189 Method no. 350/3 EU agreed (RAR, revised 2010) Validation of method 350/3 Rabe U., Mackenroth, C. 1996 BASF DocID 1996/10626
Grapes Apple - fruit Apple – processed fractions	Primary	0.05 mg/kg	HPLC-UV	Jordan, J., Riley, M.E. 1998 BASF DocID 1999/5006 Method no. 350/3 EU agreed (RAR, revised 2010) Validation of method 350/3 Rabe U., Mackenroth, C. 1996 BASF DocID 1996/10626
Wheat - grain	Primary & Confirmatory (2 mass transitions)	0.05 mg/kg	LC-MS/MS	Class, T. Senciuc, M. 2008 BASF DocID 2008/1014862 Method no. 445/0 EU agreed (RAR, revised 2010) Validation of method 445/0 Benz, A., Mackenroth, C. 2001 BASF DocID 2000/1012402 EU agreed (RAR, revised 2010
Wheat - grain	Primary & Confirmatory (2 mass transitions)	0.05 mg/kg	LC-MS/MS	Class, T. Senciuc, M. 2009 BASF DocID 2008/1018683 Method no. 445/0 EU agreed (RAR, revised 2010) Validation of method 445/0

Component of residue definition: BF 490-2				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				Benz, A., Mackenroth, C. 2001 BASF DocID 2000/1012402 EU agreed (RAR, revised 2010
Wheat - grain Soy bean Pea - dried	Primary & Confirmatory (2 mass transitions	0.05 mg/kg	LC-MS/MS	Class, T. Senciuc, M. 2009 BASF DocID 2009/1120208 Method no. 445/0 EU agreed (RAR, revised 2010) Validation of method 445/0 Benz, A., Mackenroth, C. 2001 BASF DocID 2000/1012402 EU agreed (RAR, revised 2010
Barley: Whole plant (no root) Ears Rest of plant (no roots) Grain Straw	Primary & Confirmatory (2 mass transitions	0.01 mg/kg	LC-MS/MS	Schroth, E. Martin, T. 2009 BASF DocID 2008/1090698 Method no. L0095/01 EU agreed (RAR, revised 2010 Validation of method L0095/01 Class, T. 2007 BASF DocID 2007/1037728 EU agreed (RAR, revised 2010
Wheat: Whole plant (no root) Ears Rest of plant (no roots) Grain Straw	Primary & Confirmatory (2 mass transitions	0.01 mg/kg	LC-MS/MS	Schroth, E. Martin, T. 2009 BASF DocID 2008/1090699 Method no. L0095/01 EU agreed (RAR, revised 2010 Validation of method L0095/01 Class, T. 2007 BASF DocID 2007/1037728 EU agreed (RAR, revised 2010
Animal products, food of animal origin (Residues, feeding studies) [1]				
Whole milk - bovine Skim milk - bovine	Primary	2 ppb	HPLC-UV	Redgrave, v. 1994 BASF DocID 1994/10960 Method no. 354/1 for milk matrices and 354/2 for tissue matrices EU agreed (RAR, revised
Cream - bovine		10 ppb		

Component of residue definition: BF 490-2				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Kidney - bovine				2010) Validation of method no. 354/1 and 354/2 Maxwell, J.G. 1994 BASF DocID 1994/10959 EU agreed (RAR, revised 2010)
Muscle - bovine				
Subcutaneous fat - bovine				
Peritoneal fat - bovine				
Whole milk - bovine	Primary	2 ppb	HPLC-UV	Maxwell, G. 1995 BASF DocID 1996/10146 Method no. 354/1 for milk matrices and 354/2 for tissue matrices EU agreed (RAR, revised 2010) Validation of method no. 354/1 and 354/2 Maxwell, J.G. 1994 BASF DocID 1994/10959 EU agreed (RAR, revised 2010)
Skim milk - bovine				
Cream - bovine		10 ppb		
Kidney - bovine				
Muscle - bovine				
Subcutaneous fat - bovine				
Peritoneal fat - bovine				
Animal products, food of animal origin (Residues, storage stability) [1]				
Milk -	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	**New study** KCP 5.1.2/4 xxxxxxxx. 2016 BASF DocID 2016/1235729
Kidney -				
Muscle -				
Fat -				

[1] The current residue definition for animal matrices does not include metabolite BF 490-2. Methods for the determination of BF 490-2 in animal matrices are therefore included to provide additional information only**.**

Table 5.2- 15: Validated methods for the generation of pre-authorization data (kresoxim-methyl metabolite BF 490-5)

Component of residue definition: BF 490-5				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Soil, sediment (Environmental fate)				
Soil	Primary & Confirmatory (2 mass transitions)	0.005 mg/kg	LC-MS/MS	Zangmeister W., 2008 BASF DocID 2007/1018914 Method no. L0084/01 EU agreed (RAR, revised 2010) *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.3.4)*
Water (Environmental fate)				
Drinking water, groundwater, surface water	Primary & Confirmatory (2 mass transitions)	0.03 µg/L	LC-MS/MS	Zangmeister W., 2010 BASF DocID 2010/1007230 Method no. L0156/01 EU agreed (RAR, revised 2010) *The method is also used for monitoring purposes (see KCP 5.2 and chapter 5.3.3.5)*
Drinking water, surface water	Primary & Confirmatory (2 mass transitions)	0.05 µg/L	LC-MS/MS	Zangmeister W., 2008 BASF DocID 2008/1014887 Method no. L0112/01 EU agreed (RAR, revised 2010)
Water (Ecotoxicology)				
Daphnia magna	Primary	6.25 mg/L	HPLC-MS	Janson, G.M. 2008 BASF DocID 2008/1037017 Method no. APL0574/01 EU agreed (RAR, revised 2010)

Table 5.2- 16: Validated methods for the generation of pre-authorization data (kresoxim-methyl metabolite BF 490-9)

Component of residue definition: BF 490-9				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Plants, plant products (residues, storage stability)				
Wheat – grain, green material and straw	Primary	0.05 mg/kg	GC-MS or GC-PND	Krotzky A.J., 1994 BASF DocID 1994/11176 Method no. 351/2 and 350/1 Validation of method 350/1 Mackenroth C., Krotzky A.J., 1994 Validation of method 351/2 Mackenroth C., Krotzky A.J., 1994 BASF DocID 1994/10565 Additional data for the validation of method 351/2 Mackenroth C., Krotzky A.J., 1996 BASF DocID 1996/10794 Validation of method 350/1 Mackenroth C., Krotzky A.J., 1994 BASF DocID 1994/10566
Pecan	Primary	0.05 mg/kg	HPLC-UV	Thornton, J.B. 1998 BASF DocID 1997/5339 Method no. D9611 EU agreed (RAR, revised 2010) Validation of method D9611 Movassaghi, S., Thornton, J.B. 1997 BASF DocID 1997/5282
Apple – processed fractions	Primary	0.05 mg/kg	HPLC-UV	Wofford, J.T., Movassaghi, S., Riley, M.E. 1998 BASF DocID 1998/5021 Method no. 350/3 EU agreed (RAR, revised 2010) Validation of method 350/3 Rabe U., Mackenroth, C. 1996 BASF DocID 1996/10626

Component of residue definition: BF 490-9				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Grapes Apple - fruit Apple – processed fractions	Primary	0.05 mg/kg	HPLC-UV	Movassaghi, S., Riley, M.E. 1998 BASF DocID 1998/5027 Method no. 350/3 EU agreed (RAR, revised 2010) Validation of method 350/3 Rabe U., Mackenroth, C. 1996 BASF DocID 1996/10626
Cucumber	Primary	0.05 mg/kg	HPLC-UV	Abdel-Baky, S. Riley, M.E. 1998 BASF DocID 1998/5189 Method no. 350/3 EU agreed (RAR, revised 2010) Validation of method 350/3 Rabe U., Mackenroth, C. 1996 BASF DocID 1996/10626
Grapes Apple - fruit Apple – processed fractions	Primary	0.05 mg/kg	HPLC-UV	Jordan, J., Riley, M.E. 1998 BASF DocID 1999/5006 Method no. 350/3 EU agreed (RAR, revised 2010) Validation of method 350/3 Rabe U., Mackenroth, C. 1996 BASF DocID 1996/10626
Wheat - grain	Primary & Confirmatory (2 mass transitions)	0.05 mg/kg	LC-MS/MS	Class, T. Senciuc, M. 2008 BASF DocID 2008/1014862 Method no. 445/0 EU agreed (RAR, revised 2010) Validation of method 445/0 Benz, A., Mackenroth, C. 2001 BASF DocID 2000/1012402 EU agreed (RAR, revised 2010

Component of residue definition: BF 490-9				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Wheat - grain	Primary & Confirmatory (2 mass transitions)	0.05 mg/kg	LC-MS/MS	Class, T. Senciuc, M. 2009 BASF DocID 2008/1018683 Method no. 445/0 EU agreed (RAR, revised 2010) Validation of method 445/0 Benz, A., Mackenroth, C. 2001 BASF DocID 2000/1012402 EU agreed (RAR, revised 2010
Wheat - grain Soybean Pea - dried	Primary & Confirmatory (2 mass transitions)	0.05 mg/kg	LC-MS/MS	Class, T. Senciuc, M. 2009 BASF DocID 2009/1120208 Method no. 445/0 EU agreed (RAR, revised 2010) Validation of method 445/0 Benz, A., Mackenroth, C. 2001 BASF DocID 2000/1012402 EU agreed (RAR, revised 2010
Barley: Whole plant (no root) Ears Rest of plant (no roots) Grain Straw	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	Schroth, E. Martin, T. 2009 BASF DocID 2008/1090698 Method no. L0095/01 EU agreed (RAR, revised 2010 Validation of method L0095/01 Class, T. 2007 BASF DocID 2007/1037728 EU agreed (RAR, revised 2010
Wheat: Whole plant (no root) Ears Rest of plant (no roots) Grain Straw	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	Schroth, E. Martin, T. 2009 BASF DocID 2008/1090699 Method no. L0095/01 EU agreed (RAR, revised 2010 Validation of method L0095/01 Class, T. 2007 BASF DocID 2007/1037728 EU agreed (RAR, revised 2010

Component of residue definition: BF 490-9				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Animal products, food of animal origin (Residues, feeding studies)				
Whole milk - bovine	Primary	2 ppb	HPLC-UV	Redgrave, v. 1994 BASF DocID 1994/10960 Method no. 354/1 for milk matrices and 354/2 for tissue matrices EU agreed (RAR, revised 2010) Validation of method no. 354/1 and 354/2 Maxwell, J.G. 1994 BASF DocID 1994/10959 EU agreed (RAR, revised 2010)
Skim milk - bovine				
Cream - bovine				
Liver - bovine		10 ppb		
Kidney - bovine				
Subcutaneous fat - bovine				
Peritoneal fat - bovine				
Whole milk - bovine	Primary	2 ppb	HPLC-UV	Maxwell, G. 1996 BASF DocID 1996/10146 Method no. 354/1 for milk matrices and 354/2 for tissue matrices EU agreed (RAR, revised 2010) Validation of method no. 354/1 and 354/2 Maxwell, J.G. 1994 BASF DocID 1994/10959 EU agreed (RAR, revised 2010)
Skim milk - bovine				
Cream - bovine				
Liver - bovine		10 ppb		
Kidney - bovine				
Subcutaneous fat - bovine				
Peritoneal fat - bovine				
Animal products, food of animal origin (Residues, storage stability)				
Milk -	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	**New study** KCP 5.1.2/4 xxxxxxxxx. 2016 BASF DocID 2016/1235729
Liver -				
Kidney -				
Fat -				

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 Mefentrifluconazole (KCP 5.2)

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

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

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Mefentrifluconazole	0.01 mg/kg	Reg. (EU) No 977/2019
Plant, high acid content		0.01 mg/kg	Reg. (EU) No 977/2019
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg	Reg. (EU) No 977/2019
Plant, high oil content		0.01 mg/kg	Reg. (EU) No 977/2019
Plant, difficult matrices (hops, spices, tea)		0.01 mg/kg	Reg. (EU) No 977/2019
Muscle	Mefentrifluconazole	0.01 mg/kg	Reg. (EU) No 977/2019
Milk		0.01 mg/kg	Reg. (EU) No 977/2019
Eggs		0.01 mg/kg	Reg. (EU) No 977/2019
Fat		0.01 mg/kg	Reg. (EU) No 977/2019
Liver, kidney		0.01 mg/kg	Reg. (EU) No 977/2019
Soil (Ecotoxicology)	Mefentrifluconazole	0.05 mg/kg	Common limit
Drinking water (Human toxicology)	Mefentrifluconazole	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Mefentrifluconazole	10 µg/L	21 d NOEC *Daphnia magna*
Air	Mefentrifluconazole	5.314 mg/L	LC_{50} inhal (NOAEL sys: 25 mg/kg bw/d)
Tissue (meat or liver)	Mefentrifluconazole	0.01 mg/L	Not classified as T / T+
Body fluids	Mefentrifluconazole + M750F015, M750F016, M750F017	0.01 mg/L	Not classified as T / T+

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

An overview on the acceptable methods and possible data gaps for analysis of mefentrifluconazole in plant matrices is given in the following tables.

Table 5.3-2: Validated methods for food and feed of plant origin

Component of residue definition: mefentrifluconazole				
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 (tomato, whole fruit)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	Klimmek S. et al., 2015 BASF DocID 2015/1106708 Method L0295/01 (QuEChERS) EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	Richter S.,Schmiedt S., 2015 BASF DocID 2015/1240004 Method L0295/01 (QuEChERS) EU agreed
High acid content (orange, whole fruit)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	Klimmek S. et al., 2015 BASF DocID 2015/1106708 Method L0295/01 (QuEChERS) EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	Richter S.,Schmiedt S., 2015 BASF DocID 2015/1240004 Method L0295/01 (QuEChERS) EU agreed
High oil content (soybeans, seeds)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	Klimmek S. et al., 2015 BASF DocID 2015/1106708 Method L0295/01 (QuEChERS) EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	Richter S.,Schmiedt S., 2015 BASF DocID 2015/1240004 Method L0295/01 (QuEChERS) EU agreed
High protein/high starch content (dry) (dry beans (seeds) / wheat (grain))	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS	Klimmek S. et al., 2015 BASF DocID 2015/1106708 Method L0295/01 (QuEChERS) EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Richter S.,Schmiedt S., 2015

Table 5.3-2: Validated methods for food and feed of plant origin

Component of residue definition: mefentrifluconazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	Confirmatory method not necessary (two mass transitions used for confirmation)			BASF DocID 2015/1240004 Method L0295/01 (QuEChERS) EU agreed

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

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Extraction efficiency of data generation method (L0076/01 equivalent to L0076/09) and multi methods (QuEChERS, DFG S 19, and SweEt) in commodities of plant origin were tested with radio-labeled residues and compared to the metabolism studies. The results were submitted in the context of annex I inclusion (BASF DocID 2014/1261057).
Not required, because:	-

Conclusion on extraction efficiency of plant matrices

Efficient extraction for the analytical method, BASF data generation method L0076/01 was confirmed by comparison of residue amounts extracted in the metabolism study with the amounts extracted according to extraction procedures of a residue analytical method.
Extraction efficiencies generally were 90% or higher for all matrices investigated, namely wheat forage (98%), wheat straw (111%), soybean green pod (102%) and grapevine grape (93%). In contrast, with the multi-methods, extraction efficiency was lower for forage (QuEChERS 80%, DFG S 19 63%, SweEt 56%), and for straw (QuEChERS 59%, DFG S 19 52%, SweEt 65%) while similar high extraction efficiency was observed for soybean green pod and grapevine grape (88% or higher).

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 mefentrifluconazole in animal matrices is given in the following tables.

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

Component of residue definition: mefentrifluconazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Devine C., 2015 BASF DocID 2015/1106707 Method L0272/01 EU agreed
		0.01 mg/kg	LC-MS/MS *(M750F022 only)*	Heger N.,Taraschewski I., 2016 BASF DocID 2015/1106706 Method L0309/01 EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Richter S.,Djedovic S., 2015 BASF DocID 2015/1240005 Method L0272/01 EU agreed
		0.01 mg/kg	LC-MS/MS *(M750F022 only)*	Bendig P.,Wabbel C., 2015 BASF DocID 2015/1240006 Method L0309/01 EU agreed
Eggs	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Devine C., 2015 BASF DocID 2015/1106707 Method L0272/01 EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Richter S.,Djedovic S., 2015 BASF DocID 2015/1240005 Method L0272/01 EU agreed

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

Component of residue definition: mefentrifluconazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Muscle	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Devine C., 2015 BASF DocID 2015/1106707 Method L0272/01 EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Richter S.,Djedovic S., 2015 BASF DocID 2015/1240005 Method L0272/01 EU agreed
Fat	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Devine C., 2015 BASF DocID 2015/1106707 Method L0272/01 EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Richter S.,Djedovic S., 2015 BASF DocID 2015/1240005 Method L0272/01 EU agreed
Kidney, liver	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Devine C., 2015 BASF DocID 2015/1106707 Method L0272/01 EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 mg/kg	LC-MS/MS *(parent only)*	Richter S.,Djedovic S., 2015 BASF DocID 2015/1240005 Method L0272/01 EU agreed

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:	Extraction efficiency of data generation and post-authorization methods (L0272/01 for BAS 750 F, L0309/01 for M750F022) in commodities of animal origin were tested with radio-labeled residues and compared to the metabolism studies. The results were submitted in the context of annex I inclusion (BASF DocID 2015/1161960).
Not required, because:	-

Conclusion on extraction efficiency of animal matrices

Comparison of residue amounts extracted in the metabolism study with the amounts extracted by the extraction procedures of a residue analytical method confirms efficient extraction for the analytical methods, method L0272/01 for BAS 750 F and L0309/01 for metabolite M750F022.
For BAS 750 F, extraction efficiencies generally were 80% or higher for most matrices (milk, cream, muscle, kidney, fat, egg yolk), and lower for liver (46%). For M750F022, extraction efficiencies generally were 90% or higher for most matrices (milk, cream, kidney, fat) and lower for egg yolk (66%), for muscle (61%) and for liver (46-50%).

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

An overview on the acceptable methods and possible data gaps for analysis of mefentrifluconazole in soil is given in the following table.

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

Component of residue definition: mefentrifluconazole			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.002 mg/kg	LC-MS/MS *(also Reg.No 5924326, 1,2,4-triazole and M750F003)*	Studenroth S.,Lueer D, 2015 BASF DocID 2015/1039006 Report Amendment 1: 2016/1030227 Report Amendment 2: 2016/1215646 Method L0214/01 EU agreed

Soil types used: Field soil LUFA 2.2 (USDA: loamy fine sand / ISO 11277: loamy sand (Ss)) and Field soil LUFA 2.3 (USDA: sandy loam, ISO 11277: silty sand (Su3))

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

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

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

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

Component of residue definition: mefentrifluconazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	30 ng/L	LC-MS/MS	**New study** KCP 5.2/1, not peer-reviewed Obermann M., 2017 BASF DocID 2017/1066523 Method L0359/01
	ILV Confirmatory method not necessary (two mass transitions used for confirmation	30 ng/L	LC-MS/MS	**New study** KCP 5.2/2, not peer-reviewed Stanislowski T., 2017, BASF DocID 2017/1066522 Method L0359/01
Surface water	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	30 ng/L	LC-MS/MS	**New study** KCP 5.2/1, not peer-reviewed Obermann M., 2017 BASF DocID 2017/1066523 Method L0359/01
	ILV Confirmatory method not necessary (two mass transitions used for confirmation	30 ng/L	LC-MS/MS	**New study** KCP 5.2/2, not peer-reviewed Stanislowski T., 2017, BASF DocID 2017/1066522 Method L0359/01
Surface, drinking water (Environmental fate)	Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	30 ng/L	LC-(ESI)-MS/MS	Malinsky D.S., 2016 BASF DocID 2015/7001125 Report Amendment DocID: 2016/7010048 Method D1506/01 EU agreed
	ILV Confirmatory method not necessary (two mass transitions used for confirmation	30 ng/L	LC-(ESI)-MS/MS	Guodong G., et al., 2016 BASF DocID 2015/7006199 Method D1506/01 EU agreed

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

An overview on the acceptable methods and possible data gaps for analysis of mefentrifluconazole in air is given in the following table.

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

Component of residue definition: mefentrifluconazole			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary (VAL) Confirmatory method not necessary (two mass transitions used for confirmation)	0.01 ng/L	LC-MS/MS	Obermann M.,Studenroth S., 2015 BASF DocID 2015/1111330 Method L0327/01 EU agreed

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

An overview on the acceptable methods and possible data gaps for analysis of mefentrifluconazole in body fluids and tissues is given in the following table. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

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

Component of residue definition: mefentrifluconazole + M750F015 + M750F016 + M750F017 (body fluids), Mefentrifluconazole (body tissues)			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary (VAL) Confirmatory method not necessary (two mass transitions)	0.01 mg/L	LC-MS/MS	Wiesner F., Breyer N., 2016, BASF DocID 2016/1148911 Method L0339/01 EU agreed
Primary (VAL) Confirmatory method not necessary (two mass transitions)	0.01 mg/L	LC-MS/MS	**New study** KCP 5.2/3, not peer-reviewed xxxxxxxxxxxxx 2019 BASF DocID 2019/1046404 Method L0339/02

Note: plasma and urine were the matrices used. In the case of the tissues see Table 5.3-4.

5.3.2.8 Other studies/ information

No further studies submitted.

5.3.3 Description of analytical methods for the determination of residues kresoxim-methyl (KCP 5.2)

In addition to already EU peer-reviewed residue analytical methods, the Applicant has developed and validated new analytical methods for the determination of all relevant analytes in animal matrices (and in addition, an independent laboratory validation is presented). The method is based on the determination of analyte BF 490-9 and not on the plant protection product itself. The methods are described in detail in Appendix 2. The methods allow the determination of the relevant analytes at the required limit of quantification in the matrix types as listed below.

Since Reg. (EU) 2019/1015, the residue definition for products of animal origin has changed and does no longer include BF 490-1. This is also confirmed by the present EU Regulation (2020/856). Therefore, studies previously submitted and related to BF 490-1 are therefore not included in this submission.

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

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addendum of August 2010) the current legal residue definition is identical. Regarding the residue definition in animal studies, in most recent EU documents (including the final revised DAR version of August 2010) it is proposed to use BF 490-1, expressed as parent. In earlier versions of the DAR, it used to be BF 490-9 for milk, expressed as kresoxim-methyl, BF 490-1 for beef, liver, kidney, muscle and fat, expressed as kresoxim-methyl and kresoxim-methyl for eggs.
In the current Reg. (EU) 2020/856 the residue definition for products of animal origin is BF 490-9, expressed as kresoxim-methyl (products of animal origin, except honey)

Table 5.3-10: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Kresoxim-methyl	0.2 mg/kg [0.01* mg/kg]	Reg. (EU) 2020/856
Plant, high acid content		0.5 mg/kg [0.01* mg/kg]	Reg. (EU) 2020/856
Plant, high protein/high starch content (dry commodities)		0.08 mg/kg [0.01* mg/kg]	Reg. (EU) 2020/856
Plant, high oil content		0.2 mg/kg [0.01* mg/kg]	Reg. (EU) 2020/856
Plant, difficult matrices (hops, spices, tea)		0.05* mg/kg	Reg. (EU) 2020/856
Muscle	BF 490-9, expressed as kresoxim-methyl (products of animal origin, except honey)	0.05* mg/kg	Reg. (EU) 2020/856
Milk		0.01* mg/kg	Reg. (EU) 2020/856
Eggs		0.05* mg/kg	Reg. (EU) 2020/856
Fat		0.05* mg/kg	Reg. (EU) 2020/856
Liver, kidney		0.05* mg/kg	Reg. (EU) 2020/856
Soil (Ecotoxicology)	Kresoxim-methyl, BF 490-1, BF 490-5	0.01 mg/kg	$LC_{50\ CORR}$ > 469 mg/kg dry soil, LC_{50} > 1000 mg/kg dry soil, LC_{50} > 1000 mg/kg dry soil (EFSA conclusion 2010)
Drinking water (Human toxicology)	Kresoxim-methyl, BF 490-1	0.03 µg/L covers the lowest aquatic endpoint as well as endpoints in view of human toxicology	general limit for drinking water applying a safety factor of 3
Surface water (Ecotoxicology)	Kresoxim-methyl, BF 490-1, BF 490-5		NOEC 6.6 µg/L (*Outdoor mesocosm*), EC_{50} 100 mg/L, EC_{50} 100 mg/L (EFSA conclusion 2010)
Air	Kresoxim-methyl	0.012 µg/m^3	based on the ADI of 0.4 mg/kg bw/d, applying an additional safety factor of 10
Tissue (meat or liver)	Kresoxim-methyl, BF 490-9	not required according to EFSA conclusion (2010)	not classified as T / T+
Body fluids		not required according to EFSA conclusion (2010)	not classified as T / T+

* indicates lower limit of analytical determination

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

An overview on the acceptable methods and possible data gaps for analysis of kresoxim-methyl in plant matrices is given in the following table. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-11: 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: kresoxim-methyl				
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 & Confirmatory	0.01 mg/kg	LC-MS/MS	Class, T. 2007 BASF DocID 2007/1037728 Method no. L0095/01 EU agreed (RAR, revised 2010)
High acid content				
High oil content				
High protein/ High starch content (dry)				
High water content	Primary & Confirmatory *(quantitation possible for 3 different mass transitions)*	0.05 mg/kg	LC-MS/MS	Benz A., Mackenroth C., 2001 BASF DocID 2000/1012402 Method no. 445/0 EU agreed (DAR, revised 2010)
High acid content				
High oil content				
High protein/ High starch content (dry)				
High water content	ILV	0.01 mg/kg	LC-MS/MS	Schwarz T., Class T., 2007 BASF DocID 2007/1018681 Method no. 445/0 EU agreed (DAR, revised 2010)
High acid content		0.01 mg/kg		
High oil content		0.05 mg/kg		
High protein/ High starch content (dry)		0.01 mg/kg		
High water content	Primary & Confirmatory *(quantitation possible for 3 fragment ions)*	0.01 mg/kg	GC-MS *(full mass spectrum in wheat metabolism study was recorded)*	Weeren R.D., Pelz S., 1999 BASF DocID 1999/11462 (DFG S19 based method – extended version) EU agreed (DAR, revised 2010)
High acid content		0.01 mg/kg		
High oil content		0.02 mg/kg		
High protein/ High starch content (dry)		0.01 mg/kg		
High water content	Primary & Confirmatory	0.01 mg/kg	LC-MS/MS	QuEChERS method
High acid content				
High oil content				
High protein/ High starch content (dry)				

Table 5.3-12: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Extraction efficiency in grapes was investigated in study BASF DocID 1995/5001 (Nelsen J., 1995) which is already peer-reviewed in the DAR (1997). Further metabolism studies on apples, wheat etc. presented in the DAR (1997) evaluate extraction efficiency.
Comment on extraction efficiency	Nelsen (1995) demonstrated that the extractability of residues with methanol was high (more than 90%). The studies on analytical method 445/0 (methanol: water:2N HCl – 70/25/5 v/v/v) removed comparable amounts of residues than the metabolism extraction scheme applied in the metabolism study. The extraction efficiency of the QuEChERS method was confirmed by the vast number of validated methods available on the EURL (European Reference Laboratories for Residues of Pesticides) Data Pool. No grown material was available to test extraction efficiency with method approach DFG S19 (Weeren R.D., Pelz S., 1999).

5.3.3.3 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 kresoxim-methyl metabolite BF 490-9 in animal matrices is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-13: Validated methods for food and feed of animal origin

Component of residue definition: BF 490-9				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary & Confirmatory (2 mass transitions)	0.01 mg/kg	LC-MS/MS	**New study** KCP 5.2/4, not EU peer-reviewed xxxxxxxxxxx 2019. Method no. R0062/01 BASF DocID 2019/2049829 + Report Amendment BASF DocID 2020/2095288
Eggs				
Muscle				
Fat				
Kidney				
Milk	ILV	0.01 mg/kg	LC-MS/MS	**New study** KCP 5.2/6, not EU peer-reviewed xxxxxxxxxxx 2019 Method no. R0062/01 BASF DocID 2019/2053784
Eggs				
Muscle				
Fat				
Kidney				

Table 5.3-14: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Extraction efficiency in goat was investigated in study BASF DocID 1996/10074 (Kirkpatrick D., 1996) which was already peer-reviewed in the RAR (2010).
Comment on extraction efficiency:	Acetonitrile:water (Kirkpatrick D., 1996) was applied as extraction method in the metabolism studies on goat. High levels of extractability were shown in kidney (>85%). No grown material was available to test extraction efficiency during method validation for R0062/01.

For any special comments or remarkable points concerning the analytical methods for food and feed of animal origin please refer to Appendix 2.

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

An overview on the acceptable methods and possible data gaps for analysis of kresoxim-methyl and its metabolites BF 490-1 and BF 490-5 in soil is given in the following table. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-15: Validated methods for soil

Component of residue definition: kresoxim-methyl, BF 490-1 and BF 490-5			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Primary & Confirmatory (2 mass transitions)	0.005 mg/kg	LC-MS/MS	Zangmeister W., 2008 BASF DocID 2007/1018914 Method no. L0084/01 EU agreed (DAR, revised 2010)

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

An overview on the acceptable methods and possible data gaps for analysis of kresoxim-methyl and its metabolites BF 490-1 and BF 490-5 in surface and drinking water is given in the following table. For the detailed valuation of new/additional studies it is referred to Appendix 2.

Table 5.3-16: Validated methods for water

Component of residue definition: kresoxim-methyl, BF 490-1 and BF 490-5				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Drinking water, groundwater, surface water	Primary & Confirmatory (2 mass transitions)	0.03 µg/L	LC-MS/MS	Zangmeister W., 2010 BASF DocID 2010/1007230 Method no. L0156/01 EU agreed (DAR, revised 2010)
	ILV	-	-	*ILV is not required since the product will be registered according to 545/2011*

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

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

Table 5.3-17: Validated methods for air

Component of residue definition: kresoxim-methyl			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Primary & Confirmatory (2 mass transitions)	1 µg/m^3	LC-MS/MS	Zangmeister W., 2008 BASF DocID 2008/1014885 Method no. L0111/01 EU agreed (DAR, revised 2010)

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

Kresoxim-methyl is not classified as toxic or highly toxic. The methods for the analysis of animal matrices described in Annex II, Document M, Section 4.3 of the Annex I Renewal dossier for kresoxim-methyl, which are based on GC-MS, HPLC-UV and GC-ECD, are also suitable for the analysis of human body fluids and tissues without modifications.

5.3.3.8 Other studies/ information

No new studies or information are submitted.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1/1	Ziegler, G.	2017	Validation of analytical method APL0500/03 for the determination of BAS 750 F (Reg.No. 5834378) and its metabolite M750F007 (Reg.No. 6003432) in M4-Medium, OECD-water and mixing water by LC/MS 2017/1064882 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1/2	xxxxxxxxxxxx	2016	BAS 750 F - Acute toxicity study in the fathead minnow (Pimephales promelas) 2016/1155889 xxxxxxxxxxxxxxxxxx yes Unpublished	Yes	BASF
KCP 5.1/3	Andre, M.	2017	Validation of BASF Method L0361/01 for the determination of pesticides in water by LC-MS/MS 2017/1065621 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/1	Nemitz, A.	2019	Analytical method AFL0999/01: Determination of the Active Ingredients Kresoxim-methyl and Mefentrifluconazole in BAS 765 00 F and Aqueous Solutions of BAS 765 00 F by HPLC and UPLC 2019/2071131 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1/2	Nemitz, A.	2019	Validation of the Analytical Method AFL0999/01: Determination of the Active Ingredients Kresoxim-methyl and Mefentrifluconazole in BAS 765 00 F and Aqueous Solutions of BAS 765 00 F by HPLC and UPLC 2019/2052210 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/3	Frohn, D.	2020	Analytical Method AFL 1010/01 - Determination of Dimethylformamide in Formulations containing Mefentrifluconazole {BAS 750 F) 2020/2028497 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
~~KCP 5.1.1/4~~	~~Nemitz, A.~~	~~2020~~	~~AFL0977/04: Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole~~ ~~2020/2037327~~ ~~BASF SE, Limburgerhof, Germany Fed.Rep.~~ ~~no~~ ~~Unpublished~~	~~No~~	~~BASF~~
KCP 5.1.1/4	Frohn D.	2020	Validation of the Analytical Methode AFL1010/01: "Determination of Dimethylformamide in Formulations containing Mefentrifluconazole (BAS 750 F)" 2020/2032727 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/5	Rilinger, D.	2018	Validation of the Analytical Method AFL0977/01: Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole 2018/1144190 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1/6	Rilinger, D.	2019	Analytical method AFL0977/02 - Determination of the impurity Reg.No. 87084 in formulations containing Mefentrifluconazole 2019/1009546 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 5.1.1/7	Frohn, D.	2020	Additional Validation to the Analytical Method AFL0977/02: Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole 2019/2044380 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/8	Frohn, D.	2020	Analytical Method AFL0977/03 - Determination of the impurity reg.No. 87084 in Formulations containing Mefentrifluconazole {BAS 750 F) 2019/2070568 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF
KCP 5.1.1/9	Frohn, D.	2020	Additional Validation of the Analytical Method AFL0977/03: "Determination of the impurity Reg.No. 87084 in Formulations containing Mefentrifluconazole" 2020/2034385 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/10	Harsch, M.	2017	Analytical method AFL0948/01 - Determination of Toluene in formulations containing Mefentrifluconazole (BAS 750 F) 2017/1077926 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1/11	Harsch, M.	2017	Validation of the analytical method AFL0948/01: Determination of Toluene in formualtions containing Mefentri-fluconazole (BAS 750 F) 2017/1078235 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/12	Frohn, D.	2020	Additional Validation to the Analytical Method AFL0948/01: Determination of Toluene in Formulations containing Mefentrifluconazole (BAS 750 F) 2019/2048576 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.1/13	Stegmaier, W.	2020	Validation of the Determination of Methanol and Methylchloride in BAS 765 00 F 2020/2034265 BASF SE - GKA Competence Center Analytics, Ludwigshafen, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.2/1	Andre, M.	2019	Validation of BASF Method L0361/02 for the Determination of Pesticides in Water by LC-MS/MS 2019/1039564 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.2/2	Andre, M.	2020	Amendment No.1 to Final Report - Validation of BASF Method L0361/03 for the Determination of Pesticides in Water by LC-MS/MS 2020/2090916 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.1.2/3	Kleebaum, K.	2015	Acute toxicity of BAS 490 02 F to honeybee larvae Apis mellifer L. under laboratory conditions (in vitro) 2014/1111118 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.2/4	xxxxxxxxxxxx	2016	Storage stability of metabolites of Kresoxim-methyl (BF 490-1, BF 490-2, BF 490-9) in animal tissues matrices under deep frozen conditions 2016/1235729 xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx yes Unpublished	No	BASF
KCP 5.2/1	Obermann, M.	2017	Validation of analytical method L0359/01 for the determination of BAS 750 F and its metabolites M750F003, M750F005, M750F006 (Reg.No.5863469), M750F007 (Reg.No.6003432) and M750F008 (Reg.No.6010286) in drinking and surface water by LC-MS/MS 2017/1066523 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/2	Stanislowski, T.	2017	Independent laboratory validation (IVL) of method L0359/01 for the determination of BAS 750 F and its metabolites M750F005, M750F006, M750F007 and M750F008 in drinking water and surface water by LC-MS/MS 2017/1066522 EAG Laboratories PTRL Europe, Ulm, Germany Fed.Rep. yes Unpublished	No	BASF
KCP 5.2/3	xxxxxxxxxxxxxxx	2019	Validation of BASF Analytical Method L0339/02 for the determination of M750F015, M750F016 and M750F017 in body fluids 2019/1046404 xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx yes Unpublished	No	BASF
KCP 5.2/4	xxxxxxxxxxxxxx	2019	Validation of analytical method R0062/01 - Method for the determination of BF 490-9 (Reg.No. 292932) in different matrices of animal origin 2019/2049829 xxxxxxxxxxxxxxxxxxxxxxxxxxxxxx yes Unpublished	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2/5	xxxxxxxxxxxxxxx	2019	Report Amendment No. 1 - Validation of analytical method R0062/01 - Method for the determination of BF 490-9 (Reg.No. 292932) in different matrices of animal origin 2020/2095288 xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx yes Unpublished	No	BASF
KCP 5.2/6	xxxxxxxxxxxxxxxxxxxx	2019	Independent Laboratory Validation of an Analytical Method for the Determination of Kresoxim-methyl Metabolite BF 490-9 in Different Matrices of Animal Origin 2019/2053784 xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx yes Unpublished	No	BASF

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

Please refer to Part A

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for Mefentrifluconazole

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

Comments of zRMS:	The validation of the LC-MS method APL500/03 has been accepted. The method is for the determination of BAS 750 F and its metabolite M750F007 in water (M4 Medium etc.). The LOQ for both analytes is defined by the lowest fortification level successfully tested, hence 0.001 mg/L. All average recovery values (mean of 5 replicates per fortification level (each of 2), analyte and matrix) were between 86% and 109%, RSD < 20%. The method meets the requirements with regard to linearity, specificity, repeatability, LOQ and recoveries and is therefore suitable to the assigned purposes – to correctly determine residues of BAS 750 F and M750F007 in water samples.

Reference:	CP 5.1/1
Report	Validation of analytical method APL0500/03 for the determination of BAS 750 F (Reg.No. 5834378) and its metabolite M750F007 (Reg.No. 6003432) in M4-Medium, OECD-water and mixing water by LC/MS, Ziegler, G., 2017 report No 838449 BASF DocID 2017/1064882 Authority registration No
Guideline(s):	EFSA Panel on Plant Prptection Products and their Residues (PPR), OECD-ENV/JM/MONO/(2007)17, SANCO/3029/99 rev. 4
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany),
Acceptability:	Yes

Principle of the Method

Samples are diluted with acetonitrile, acidified with formic acid, and analysed by LC-MS. Separation is achieved by a YMC Pro C18 column (50 mm x 3 mm, 3 µm for mefentrifluconazole and 150 mm x 4.6 mm, 3µm for M750F007) and a gradient mixture of water/formic acid (1000/1, v/v) and acetonitrile/formic acid (1000/1, v/v) at a flow rate of 0.7 mL/min. Detection is accomplished by MS measurement in ESI positive mode.

Recovery Findings

The method proved to be suitable to determine mefentrifluconazole and M750F007 in water. Samples were spiked with the analytes at LOQ and 10x LOQ. All average recovery values (mean of 5 replicates per fortification level, analyte and matrix) were between 70% and 110%. The detailed

results are given in the table below (Table A 1).

Table A 1 Results of the Method Validation for the Determination of Mefentrifluconazole and M750F007 in Water

Analyte	Matrix	Fortification Level [mg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Mefentrifluconazole	M4-Medium	0.001	5	108	1.0	108	2.2
		0.01	5	109	3.2		
	Mixing-Water	0.001	5	106	0.5	105	2.0
		0.01	5	104	2.5		
	OECD-Medium	0.001	5	103	0.7	103	0.8
		0.01	5	103	0.9		
M750F007	M4-Medium	0.001	5	86	1.4	96	1.3
		0.01	5	86	1.3		
	Mixing-Water	0.001	5	93	1.6	95	2.6
		0.01	5	97	2.0		
	OECD-Medium	0.001	5	93	1.8	96	3.9
		0.01	5	98	3.3		

RSD = Relative standard deviation

Linearity Linearity of detector response was tested using five calibration standard concentrations in the range of 0.25 ng/mL to 5 ng/mL with correlation coefficients of > 0.995. The calibration standards were prepared in water/acetonitrile/formic acid (80:20:0.1, v/v/v).

Specificity The method is specific for analysis of the test items in water. Quantification was done by reversed phase HPLC using MS detection at *m/z* 398 $[M+H]^+$ mefentrifluconazole and at *m/z* 338 $[M+H]^+$ for M750F007 and external calibration calculated from a linear regression line. The retention times of the test items in samples matched the retention times in calibration solutions. No peak interferences occurred at the retention times of mefentrifluconazole and its metabolite M750F007.

Matrix Effects Solvent standards as well as matrix-matched standards were analysed to assess potential matrix effects. As no significant matrix effects were identified, solvent-standards, prepared in water/acetonitrile/formic acid (80:20:0.1, v/v/v), were used for calibration and quantification of the analyte mefentrifluconazole and its metabolite M750F007.

Interference No significant interferences (> 30% LOQ) were observed at the appropriate retention time and using the given detector.

Limit of Quantification The method has a limit LOQ of 0.001 mg/L, corresponding to the lowest fortification level successfully tested.

Limit of Detection The method has a limit of detection (LOD) of 0.00025 mg/L, corresponding to the lowest calibration level used.

Stability Working Solutions Stability of working solutions was not determined within this validation study hence calibration solutions and matrix matched standard solutions were prepared freshly before the analytical determination.

Repeatability The relative standard deviations (RSD, %) for all fortification levels were

< 20%.

Reproducibility Reproducibility of the method was not determined within the validation study.

Conclusion It could be demonstrated that the analytical method APL500/03 fulfils the requirements with regard to linearity, specificity, repeatability, LOQ and recoveries and is therefore applicable to correctly determine residues of mefentrifluconazole and its metabolite M750F007 in M4-medium, OECD-water and mixing water with a LOQ of 0.001 mg/L.

Comments of zRMS:	The study has been accepted. In this study the analytical modified method APL0500/03 was used to determine BAS 750 F in test water. The method was validated within the current study with regard to recovery, repeatability, limit of quantification, specificity and linearity. Two fortification levels of about 0.001 mg/L and 5.0 mg/L BAS 750 F in test water with 5 replicates each cover the range of the analyzed samples. Mean recovery rates of 106 % for the lower fortification level and 103% for the higher fortification level were found. The relative standard deviation (RSD) was < 10 % for both levels investigated. Linearity was shown over a range of 0.0002 mg/L – 0.004 mg/L BAS 750 F with correlation coefficients (r) ≥ 0.99. The LOQ for BAS 750 F is 0.001 mg/L, the LOD is 0.002 mg/L. Significant peak interference was not observed in the control samples at the retention time of BAS 750 F. Blank control samples (untreated test water) were analyzed within this study. Residues were < LOQ. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4. The method applied in the study is suitable to the assigned purposes.

Reference:	CP 5.1/2
Report	BAS 750 F - Acute toxicity study in the fathead minnow (Pimephales promelas), xxxxxxxxxxxx., 2016 report No 805877 BASF DocID 2016/1155889 Authority registration No
Guideline(s):	EC 440/2008 C.1, EPA 72-1, EPA 850.1075, OECD 203
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Principle of the Method The method used for the determination of BAS 750 F in test water is based on BASF method APL0500/03 and was validated within the current study. Fortified samples and test samples were directly dissolved in 0.5 % formic acid in acetonitrile and if necessary, further diluted with a mixture of test water/acetonitrile/formic (80:20:0.1, v/v/v) into the range of the calibration solutions.
Quantification of residues of mefentrifluconazole (BAS 750 F) was done by

reversed phase UHPLC on a BEH C18 column using MS-detection at m/z 398 ($[M+H]^+$) and external calibration calculated from a linear regression line. The identity of the test item was confirmed by comparison of the mean retention time of the reference item with the mean retention time of the corresponding peak of the test item during UHPLC-MS analysis.

Recovery findings

The analytical method APL0500/03 was used and slightly modified with respect to the chromatographic conditions to determine BAS 750 F in test water. The modified method was validated with regard to recovery, repeatability, limit of quantification, linearity and specificity. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4 (11/07/2000). Mean recovery rates of 106 % for the lower fortification level (0.001 mg/L) as well as 103 % for the higher fortification level (5.0 mg/L) were found. The relative standard deviation (RSD) was <10 % for both levels investigated. This confirms the validity of the method for the determination of the test item in test water.

Table A 2: Recovery results from method validation of mefentrifluconazole using the analytical method

Matrix	Analyte	Fortification level (mg/L)	Number of Replicates	Mean recovery (%)	RSD (%)	Overall recovery [%]	RSD [%]	Comments
Test water	BAS 750 F	0.000972	2	111	0	106	4.3	Quantitation m/z 398 ([M+H]+)
			2	109	1.4			
			2	108	0			
		0.000976	2	101	1.3			
			2	101	1.3			
		4.86	2	104	0.5	103	1.8	
			2	101	0			
			2	105	0			
		4.88	2	101	0			
			2	102	0			

RSD = Relative standard deviation

Linearity

Calibration standards, ranging from 0.0002 mg/L – 0.004 mg/L, were prepared in test water/acetonitrile/formic acid mixture (80:20:0.1, v/v/v). Five calibration points were used and individual calibration data was presented. Linear correlations with coefficients r ≥0.99 were obtained, thus demonstrating satisfactory linearity.

Specificity

Significant peak interference (>30% of the LOQ) was not observed in the control samples at the retention time of BAS 750 F.

Matrix Effects

Not relevant for water matrix.

Interference

No significant interferences (> 30% LOQ) were observed at the appropriate retention time and using the given detector.

Limit of Quantification	The limit of quantification (LOQ) representing the lowest validated level with sufficient recovery and precision was 0.001 mg/L.
Limit of Detection	The limit of detection (LOD) is 0.0002 mg/L corresponding to the lowest calibration standard.
Stability Working Solutions	Stability of working solutions was not determined within this validation study hence calibration solutions and matrix matched standard solutions were prepared freshly before the analytical determination.
Extract Stability	Not relevant as no extract available (only direct dissolving of water in several solvents).
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were < 20%.
Reproducibility	Reproducibility of the method was not determined within the validation study.
Conclusion	The method uses highly specific UHPLC-MS for final determination of mefentrifluconazole with a limit of quantitation of 0.001 mg/kg. Thereby, it could be demonstrated that the method fulfils the requirements with regards to recovery, repeatability, limit of quantitation, linearity and specificity.

Comments of zRMS:	The analytical study has been accepted. The validation of the method L0361/01 has been accepted. For BAS 750 F the LOQ of 0,1 µg/L was set. 2 mass transitions were monitored for all analytes. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary. Recovery data were reported for each mass transition and matrix considered. The mean recovery values of the validation experiments over all tested analytes were between 83% and 106%, which fulfils the legal requirements for mean recovery values (RSD < 20%). Good linearity (r > 0.998) was observed in the range of 0.1 ng/mL to 3 ng/mL for both mass transitions of BAS 684 H, BAS 395 I and Prothioconazole and in the range of 0.01 ng/mL to 0.8 ng/mL for both mass transitions of the other analytes. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of the analytes. It could be demonstrated that LC-MS/MS analytical method L0361/01 fulfils the requirements with regard to specificity, linearity, repeatability, limit of quantification and recoveries and is therefore suitable to determine residues of BAS 500 F (Pyraclostrobin), BAS 510 F (Boscalid), BAS 550 F (Dimethomorph), BAS 700 F (Fluxapyroxad), **BAS 750 F (Mefentrifluconazole)**, BAS 656 H (Dimethenamid-P), BAS 684 H (Cinmethylin), BAS 720 H (Imazamox), BAS 9178 H (Clomazone), BAS 395 I (Dinotefuran), BAS 550 I (Reg. No. 5845955) and Prothioconazole in tap water and M4-medium.

Reference:	CP 5.1/3
Report	Validation of BASF Method L0361/01 for the Determination of Pesticides in Water by LC-MS/MS, Andre, M., 2017 report No EU-IF-17/04022633, EU-783160, IF-17/04022633 BASF DocID 2017/1065621

	Authority registration No
Guideline(s):	EFSA Panel on Plant Protection Products and their Residues (PPR), OECD-ENV/JM/MONO/(2007)17, SANCO/3029/99 rev. 4 (11 July 2000)
Deviations:	No
GLP:	yes (certified by Hess. Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz, Wiesbaden, Germany)
Acceptability:	Yes

Principle of the Method

A 5 g tap water or M4-medium specimen aliquot is extracted by shaking with Acetonitrile/Water/HCOOH, 400/600/2, v/v/v. An aliquot of the extract is then used for determination by LC-MS/MS. Analysis was accomplished using a Pinnacle DB AQ C18 column and a water-acetonitrile gradient with formic acid as modifier at a flow rate of 600 µL/min. Samples were analysed at mass transition *m/z* 398 → 70 for quantitation and *m/z* 398 → 182 for confirmation for mefentrifluconazole.

Recovery findings

Fortification levels of 0.1 µg/L, 1.0 µg/L and 10 µg/L were validated for BAS 750 F. Method validation acceptance criteria were fully met with mean recovery values between 94% and 106% in all matrices tested.

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

Matrix	Analyte	Fortification level (µg/L)	Number of replicates	Mean recovery [%]	RSD (%)	Overall recovery [%]	RSD (%)	Comments
Tap water	BAS 750 F	0.1	5	94	4.3	95	2.8	Mass transition m/z 398→70*
		1.0	5	96	2.0			
		10	5	97	0.6			
		0.1	5	99	5.2	96	3.9	Mass transition m/z 398→182
		1.0	5	94	2.4			
		10	5	96	1.5			
M4-medium		0.1	5	101	2.9	100	2.5	Mass transition m/z 398→70*
		1.0	5	101	2.1			
		10	5	99	2.4			
		0.1	5	106	3.9	104	3.3	Mass transition m/z 398→182
		1.0	5	105	2.3			
		10	5	101	1.8			

*used as quantification transition
RSD = Relative standard deviation

Linearity	Good linearity (r > 0.9995) was observed in the range of 0.01 ng/mL to 0.8 ng/mL for the two mass transitions of BAS 750 F At least six calibration levels, prepared as matrix matched standards, distributed over the tested concentration range were used.
Specificity	The method allows the specific determination of BAS 750 F in tap water and M4-Medium using LC-MS/MS. Detection is accomplished by high selective MS/MS-detection using two mass transitions.
Matrix Effects	The results demonstrate that the matrix-load in the tested matrix-matched standards had negligible influence on the detection. But as the matrices were used for fortification and control specimens, the matrices were used also for preparation of standard solutions.
Interference	No significant interferences (> 30% LOQ) were observed at the appropriate retention time and using the given detector.
Limit of Quantification	The limit of quantification (LOQ) representing the lowest validated level with sufficient recovery and precision was 0.1 µg/L.
Limit of Detection	The limit of detection (LOD) is 0.02 µg/L corresponding to the lowest calibration standard.
Stability Working Solutions	Stability tests confirmed that the analytes were stable for at least 28 days in calibration solutions in tap water matrix and 29 days in calibration solutions in M4-medium matrix when stored refrigerated at approximately 2 – 8 °C in the dark. For fortification solutions stability was proven for 28 days. Mean uncorrected recoveries for all analytes were in an acceptable range 85% to 110% for calibration solutions over the tested time period. As the stability was confirmed over all concentrations investigated, it can be concluded that concentration dependency is not given.
Extract Stability	The stability of specimen final volumes was not investigated during this study, as storage stability of matrix matched standards was proven and composition of matrix matched standards and specimen final volume is equal.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were < 20%.
Reproducibility	Reproducibility of the method was not determined within the validation study.
Conclusion	It could be demonstrated that analytical method L0361/01 fulfils the requirements with regard to specificity, linearity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of BAS 750 F (Mefentrifluconazole).

A 2.1.1.1.1.1 Confirmatory method

A confirmatory technique is not required since the detection by MS/MS with two characteristic mass transitions is regarded to be highly specific.

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

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

A 2.1.2.4.1 Analytical method L0359/01 for the determination of mefentrifluconazole in water

A 2.1.2.4.1.1 Method validation 1

Comments of zRMS:	The validation of analytical method L0359/01 has been accepted. Requirements of SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 were met. The analytical method L0359/01 fulfils the requirements with regard to specificity, linearity, repeatability, LOQ (0.03 µg/L) and recoveries and is therefore suitable to the assigned purposes.

Reference:	CP 5.2/1
Report	Validation, analytical method L0359/01, BAS 750 F (Reg.No.5834378) and metabolites M750F003 (Reg.No.5924326), M750F005 (Reg.No.6003433), M750F006 (Reg.No.5863469), M750F007 (Reg.No.6003432) and M750F008 (Reg.No.6010286) in drinking and surface water by LC-MS/MS Obermann, M., 2017 report No 836940 BASF DocID 2017/1066523
Guideline(s):	EPA 850.6100 (2012), SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Please note that the method was validated for mefentrifluconazole and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008. Only results for parent are presented in the summary below as this is the only compound relevant for residue definition.

Materials and methods

Residues of mefentrifluconazole (BAS 750 F) are extracted from water with ethyl acetate. An aliquot of the organic phase is evaporated to dryness using a nitrogen evaporator at 40°C and the obtained residues are reconstituted in acetonitrile/water (50/50, v/v) prior to final determination by LC-MS/MS. Analysis was accomplished using a Waters Xbridge C18 column and a water-acetonitrile gradient with formic acid as modifier at a flow rate of 800 µL/min. Samples were analysed at mass transition 398 → 70 for quantitation and 400 → 70 for confirmation for mefentrifluconazole.

Results and discussions

Method validation acceptance criteria were fully met with mean recovery values between 70% and 110% in all matrices tested. The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%. Method validation data are summarised in the table below.

Table A 4: Recovery results from method validation of mefentrifluconazole using the analytical method

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Ground water	BAS 750 F	0.03 (n=5)	95	2.1	Quantitation
		0.3 (n=5)	98	2.0	m/z 398→70
		0.03 (n=5)	96	2.7	Confirmation
		0.3 (n=5)	98	2.8	m/z 400→70
Surface water	BAS 750 F	0.03 (n=5)	103	1.3	Quantitation
		0.3 (n=5)	102	1.5	m/z 398→70
		0.03 (n=5)	101	3.2	Confirmation
		0.3 (n=5)	98	2.7	m/z 400→70

Table A 5: Characteristics for the analytical method used for validation of mefentrifluconazole residues in water

	Mefentrifluconazole
Specificity	The method L0359/01 determines residues of mefentrifluconazole in water. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered.
Calibration (type, number of data points)	Calibration standards were prepared in acetonitrile / water (50/50, v/v). Six calibration points were used and individual calibration data was presented. Linear correlations with coefficients ≥0.99 were obtained.
Calibration range	Calibration points distributed over a concentration range of 0.03 to 1 ng/mL were used. This covers the tested concentration range.
Assessment of matrix effects is presented	Solvent- as well as matrix-matched standards were analysed to assess potential matrix effects. As no significant matrix effects were identified, solvent standards, prepared in acetonitrile/water (50/50, v/v), were used for calibration and quantification of BAS 750 F.
Limit of determination/quantification	The limit of quantification (LOQ) representing the lowest validated level with sufficient recovery and precision was 0.03 µg/L.
Standard stability	BAS 750 F was stable for a maximum duration of 30 days in stock and calibration solutions, when stored refrigerated at approximately 4°C in the dark. Stock solutions were prepared in acetonitrile, while calibration solutions were prepared in acetonitrile/water (50/50, v/v). BAS 750 F was stable in final water-sample extracts, prepared in acetonitrile/water, 50/50, v/v), over a time period of

	Mefentrifluconazole
	7 days in case of surface water and 8 days in case of ground water, when stored refrigerated at approximately 4°C in the dark.

Conclusion

The method uses highly specific LC-MS/MS for final determination of mefentrifluconazole with a limit of quantitation of 0.03 µg/L. Thereby, it could be demonstrated that the method fulfils the requirements with regards to specificity, linearity, repeatability, limit of quantitation and recoveries.

A 2.1.2.4.1.2 Independent laboratory validation 1

Comments of zRMS:	The ILV of the method L0359/01 has been accepted. The method L0359/01 was successfully independently validated for BAS 750 F, M750F003, M750F005, M750F006, M750F007 and M750F008 in drinking and surface water by LC-MS/MS, demonstrating the LOQ of 0.03 µg/L. It is concluded that this method fulfils the reproducibility requirements as defined in SANCO/825/00 rev. 8.1 and is, therefore, applicable as residue and enforcement method.

Reference:	CP 5.2/2
Report	Independent laboratory validation (~~IVL~~ ILV) of method L0359/01 for the determination of BAS 750 F and its metabolites M750F005, M750F006, M750F007 and M750F008 in drinking water and surface water by LC-MS/MS Stanislowski, T., 2017 report No EU-836906,P 4262 G BASF DocID 2017/1066522
Guideline(s):	EPA 850.6100, EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 8.1 (16 November 2010)
Deviations:	No
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)
Acceptability:	Yes

Please note that the ILV was performed for mefentrifluconazole and its metabolites M750F003, M750F005, M750F006, M750F007 and M750F008. Only results for parent are presented in the summary below as this is the only compound relevant for residue definition.

Materials and methods

There were no significant deviations from the primary method.

Results and discussions

Method validation acceptance criteria were fully met with mean recovery values between 70% and 110% in all matrices tested. The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%. Method validation data are summarised in the table below.

Table A 6: Recovery results from independent laboratory validation of mefentrifluconazole using the analytical method

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Drinking water	BAS 750 F	0.03 (n=5)	87.7	5.6	Quantitation m/z 398→70
		0.3 (n=5)	102	10	
		0.03 (n=5)	92.2	2.6	Confirmation m/z 400→70
		0.3 (n=5)	108	7.4	
Surface water	BAS 750 F	0.03 (n=5)	108	7.8	Quantitation m/z 398→70
		0.3 (n=5)	108	8.3	
		0.03 (n=5)	110	2.8	Confirmation m/z 400→70
		0.3 (n=5)	108	2.3	

Table A 7: Characteristics for the analytical method used for independent laboratory validation of mefentrifluconazole residues in water

	Mefentrifluconazole
Specificity	The method L0359/01 determines residues of mefentrifluconazole in water. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered.
Calibration (type, number of data points)	Calibration standards were prepared in acetonitrile/water (50:50, v/v). Six (or three injected in at least duplicate for storage stability determination) calibration points were used and individual calibration data was presented. Linear correlations with coefficients ≥0.99 were obtained.
Calibration range	Calibration points distributed over a concentration range of 0.03 to 1 ng/mL were used. This covers the tested concentration range.
Assessment of matrix effects is presented	The matrix effect was tested for each matrix. No significant matrix effect was observed.
Limit of determination/quantification	The limit of quantification (LOQ) representing the lowest validated level with sufficient recovery and precision was 0.03 µg/L.
Standard stability	BAS 750 F indicated sufficient stability (less than 10 % difference) in stock solution (acetonitrile) for 16 days as well as in acetonitrile/water (1/1, v/v) solutions used for fortification and calibration (<20% difference for BAS 750 F) when stored refrigerated in the dark. Final sample extracts in acetonitrile/water (1/1, v/v) were re-injected after 11 (for surface water) or 15 days (for drinking water) of storage under refrigerated conditions. No

	Mefentrifluconazole
	significant decrease (80.4-98.6% of initial value) or increase (101-114% of initial value) in recovery in the stored final extracts was observed when the results were evaluated with freshly prepared calibration solutions in solvent. Thus, stability of final extracts is considered sufficiently proven for at least 11 or 15 days under refrigerated storage conditions.

Conclusion

The method uses highly specific LC-MS/MS for final determination of mefentrifluconazole with a limit of quantitation of 0.03 µg/L. Thereby, it could be demonstrated that the method fulfils the requirements with regards to specificity, linearity, repeatability, limit of quantitation and recoveries. The method is acceptable as ILV for the primary method.

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

No new or additional studies have been submitted

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

Comments of zRMS:	Validation of the analytical method L0339/02 for the determination of M750F015, M750F016 and M750F017 in body fluids using LC-MS/MS has been accepted. Requirements of SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 were met. The analytical method L0339/02 fulfils the requirements with regard to specificity, linearity, repeatability, LOQ and recoveries and is therefore suitable to the assigned purposes. Earlier, immediately after completion of the study, according to the applicant information, this 2019/1046404 study was available to be submitted by request.

Reference:	CP 5.2/3
Report	Validation of BASF Analytical Method L0339/02 for the determination of M750F015, M750F016 and M750F017 in body fluids, xxxxxxxxxxxxx 2019 report No EU-20180309, EU-867704, 20180309 BASF DocID 2019/1046404 Authority registration No
Guideline(s):	EPA 860.1340 (1996), OECD-ENV/JM/MONO/(2007)17, SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010)
Deviations:	No
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)
Acceptability:	Yes

Materials and methods

The analytical method L0339/02 was validated for the determination of M750F015, M750F016 and M750F017 (metabolites of BAS 750 F) in body fluids (bovine plasma and human urine) by LC-MS/MS.

Residues of M750F015, M750F016 and M750F017 are extracted from body fluids with acetonitrile. A salt mixture containing magnesium sulfate, sodium chloride and sodium citrate is added, and the extract is shaken. After centrifugation, an aliquot of the acetonitrile phase is cleaned up using primary secondary amine (PSA) and magnesium sulphate mixture. The final determination of M750F015, M750F016 and M750F017 is performed by LC-MS/MS, monitoring two mass transitions for each analyte in positive ion ESI mode. For quantification, the mass transition m/z 414→70 (M750F015, M750F016 and M750F017) is proposed and for confirmation, the mass transitions m/z 414→143 (M750F015 and M750F017) and m/z 414→182 (M750F016) are proposed. Analysis is accomplished on a Waters Acquity C18 BEH column (150 mm x 2.1 mm, 1.7 µm) applying a gradient mixture of water and acetonitrile with 0.1% formic acid as modifier at a flow rate of 0.4 mL/min.

Results and discussions

The results show that the method is suitable to determine residues of M750F015, M750F016 and M750F017 in body fluids. Samples were spiked with the analytes at the limit of quantification (0.01 mg/L) and 10x LOQ (0.1 mg/L). The overall recovery values (mean of five replicates per fortification level, matrix, analyte and mass transition) were between 70% and 110%. The detailed results are given in the table below.

Table A 8: Results of the method validation for the determination of M750F015, M750F016 and M750F017 in body fluids

Analyte	Matrix	m/z	Fortification level [mg/L]	Number of replicates	Mean recovery [%]	RSD [%]	Overall recovery [%]	Overall RSD [%]
M750F015	Plasma	414→70	0.010	5	94.7	1.7	92.2	3.2
			0.10	5	89.8	1.6		
		414→143	0.010	5	99.5	2.8	97.1	3.5
			0.10	5	94.7	2.0		
	Urine	414→70	0.010	5	95.4	1.9	92.7	3.6
			0.10	5	90.0	2.1		
		414→143	0.010	5	102	3.1	98.2	4.7
			0.10	5	94.6	2.5		
M750F016	Plasma	414→70	0.010	5	87.4	3.2	85.4	3.9
			0.10	5	83.4	3.3		
		414→182	0.010	5	86.8	2.7	85.2	3.5
			0.10	5	83.7	3.5		
	Urine	414→70	0.010	5	97.6	2.0	95.3	3.0
			0.10	5	93.1	1.7		
		414→182	0.010	5	100	3.9	96.2	5.0
			0.10	5	92.5	1.9		
M750F017	Plasma	414→70	0.010	5	91.5	1.3	89.7	2.8
			0.10	5	87.9	2.6		
		414→143	0.010	5	90.5	1.8	88.1	3.2
			0.10	5	85.8	1.6		
	Urine	414→70	0.010	5	98.8	2.0	95.9	3.6
			0.10	5	93.0	1.6		
		414→143	0.010	5	99.1	2.4	95.2	4.9
			0.10	5	91.3	2.4		

RSD = Relative standard deviation

Table A 9: Characteristics for the analytical method used M750F015, M750F016, M750F017 in body fluids

	M750F015, M750F016, M750F017
Specificity	The method L0359/02 determines residues of mefentrifluconazole metabolites in body fluids. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered. LC-MS/MS is a highly specific self-confirmatory technique. Under the described conditions the method is specific for the determination of M750F015, M750F016 and M750F017 in plasma and urine matrices.
Calibration (type, number of data points)	Good linearity of $r \geq 0.99$ was observed in the calibration range of 0.10 ng/mL to 10 ng/mL for all analytes. Seven calibration standards, prepared in acetonitrile/water (1/1, v/v), distributed over the tested concentration range were used. The LOQ falls within the calibration range determined.
Calibration range	Calibration points distributed over a concentration range of 0.10 ng/mL to 10 ng/mL were used. This covers the tested concentration range.
Assessment of matrix effects is presented	No significant matrix effects (i.e > ±20% signal suppression or signal enhancement) were observed for M750F015, M750F016 and M750F017 in any of the body fluid matrices tested. Therefore, solvent calibration standards were used for the quantification for all matrices.
Limit of determination/quantification	The method has a limit of quantification (LOQ) of 0.01 mg/L, corresponding to the lowest fortification level successfully tested. The limit of detection (LOD) is 0.10 ng/mL, corresponding to the lowest calibration standard.
Standard stability	Stability tests showed that M750F015, M750F016 and M750F017 stock, fortification and calibration solutions in acetonitrile and acetonitrile/water (1/1, v/v) were stable for 11 days, when stored refrigerated (2 – 8°C) in the dark. Raw extracts and final volume samples fortified at LOQ and 10x LOQ were shown to be stable for 8 days when stored refrigerated (2 – 8°C) in the dark for all body fluid matrices tested. Final volume samples were re-injected after 8 days of storage and raw extracts were carried through the complete work-up procedure and injected after 8 days of storage.

Conclusion

The method for analysis of M750F015, M750F016 and M750F017 in body fluids uses LC-MS/MS for final determination, which is a highly specific technique.
It could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of M750F015, M750F016 and M750F017 in body fluids.

A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted

A 2.2 Analytical methods for kresoxim-methyl

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

A 2.2.1.1 Description of analytical methods for the determination of residues in Water (Ecotoxicology) (KCP 5.1.2)

A 2.2.1.1.1 Method validation

Comments of zRMS:	Validation of BASF Method L0361/03 has been accepted. The requirements of SANCO/3029/99 rev. 4 are met. The objective of this validation study was to validate the analytical method L0361/03 for the determination of BAS 351 H (Bentazone), BAS 455 H (Pendimethalin), BAS 490 F (**Kresoxim-methyl**), BAS 517 H (Cycloxydim), BAS 635 H (Tritosulfuron), BAS 830 F and BAS 9164 F (Azoxystrobin) in tap water, OECD- and M4-medium by LC-MS/MS. The LOQ at 0.1 µg/L is set. Fortification levels of 0.1 µg/L and 1.0 µg/L were validated for all analytes. For each fortification level and matrix, five replicates were prepared and analysed. Additionally, at least two replicates of unfortified samples were analysed (untreated controls). Two mass transitions were monitored and evaluated for all analytes. The evaluated study was amended for the minor reasons (3) not affecting the study (see the reference CP 5.1.2/2 below).

Reference:	CP 5.1.2/1
Report	Validation of BASF Method L0361/03 for the Determination of Pesticides in Water by LC-MS/MS, Andre, M., 2019 report No 863158, IF18-04586604 BASF DocID 2019/1039564 Authority registration No
Guideline(s):	EFSA Journal (2013), EFSA Panel on Plant Prptection Products and their Residues (PPR), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07, SANCO/3029/99 rev. 4 (11 July 2000)
Deviations:	No
GLP:	yes (certified by Hess. Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz, Wiesbaden, Germany)
Acceptability:	Yes
Reference:	CP 5.1.2/2
Report	Amendment No. 1 to Final Report - Validation of BASF Method L0361/03 for the Determination of Pesticides in Water by LC-MS/MS, Andre, M., 2020 report No 863158, IF18-04586604 BASF DocID 2020/2090916 Authority registration No
Guideline(s):	EFSA Journal (2013), EFSA Panel on Plant Prptection Products and

	their Residues (PPR), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07, SANCO/3029/99 rev. 4 (11 July 2000)
Deviations:	No
GLP:	yes (certified by Hess. Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz, Wiesbaden, Germany)
Acceptability:	Yes

Materials and methods

Kresoxim-methyl residues are extracted from tap water, OECD- and M4-medium specimen by shaking with acetonitrile/water/HCOOH, 400/600/2, v/v/v. An aliquot of the extract is then used for determination by LC-MS/MS. Analysis was accomplished using a Pinnacle DB AQ C18 column and a water-acetonitrile gradient with formic acid as modifier at a flow rate of 600 µL/min. Samples were analysed at mass transition *m/z* 314 → 116 for quantitation and *m/z* 314 → 131 for confirmation for kresoxim-methyl.

Results and discussions

Method validation acceptance criteria were fully met with mean recovery values between 93% and 100% in all matrices tested. The relative standard deviations (RSD, %) for all commodities and fortification levels were <20%. Method validation data are summarised in the table below.

Table A 10: Recovery results from method validation of kresoxim-methyl using the analytical method

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Overall recovery (%)	RSD (%)	Comments
Tap water	**BAS 490 F**	0.1 (n=5)	93.7	4.4	95.1	3.6	Mass transition m/z 314→116*
		1.0 (n=5)	96.6	2.2			
		0.1 (n=5)	98.3	2.9	97.3	2.6	Mass transition m/z 314→131
		1.0 (n=5)	96.3	2.0			
OECD-medium		0.1 (n=5)	93.3	4.1	95.7	4.0	Mass transition m/z 314→116*
		1.0 (n=5)	98.2	1.7			
		0.1 (n=5)	97.5	2.1	98.6	2.0	Mass transition m/z 314→131
		1.0 (n=5)	100	1.3			
M4-medium		0.1 (n=5)	96.9	2.6	96.9	2.0	Mass transition m/z 314→116*
		1.0 (n=5)	96.9	1.5			
		0.1 (n=5)	97.4	1.7	97.4	1.9	Mass transition m/z 314→131
		1.0 (n=5)	97.5	2.3			

*proposed as quantification transition

Table A 11: Characteristics for the analytical method used for validation of kresoxim-methyl residues in test matrices

	Kresoxim-methyl
Specificity	The method L0361/03 determines residues of BAS 490 F (Kresoxim-methyl) in tap water, OECD- medium and M4-medium. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of the analytes.
Calibration (type, number of data points)	Calibration solutions were prepared in a mixture of control matrix extract and ACN/H_2O/HCOOH (400/600/2, v/v/v). At least five calibration levels were used and individual calibration data was presented. Linear correlations with coefficients >0.9995 were obtained.
Calibration range	Calibration points distributed over a concentration range of 0.01 to 0.65 ng/mL were used. This covers the tested concentration range.
Assessment of matrix effects is presented	It was demonstrated that the matrix-load in the tested matrix-matched standards had no significant influence on the detection of the analyte. But as the matrices were used for fortification and control specimens, the matrices were used also for preparation of standard solutions.
Limit of determination/quantification	The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested, hence 0.1 µg/L is the LOQ. The limit of detection (LOD) is defined as lowest calibration level used for the respective validation set and is set at 20 % of the LOQ (0.02 µg/L).
Standard stability	BAS 490 F was stable for a maximum duration of at least 33 days in fortification and calibration solutions, when stored refrigerated at 2-8°C in the dark. Stock solutions were prepared in methanol, whereas calibration solutions were prepared in a mixture of control matrix extract and ACN/H_2O/HCOOH (400/600/2, v/v/v). BAS 490 F was stable in final volumes of each matrix, prepared in methanol, over a time period of 6 days in case of M4-Medium, 7 days in case of OECD-medium and 8 days in case of tap water, when stored at 2-8°C.

Conclusion

The method uses highly specific LC-MS/MS for final determination of kresoxim-methyl with a limit of quantitation of 0.1 µg/L. Thereby, it could be demonstrated that method L0361/03 fulfils the requirements with regard to specificity, linearity, repeatability, limit of quantification and recoveries.

A 2.2.1.1.1.1 Confirmatory method

A confirmatory technique is not required since the detection by MS/MS with two characteristic mass transitions is regarded to be highly specific.

A 2.2.1.2 Description of analytical methods for the determination of residues in stock solutions used in ecotoxicological studies (KCP 5.1.2)

A 2.2.1.2.1 Method validation

Comments of zRMS:	The HPLC with UV-detection analytical method employed in the study has been accepted. The method meets requirements of SANCO/3029/99 rev.4 and is suitable for the determination of kresoxim-methyl in aqueous solutions.

Reference:	CP 5.1.2/3
Report	Acute toxicity of BAS 490 02 F to honeybee larvae *Apis mellifera* L. under laboratory conditions (in vitro) Kleebaum, K., 2015 report No EU-141048063B, EU-702199,14 10 48 063 B BASF DocID 2014/1111118
Guideline(s):	OECD 237 (2013) Honeybee (Apis mellifera) larval toxicity test single exposure
Deviations:	No
GLP:	yes (certified by Staatsministerium Für Umwelt und Landwirtschaft, Sachsen, Germany)
Acceptability:	Yes

Materials and methods
Validation stock solutions of 16837 mg a.i./L and 8414 mg a.i./L were prepared by dissolving appropriate amounts of the test item (BAS 490 02 F) in acetone and diluting first with dilution medium (50/50 methanol/water) and then with water. Samples were analysed using a Shimadzu LC-10 HPLC system fitted with a Nucleoshell RP18, 2.7 mm × 100 mm, 2.7 µm column (Macherey-Nagel) and connected to a Shimadzu SPD-M10 UV diode array detector. Quantitative determination of the target analyte was achieved with the aid of a 5 level calibration curve.

Results and discussion
An HPLC-UV method for the determination of BAS 490 F in stock solutions was validated according to the requirements of SANC/3029/99 rev. 4 guidelines. The response of the detector was found to be linear over the range 4.43 – 13.43 mg/L with an associated correlation coefficient (r^2) > 0.99. The limit of quantification of the method was defined as the lowest successfully validated fortification level, i.e. 8414 mg a.i./L. Accuracy and precision values were found to be within the requirements of SANCO/3029/99 rev. 4 guidelines and are detailed in the table below. No interference was observed in any controls and target analyte concentrations in controls were less than 30% of the lowest validated concentration The specificity of the method was confirmed by comparing UV spectra (200 – 300 nm) recorded during the analysis of the validation samples to those of reference spectra. Example chromatograms and absorption spectra are provided in the study report.

Table A 12: Recovery results from the method validation for kresoxim-methyl

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Aqueous stock solution	Kresoxim-methyl	8414	100	0.5	-
		16837	101	0.5	

Table A 13: Characteristics of the analytical method validated for the determination of kresoxim-methyl in aqueous stock solutions

	Kresoxim-methyl
Specificity	Kresoxim-methyl is determined by HPLC-UV with diode array detection. The specificity of the method was confirmed by comparing UV spectra (200 – 300 nm) recorded during the analysis of the validation samples to those of reference spectra. No significant interference was observed at the elution time of kresoxim-methyl in control samples (interference <30% LOQ).
Calibration (type, number of data points)	The standards used for calibration were prepared in methanol/water. Five standard concentrations were injected and the response was plotted against the concentration. A linear correlation with a coefficient (r^2) of 0.99 was obtained.
Calibration range	4.43 – 13.43 mg/L
Limit of determination/quantification	The limit of quantitation was defined as the lowest fortification level successfully tested and was 8414 mg a.i/L.

Conclusion
An HPLC-UV method with diode array detection for the determination of kresoxim-methyl in aqueous solutions was validated according to the requirements of SANCO/3029/99 rev.4 guidelines. All validation data meet the requirements of the guidelines and the method is therefore suitable for the determination of kresoxim-methyl in aqueous solutions.

A 2.2.1.2.1.1 Confirmatory method

A confirmatory technique is not required as the method detailed above used diode array detection to compare absorption spectra obtained at the retention time of the target analyte in validation samples (approximately 6 min) to absorption spectra produced by a reference standard.

A 2.2.1.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.1.2)

A 2.2.1.3.1 Method validation

Comments of zRMS:	The study has been accepted. The method applied for the study purpose was BASF method L0177/01 with a LOQ of 0.01 mg/kg. The method in animal matrices was validated according to the requirements of SANCO/3029/99 rev.4. The study results show that metabolites BF 490-1, BF 490-2, BF 490-9 are stable under deep-frozen conditions (≤ -18°C) in the tested matrices (milk, liver, kidney, muscle, fat) for at least 15 months of storage.

Reference:	CP 5.1.2/4
Report	Storage stability of metabolites of kresoxim-methyl (BF 490-1, BF 490-2. BF 490-9) in animal tissues matrices under deep frozen conditions xxxxxxxxxxxx 2016 report No EU-S14-05353 BASF DocID 2016/1235729
Guideline(s):	EC 1107/2009, EEC 7032/VI/95 rev. 5, OECD 506, SANCO/3029/99 rev. 4
Deviations:	No
GLP:	yes (certified by Landesanstalt für Umwelt, Messungen und Naturschutz, Baden-Wuerttemberg, Karlsruhe, Germany)
Acceptability:	Yes

Materials and methods

Stock solutions of 1000 ng/mL were prepared by individually dissolving appropriate amounts of target analyte standards in separate volumes of methanol. Stock solutions were further diluted for use as fortification solutions for recovery experiments and for the preparation of matrix-matched calibration solutions. Matrix-matched calibration solutions were prepared for each matrix-analyte combination using final sample extracts of untreated controls fortified with appropriate amounts of the diluted stock solutions. For each matrix, 5 g homogenised sample material were weighed into a 100 mL glass bottle and fortified with the fortification solutions to provide recovery samples for each matrix/analyte combination at 0.01 mg/kg and 0.1 mg/kg. Residues were extracted with methanol using a Miccra homogeniser. Following centrifugation, the supernatants were decanted and the extraction procedure was repeated twice for fat and once for all other matrices. The extracts from individual matrices were then combined to provide a single extract for each matrix and made up to 100 mL with methanol. An aliquot of the extract from milk, egg, meat, kidney and liver was then analysed using HPLC-MS/MS.
For fat, the 100 mL extract was evaporated to dryness under a stream of nitrogen, residues were re-dissolved in 5 mL n-hexane (saturated with acetonitrile) and transferred to a 50 mL polyethylene vial. 10 mL acetonitrile (saturated with n-hexane) were added, the solution was shaken for 2 min and then centrifuged. The upper n-hexane phase was transferred to a new vial and the partitioning process was repeated twice. The n-hexane extracts were combined and 15 mL acetonitrile (saturated with n-hexane) were added. The solution was then shaken and centrifuged. The acetonitrile extracts were combined, evaporated to dryness

and reconstituted in in 5 mL acetonitrile/water 1:1 v/v + 0.1% acetic acid. An aliquot of the final extract was then analysed using HPLC-MS/MS.
HPLC analysis was achieved using an Agilent 1290 Infinity I HPLC fitted with a Thermo Aquasil C18, 150 × 3 mm, 3 µm column connected to a AB Sciex API 6500 Triple Quadrupole Mass Spectrometer. Quantitative determination of the target analyte was achieved with the aid of a 7 level calibration curve constructed using matrix-matched standards for each matrix/analyte combination.

Results and discussion
An HPLC-MS/MS method for the determination of kresoxim-methyl metabolites BF 490-1, BF 490-2 and BF 490-9 in animal matrices was validated according to the requirements of SANCO/3029/99 rev. 4 guidelines. The response of the detector was found to be linear for all target analyte/matrix combinations, over the range 0.1 – 100 ng/mL (equivalent to 0.002 – 2 mg/kg) with associated correlation coefficients (r^2) > 0.99. The limit of quantification of the method is 0.01 mg/kg for all target analyte/matrix combinations. Accuracy and precision values were found to be within the requirements of SANCO/3029/99 rev. 4 guidelines for all target analyte/matrix combinations and are detailed in the table below. No interference was observed in any controls and none of the target analytes was detected in any controls (LOD = 0.003 mg/kg). The method is highly specific with two mass transitions monitored for each target analyte. Example chromatograms are provided in the study report.

Table A 14: Recovery results from the method validation for metabolites BF 490-1, BF 490-2 and BF 490-9 in animal matrices

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Milk	BF 490-2	0.01	100	14	-
		0.1	106	9	
	BF 490-9	0.01	99	7	-
		0.1	102	8	
Liver	BF 490-1	0.01	101	10	-
		0.1	105	12	
	BF 490-9	0.01	96	12	-
		0.1	103	11	
Muscle	BF 490-1	0.01	95	13	-
		0.1	94	13	
	BF 490-2	0.01	81	16	-
		0.1	94	10	
Kidney	BF 490-1	0.01	94	8	-
		0.1	91	14	
	BF 490-2	0.01	87	11	-
		0.1	91	10	
	BF 490-9	0.01	85	16	-
		0.1	87	12	
Fat	BF 490-1	0.01	101	12	-
		0.1	107	11	
	BF 490-2	0.01	90	8	-

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
		0.1	107	11	
	BF 490-9	0.01	96	11	-
		0.1	106	9	

Table A 15: Characteristics of the analytical method validated for the determination of metabolites BF 490-1, BF 490-2 and BF 490-9 in animal matrices

	BF 490-1, BF 490-2 and BF 490-9
Specificity	BF 490-1, BF 490-2 and BF 490-9 are determined by HPLC-MS/MS with two transitions monitored for each analysis. No significant interference was observed at the elution time of any of the target analytes in any control samples. The target analytes were not detected in any control samples
Calibration (type, number of data points)	Matrix-matched calibration standards were used to construct all calibration curves. In each case seven standard concentrations were injected and the response plotted against concentration. Linear correlations with coefficients (r^2) of 0.99 were obtained for each target analyte/matrix combination.
Calibration range	0.1 – 100 ng/mL (equivalent to 0.002 – 2 mg/kg)
Limit of determination/quantification	The limit of quantitation was 0.01 mg/kg

Conclusion
An HPLC-MS/MS method for the determination of kresoxim-methyl metabolites BF 490-1, BF 490-2 and BF 490-3 in animal matrices was validated according to the requirements of SANCO/3029/99 rev.4 guidelines. All validation data meet the requirements of the guidelines and the method is therefore suitable for the determination of methyl metabolites BF 490-1, BF 490-2 and BF 490-3 in animal matrices.

A 2.2.1.3.1.1 Confirmatory method

A confirmatory technique is not required as the method detailed above is highly specific with two mass transitions monitored for each analysis.

A 2.2.1.4 Description of analytical methods for the determination of residues in soil (KCP 5.1.2)

No new or additional studies have been submitted.

A 2.2.1.5 Description of analytical methods for the determination of residues in water (KCP 5.1.2)

No new or additional studies have been submitted.

A 2.2.1.6 Description of analytical methods for the determination of residues in air (KCP 5.1.2)

No new or additional studies have been submitted.

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

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

No new or additional studies have been submitted

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

A 2.2.2.2.1 Validation of BASF method R0062/01: Determination of BF 490-9 (Reg.No. 292 932) in different matrices of animal origin by LC-MS/MS

A 2.2.2.2.1.1 Method validation

Comments of zRMS:	Validation of the method R0062/01 in animal matrices consistently with SANCO/825/00 rev. 8.1 has been accepted. The entire evaluated study was amended (see ref. CP 5.2/5 below) because the amount of final volume given in the tables about preparation of standard solutions for calibration was not correct and it was corrected to 1000 µL in each table (see e.g. old page 25 and new 26). It was proved in the study that the analytical method R0062/01 is suitable for the determination of residues of BF 490-9 in milk, egg, fat, meat and kidney. The mean recovery values ranged between 71 % and 103 % of the nominal values for each matrix and each mass transition. The relative standard deviations (RSD, %) for both fortification levels, each matrix and each mass transition were below 20 %. Due to the high selectivity and specificity of HPLC-MS/MS an additional confirmatory technique was not necessary. The LOQ of the method was 0.01 mg/kg. The method is applicable to correctly determine residues of BF 490-9 in matrices of animal origin (milk, egg, fat, meat and kidney).

Reference:	CP 5.2/4
Report	Validation of analytical method R0062/01 - Method for the determination of BF 490-9 (Reg.No. 292932) in different matrices of animal origin xxxxxxxxxxxxxxxx., 2019 report No 883049, S19-03618 BASF DocID 2019/2049829
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010)
Deviations:	No
GLP:	Yes (certified by Landesanstalt fuer Umwelt, Baden-Wuerttemberg, Karlsruhe, Germany)
Acceptability:	Yes

Reference:	CP 5.2/5
Report	Report Amendment No. 1 - Validation of analytical method R0062/01 - Method for the determination of BF 490-9 (Reg.No. 292932) in different matrices of animal origin, xxxxxxxxxxxxx 2019 report No 883049, S19-03618 2020/20952x88 Authority registration No
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010)
Deviations:	No
GLP:	yes (certified by Landesanstalt fuer Umwelt, Baden-Wuerttemberg, Karlsruhe, Germany)
Acceptability:	Yes

Materials and methods

For milk, egg and fat: To an aliquot of homogenized sample material, water and acetonitrile + 1% formic acid was added, and the mixture was shaken on a flatbed shaker. Afterwards, liquid/liquid partition was achieved, by addition of magnesium sulphate and sodium chloride. After centrifugation, an aliquot of the upper acetonitrile phase was frozen out at –18°C overnight. The extract was then diluted with acetonitrile/water (1/1, v/v) + 0.1% acetic acid and analyzed by HPLC-MS/MS.
For meat: An aliquot of homogenized sample material was extracted twice with acetonitrile/water (50/50, v/v) on a flatbed shaker. After centrifugation, the combined extracts were diluted with water containing 0.1% acetic acid and analyzed by HPLC-MS/MS.
For kidney: To an aliquot of homogenized sample material, water and 5 N sodium hydroxide solution was added, and the mixture was shaken on a flatbed shaker. After adding 5 N sulphuric acid, shaking by hand, then adding acetonitrile + 1% formic acid, the mixture was shaken on a flatbed shaker. Afterwards, liquid/liquid partition was achieved, by addition of magnesium sulphate and sodium chloride. After centrifugation, an aliquot of the upper acetonitrile phase was frozen out at –18°C overnight. The extract was purified with primary secondary amine (PSA) and magnesium sulphate. After centrifugation, the purified extract was diluted with water containing 0.1% acetic acid and analyzed by HPLC-MS/MS.

Results and discussions

In all matrices tested, the mean recovery values were between 71% and 103%. The relative standard deviations (RSD, %) for both fortification levels, each matrix and each mass transition were below 20%. The detailed results are given in the tables below.

The analyte BF 490-9 was stable for a maximum duration of at least 86 days in stock and 30 days in fortification solutions and for at least 7 days in calibration solutions when stored refrigerated at approximately 1-10°C in the dark. Stock solutions of BF 490-9 were prepared in acetonitrile and fortification solutions of BF 490-9 were prepared in acetonitrile/water (1/1, v/v) containing 0.1% acetic acid. Solvent calibration solutions of the analyte were prepared in acetonitrile containing 1% formic acid diluted by factor 10 (for milk, egg) or diluted by factor 4 (for fat) with acetonitrile/water (1/1, v/v) containing 0.1 % acetic acid. For meat, solvent calibration solutions of the analyte were prepared in acetonitrile/water (25/75, v/v) containing 0.05% acetic acid. For kidney, solvent calibration solutions of the analyte were prepared in acetonitrile containing 1% formic acid diluted by factor 10 with water containing 0.1% acetic acid.

The analyte BF 490-9 was stable in extracts over a time period of 16 days (milk), 11 days (egg), 10 days (at least; fat) and 7 days (meat), when stored refrigerated at 1-10 °C in the dark.
The analyte BF 490-9 was not stable in extracts over a time period of seven days in kidney, when stored refrigerated at 1-10 °C in the dark. Validation samples were analysed directly after extraction.
An independent laboratory validation has been successfully conducted and is reported below.

Table A 16: Recovery results from method validation of kresoxim-methyl metabolite BF 490-9 using the analytical method R0062/01

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Milk	BF 490-9	0.01	101	4	Quantitation m/z 314 → 238
		0.1	99	2	
		0.01	102	3	Confirmation m/z 314 → 116
		0.1	98	3	
Eggs	BF 490-9	0.01	103	3	Quantitation m/z 314 → 238
		0.1	101	2	
		0.01	101	3	Confirmation m/z 314 → 116
		0.1	101	2	
Fat	BF 490-9	0.01	95	2	Quantitation m/z 314 → 238
		0.1	93	4	
		0.01	95	6	Confirmation m/z 314 → 116
		0.1	92	5	
Meat	BF 490-9	0.01	78	12	Quantitation m/z 314 → 238
		0.1	77	1	
		0.01	85	13	Confirmation m/z 314 → 116
		0.1	78	7	
Kidney	BF 490-9	0.01	74	13	Quantitation m/z 314 → 238
		0.1	71	8	
		0.01	76	12	Confirmation m/z 314 → 116
		0.1	71	8	

Table A 17: Characteristics for the analytical method used for validation of BF 490-9 residues in animal matrices

	BF 490-9
Specificity	Quantitation is performed by HPLC-MS/MS monitoring two highly specific. Mass spectra of BF 490-9 are provided in the study report. Target analyte concentrations in controls were < 30% of the method LOQ. No interefrence from co-eluting compounds at the retention time of the target analyte.

	BF 490-9
Calibration (type, number of data points)	Linear LC-MS/MS calibration functions were generated with good correlations ($r > 0.995$), using matrix-matched standards with ≥ 6 concentration levels.
Calibration range	0.10 ng/mL to 100 ng/mL for milk, eggs and fat 0.15 ng/mL to 100 ng/mL for meat 0.075 ng/mL to 10 ng/mL for kidney
Assessment of matrix effects is presented	Yes, an assessment of matrix effects is presented in the study report, please refer to pages 45 to 47.
Limit of determination/quantification	The limit of quantitation of the analytical method is 0.01 mg/kg. The limit of detection (LOD) is 0.002 mg/kg for milk, eggs and fat and 0.003 mg/kg for meat and kidney.

Conclusion

The HPLC-MS/MS based analytical BASF Method No. R0062/01 was successfully developed and validated for the determination of BF 490-9 in bovine whole milk, poultry egg, bovine meat, bovine kidney and bovine fat with an LOQ of 0.01 mg/kg.

A 2.2.2.2.1.2 Independent laboratory validation

Comments of zRMS:	The ILV of the method R0062/01 has been accepted. The method was independently validated, as previously at two fortification levels (0.01 mg/kg and 0.1 mg/kg) for five different matrices (milk, eggs, fat, meat and kidney). For each fortification level and matrix, five replicates were prepared and analysed. Additionally, at least two replicates of unfortified samples (untreated control samples) and one reagent blank were analysed for each matrix. Two mass transitions were evaluated for quantification and confirmation of BF 490-9. It was proved that the method is applicable to correctly determine residues of BF 490-9 in matrices of animal origin with LOQ 0.01 mg/kg for all matrices.

Reference:	CP 5.2/6
Report	Independent Laboratory Validation of an Analytical Method for the Determination of Kresoxim-methyl Metabolite BF 490-9 in Different Matrices of Animal Origin xxxxxxxxxxxxx 2019 report No 883049, P5325G DocID 2019/2053784
Guideline(s):	SANCO/825/00 rev. 8.1 (16 November 2010)
Deviations:	No
GLP:	Yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)
Acceptability:	Yes

Materials and methods

The independent validation study followed the analytical steps of the primary method.

Results and discussions

The results of the recovery experiments indicate that the independent laboratory validation was successfully completed. In all matrices tested, the mean recovery values were between 73% and 109%. The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed results are given in the tables below.

Table A 18: Recovery results from independent laboratory validation of kresoxim-methyl metabolite BF 490-9 using the analytical method R0062/01

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Milk	BF 490-9	0.01	85	7	Quantitation m/z 314 → 238
		0.1	78	9	
		0.01	89	10	Confirmation m/z 314 → 116
		0.1	77	8	
Eggs	BF 490-9	0.01	102	6	Quantitation m/z 314 → 238
		0.1	97	4	
		0.01	105	6	Confirmation m/z 314 → 116
		0.1	98	4	
Fat	BF 490-9	0.01	106	2	Quantitation m/z 314 → 238
		0.1	109	8	
		0.01	92	8	Confirmation m/z 314 → 116
		0.1	91	8	
Meat	BF 490-9	0.01	95	9	Quantitation m/z 314 → 238
		0.1	75	3	
		0.01	88	5	Confirmation m/z 314 → 116
		0.1	73	2	
Kidney	BF 490-9	0.01	77	7	Quantitation m/z 314 → 238
		0.1	85	3	
		0.01	85	8	Confirmation m/z 314 → 116
		0.1	82	4	

Table A 19: Characteristics for the analytical method used for independent laboratory validation of BF 490-9 residues in animal matrices

	BF 490-9
Specificity	Quantitation is performed by HPLC-MS/MS monitoring two highly specific ion transitions. Mass spectra of BF 490-9 are provided in the study report. Target analyte concentrations in controls were < 30% of the method

	BF 490-9
	LOQ. No interefrence from co-eluting compounds at the retention time of the target analyte.
Calibration (type, number of data points)	Linear LC-MS/MS calibration functions were generated with good correlations ($r \geq 0.99$), using matrix-matched standards with ≥5 concentration levels.
Calibration range	0.10 ng/mL to 100 ng/mL for milk, eggs and fat 0.15 ng/mL to 100 ng/mL for meat 0.075 ng/mL to 10 ng/mL for kidney
Assessment of matrix effects is presented	Yes, an assessment of matrix effects is presented in the study report, please refer to pages 44 to 49.
Limit of determination/quantification	The limit of quantitation (LOQ) of the method was 0.01 mg/kg in animal matrices milk, egg, muscle, liver, kidney and fat.

Conclusion

The results of the independent laboratory validation confirm the results of the validation study reported above (DocID 2019/2049829) and demonstrate that analytical method R0062/01 is suitable to determine residues of BF 490-9 in bovine whole milk, poultry egg, bovine meat, bovine kidney and bovine fat with an LOQ of 0.01 mg/kg.

A 2.2.2.2.1.3 Confirmatory method

A confirmatory technique is not required as method R0062/01 uses two different mass transitions of BF 490-9 for quantitation and confirmation.

A 2.2.2.2.1.4 Extraction efficiency

BF 490-9 is extracted from milk, egg and fat with water and acetonitrile + 1% formic acid. Afterwards, liquid/liquid partition was achieved, by addition of magnesium sulphate and sodium chloride. The upper acetonitrile phase was frozen out at –18°C overnight and diluted with acetonitrile/water (1/1, v/v) + 0.1% acetic acid.
For meat, sample material was extracted twice with acetonitrile/water (50/50, v/v) and combined extracts were diluted with water containing 0.1% acetic acid.
For kidney, water and 5 N sodium hydroxide, 5 N sulphuric acid and acetonitrile + 1% formic acid was added and mixed. Afterwards, liquid/liquid partition was achieved, by addition of magnesium sulphate and sodium chloride. The upper acetonitrile phase was frozen out at –18°C overnight. The extract was purified with primary secondary amine (PSA) and magnesium sulphate. After centrifugation, the purified extract was diluted with water containing 0.1% acetic acid.
As an extraction procedure using acetonitrile was applied in the goat metabolism study, no separate assessment of extraction efficiency is required. For more information see section 5.3.3.3.

Accepted.

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.2.2.7 Other Studies/ Information

No new or additional studies have been submitted