REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: GF-3308

Product name: Questar

Chemical active substance:

Fenpicoxamid (XDE-777), 50 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Corteva Agriscience

Submission date: May 2021

MS Finalisation date: March 2022 (initial Core Assessment)

August 2022 (final Core Assessment)

Version history

When	What
May 2021	New submission of GF-3308 in the Central Zone.
March 2022	Initial assessment by the zRMS. The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through and shaded for transparency~~.
August 2022	Final report (Core Assessment updated following the commenting period). Additional information/assessments included by the zRMS in the report in response to comments received from the cMS and the Applicant are highlighted in yellow. Information no longer relevant ~~is struck through and shaded~~.

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5.1 Conclusion and summary of assessment

zRMS-PL conclusions:
EFSA in EFSA Journal 2018;16(1):5146 concluded:
"*Fenpicoxamid residues and also its metabolite X642188 can be monitored in food and feed of plant origin by liquid chromatography with tandem mass spectrometry (LC–MS/MS) with limit of quantifications (LOQs) of 0.01 mg/kg in all plant commodity groups for each analyte. Monitoring residues of fenpicoxamid and metabolite X642188 in milk, meat, liver, fat and poultry egg can be performed using LC–MS/MS with LOQs of 0.01 mg/kg all matrices for both compounds. The residue definition for monitoring in soil and water was defined as fenpicoxamid and its metabolite X642188.*
Appropriate LC–MS/MS methods exist for monitoring fenpicoxamid and metabolite X642188 in soil and water with LOQs of 0.05 mg/kg and LOQs of 0.05 lg/L, respectively, for both analytes. Fenpicoxamid residues in air can be determined by LC–MS/MS with a LOQ of 1.39 µg/m³.
Determination of residues of fenpicoxamid in urine and blood can be done by LC–MS/MS with a LOQ of 0.05 mg/L."

List of End-point (UK, 2017):

Analytical methods for residues (Regulation (EU) N° 283/2013, Annex Part A, point 4.2 & point 7.4.2)
Residue definitions for monitoring purposes

Food of plant origin		XDE-777
Food of animal origin		No residue definition is proposed.
Soil		XDE-777 and metabolite X642188
Sediment		No data has been provided by the applicant and therefore it is not possible to set residue definition for sediment.
Water	surface	XDE-777 and metabolite X642188
	drinking/ground	XDE-777 and metabolite X642188
Air		XDE-777
Body fluids and tissues		XDE-777

Monitoring/Enforcement methods

Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)	LC/MS/MS (ESI+) LOQ = 0.01 mg/kg for XDE-777 and its metabolite X642188 in plants (rye, lettuce, lemon and oilseed rape). LC/MS/MS (ESI+) LOQ = 0.01 mg/kg for XDE-777 and its metabolite X642188 in plants and processed fractions (cereal grain and straw, lettuce, cabbage, orange, grapefruit, oil seed rape seed, olive, bran, flour, bread).
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)	LC/MS/MS (ESI+) LOQ = 0.01 mg/kg for XDE-777 in animal (bovine milk, meat, liver and fat and poultry egg) LOQ = 0.01 mg/kg for the metabolite X642188 in animal (bovine milk, meat, liver and fat and poultry egg). LOQ = 0.01 mg/kg for the metabolite X12326349 in animal (bovine milk, liver and fat and poultry egg).
Soil (analytical technique and LOQ)	LC/MS/MS (ESI+) LOQ = 0.05 mg/kg for XDE-777 and its metabolite X642188 in the four types of soil and in one type of sediment
Water (analytical technique and LOQ)	LC/MS/MS (ESI+) LOQ = 0.05 µg/L for XDE-777 and its metabolite X642188 in surface, ground and drinking water.
Air (analytical technique and LOQ)	LC/MS/MS (ESI+) LOQ = 0.5 µg for XDE-777 equivalent to 1.39 µg/m³ of ambient air and warm and humid air.
Body fluids and tissues (analytical technique and LOQ)	LC/MS/MS (ESI+) LOQ = 0.05 mg/L for XDE-777 in urine and blood

Applicant submitted several new methods used in support of ecotoxicology studies. An overview of these methods and their evaluations are presented in Appendix 2 of Part B5.

Sufficiently sensitive and selective analytical methods for post-authorization control and monitoring purposes are available for all analytes included in the residue definitions.
In SANTE/2020/12830, Rev.1 it is stated that analytical methods for monitoring residues in body fluids and tissues must be validated with the following matrix groups:
- Body fluids (either blood, serum, plasma or urine),
- Body tissues (either meat, liver or kidney).
For body tissues, a method for the determination of XDE-777 in bovine milk, meat, liver and fat and poultry egg with LOQ=0.01 mg/kg is available. This is acceptable.
For body fluids, a method for the determination of XDE-777 in urine and blood with LOQ = 0.05 mg/L is available. However, according to the SANTE/2020/12830, Rev.1 (24. February 2021), a lower LOQ is required for analytical methods for body fluids, the LOQ should be 0.01 mg/L instead of 0.05 mg/L (SANCO/825/00 rev. 8.1).
Information submitted by Applicant (February 2022):
"*Since SANTE/2020/12830, Rev.1 was published on 24-February-2021, Corteva did not have the opportunity to validate a new body fluids method prior to submission date for this plant protection product (June 2021). We recognize the need to update the body fluids method to lower the LOQ to 0.01 mg/L and have a study planned for 2023. The new body fluids method will be presented as part of the active substance renewal dossier in 2025*."

Additionally, new study concerning extraction efficiency, conducted with using 3 different solvent systems, was submitted in the framework of this application (Study No. S20-01536; DAS Study No. 200456).
This study has proven the satisfactory extraction efficiency of the extraction used in the analytical methods (MOR Method/ DAS #120615, MRM Method/DAS # 120998) for the quantitative determination of residues of XDE-777 when compared with the NOR Method/DAS #110334 for fenpicoxamid (XDE-777) in banana, barley grain and oilseed rape seed matrices.
The study is acceptable. Summary is presented in Appendix 2.

No additional data are required to support the intended uses for GF-3308.

Sufficiently sensitive and selective analytical methods are available for the active substance in the plant protection product.
Noticed data gaps are: none

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.
Noticed data gaps are: none

Commodity/crop	Supported/ Not supported
Wheat, rye, triticale, spelt	Supported

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

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

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

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

Comments of zRMS:	The method is considered to be sufficient for the determination of fenpicoxamide in GF-3308 - the method has been validated according to the SANCO 3030/99 rev 4 guideline.

Reference:	KCP 5.1.1/1
Report	Analytical Method and Validation for the Determination of Fenpicoxamid in GF-3308 Formulation, Jones, J. Evenson, M., 2017, DAS-AM-G-161106

Guideline(s):	Yes, U.S. EPA OPPTS Test Guideline 830.1800 EEC Guideline SANCO/3030/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Internal standard composed of dibutylphthalate in acetonitrile is prepared. Standard solutions are prepared by dissolving the analytical standards with 10 mL of internal standard solution and 40 mL of acetonitrile. Samples are prepared by weighing aliquots into a glass jar and adding 10 mL of internal standard solution and 40 mL of acetonitrile. Solutions are then sonicated. The concentrations of fenpicoxamid are determined using internal standard calibration using peak areas.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substance Fenpicoxamid in plant protection product GF-3308

	Fenpicoxamid
Author(s), year	Jones, J. Evenson, M, 2017
Principle of method	Analytical method for determination of Fenpicoxamid in GF-3308 formulation. A high pressure liquid chromatographic (HPLC) method was validated using an Ascentis Express C18 column, 5 cm x 3.0 mm, 2.7 micron, with an ultra-violet detector set at 240 nm. Concentrations were determined using internal standard calibration.
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	The detector response was shown to be linear for Fenpicoxamid over a range of 0.344 – 0.644 mg/mL (R2 = 0.9939). The detector response was shown to be linear for Internal Standard over a range of 0.40 – 1.44 mg/mL (R2 = 1.0000).
Precision – Repeatability Mean n = 10 (%RSD)	The relative standard deviation was 0.72% at an average concentration of 4.62% of Fenpicoxamid.
Accuracy n = 7 (% Recovery)	Recovery data were obtained over the range of 3.408 – 6.165% Fenpicoxamid, at an average recovery of 99.6%
Interference/ Specificity	No significant interferences were detected between the solvent blank, formulation blank, internal standard and technical grade active ingredient.
Comment	No comment.

Conclusion

This method has been successfully validated for Fenpicoxamid active substance in GF-3308.

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

No impurity has been considered as relevant in the technical active substance, so no relevant impurity is expected in the formulation, hence no analytical method is required.

Analytical method for breakdown products:

Comments of zRMS:	The method is considered to be sufficient for the determination of X12019520, X12393285, X12335723 and X12314005 in GF-3308 - the method has been validated according to the SANCO 3030/99 rev 4 guideline.

Reference:	KCP 5.1.1/2
Report	Method Validation for the Determination of Degradants as Impurities (X12314005, X12019520, X12393285, X12335723) in GF-3308, Jacobson, P., 2018, DAS-AM-G-180924
Guideline(s):	Yes, U.S. EPA OPPTS Test Guideline 830.1800 EEC Guideline SANCO/3030/99 rev.4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Standard solutions are prepared by dissolving the analytical standards in 100-mL of acidified DMF. Samples are prepared by weighing aliquots into a 25-mL volumetric flask and diluting to volume with acidified DMF. The concentration of each degradant is determined using quadratic regression calibration.

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of active substance Fenpicoxamid in plant protection product GF-3308

Principle of method	Analytical method for determination of degradant impurities in GF-3308 formulation. A liquid chromatography system with tandem mass spectrometer detector (LC-MS/MS) method was validated using an Ascentis Express C8 column, 2.1 cm x 100 mm, 2.7 micron. Concentrations were determined using quadratic regression calibration.				
Linearity (n ≥ 7)	The detector response was shown to be linear for the degradant impurities.				
	Component	X12314005	X12019520	X12393285	X12335723
	Range (wt%)	0.0058-0.66	0.0046-0.52	0.0049-0.55	0.0045-0.51
	Coefficient of Determination (r^2)	0.9970	0.9986	0.9986	0.9990
Precision n = 5 (%RSD)	Component	X12314005	X12019520	X12393285	X12335723
	Avg wt%	0.086	0.0087	0.016	0.096
	%RSD	3.76	1.35	1.48	0.25
Accuracy n = 6 (% Recovery)	Component	X12314005	X12019520	X12393285	X12335723
	Equivalent Wt%	0.045-0.38	0.038-0.30	0.039-0.30	0.029-0.25
	Avg Recovery %	100.0	98.0	102.3	93.6
LOQ	Avg wt %	0.0068	0.0062	0.0056	0.0065
	% RSD	3.71	1.55	1.49	1.20
	Avg Recovery %	77.5	84.9	78.0	107.4
LOD	Calculated LOD wt %	0.0031	0.0024	0.0021	0.0024
Interference/ Specificity	No significant interferences were detected between the degradants, formulation bland and diluent blank.				

Conclusion

This method has been successfully validated for degradant impurities active substance in GF-3308.

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

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

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There is currently no CIPAC method available for the determination of Fenpicoxamid.

5.2.2 Methods for the determination of residues, Fenpicoxamid (KCP 5.1.2)

An overview of the acceptable methods and possible data gaps for analysis of residues of Fenpicoxamid for the generation of pre-authorization data is given in the following table. These studies have already been evaluated during the EU approval process of the active substance (EFSA 2018). For the detailed evaluation of new/additional studies, refer to Appendix 2.

The residue definition for risk assessment for food of plant origin is fenpicoxamid (EFSA Journal 2018;16(1):5146). The crop method used to analyze for fenpicoxamid residues in wheat studies 150650, 140648, 140649, 150649, 160393, 110414, 120434, 140650, and 120435 (KCA 6.3.1/01 – KCA 6.3.1/08 and KCA 6.5.3/01) was the EU agreed Method No. 120615 (Watson, G., 2012). The crop method used to analyze for fenpicoxamid residues in wheat study 140696 (KCA 6.5.3/02) was EU agreed Method No. 140696 which was validated within the study (Eversfield, S., 2017). The extraction efficiency of Method No. 120615 was successfully evaluated using incurred radiolabeled wheat samples (grain, hay, straw, forage) from the metabolism study. Fenpicoxamid residue levels determined using the manual extraction procedure outlined in the crop analytical method (acetonitrile/water (90/10, v/v)) were comparable (differed by no more than 30%) to residue levels determined using the accelerated solvent extraction (ASE) procedure outlined in the wheat nature of residue (NOR) study (Li, Q., Dixit, V., 2013). The solvent used in Method No. 140696 (acetonitrile/water/phosphoric acid (90/10/0.1, v/v/v)) differs in composition by no more than 20 vol% compared to the solvent used in analytical method 120615 (acetonitrile/water (90/10, v/v)). Therefore, the extraction efficiency of method 140696 for fenpicoxamid has also been successfully demonstrated.

Table 5.2-3: Validated methods for the generation of pre-authorization data

Component of residue definition: Fenpicoxamid					
Matrix type	**Method No.**	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content, high acid content, high oil content, high protein/high starch content (dry) (Residues)	120615*	Primary	0.01 mg/kg	LC-MS/MS	Watson, G., 2012, EU agreed
High water content, high protein/high starch content (dry) (Residues)	140696	Primary	0.01 mg/kg	LC-MS/MS	Eversfield, S., 2017, EU agreed
Animal products (feeding study)	130949	Primary	0.01 mg/kg	LC-MS/MS	Rawle, N.W., 2013, EU agreed
Soil (Environmental fate: TFD study)	141042	Primary	0.012 mg/kg	LC-MS/MS	Li, Q., Hastings, M., Slinkard, E.W., 2015, EU Agreed
Water (Ecotoxicology)	160103	Primary	0.985 mg GF-3308/L	LC-MS/MS	Bergfield, A., 2016

Component of residue definition: Fenpicoxamid					
Matrix type	**Method No.**	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	160101		0.070 ng/mL		xxx., 2016a
	160102		0.066 ng/mL		Goudie, O., 2016b
	191366		7.05 µg/L		Goudie, O., 2020
	202284		19.7 ng/L		Goudie, O., 2021
	140489		0.0217 ng/mL		Hadsell, R., 2014, revised 2018
	160126		0.000050 mg/L		Hicks, S., 2016
	160125		0.0500 µg/L		Hicks, S., 2017
Honey Bee (Ecotoxicology)	190305	Primary	0.600 mg/kg (larval diet) 6.00 mg/L (water)	LC-MS/MS	Verge, E., 2020
	160522		1.20 mg/L (feeding solution)		Verge, E., 2017
	160515		0.01 mg/kg (pollen, nectar)		Kleinhenz, M., 2017

*Also used as a post-registration enforcement method

Component of residue definition: Metabolite X642188					
Matrix type	**Method No.**	**Method Type**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Water (Ecotoxicology)	180562	Primary	0.02 µg/L	LC-MS/MS	Goudie, O., 2018
	160126		0.0000040 mg/L		Hicks, S., 2016
Sediment, Water (Ecotoxicology)	180563	Primary	0.02 µg/L (overlaying water) 14 mg/L (porewater) 0.046 mg/kg (sediment)	LC-MS/MS	Beasley, J., 2018
	180639		0.33 µg/L (water) 0.046 mg/kg (sediment)		Dinehart, S., 2019
Soil (Environmental fate: TFD study)	141042	Primary	0.012 mg/kg	LC-MS/MS	Li, Q., Hastings, M., Slinkard, E.W., 2015, EU Agreed

Component of residue definition: Metabolite X12326349					
Matrix type	**Method No.**	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Animal products (feeding study)	130949	Primary	0.01 mg/kg	LC-MS/MS	Rawle, N.W., 2013, EU agreed

Component of residue definition: Metabolite X12019520					
Matrix type	**Method No.**	**Method Type**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Water (Ecotoxicology)	180560	Primary	4.9 mg/L	LC-MS/MS	xxx, 2018a

Component of residue definition: Metabolite X12446477					
Matrix type	**Method No.**	**Method Type**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Water (Ecotoxicology)	180561	Primary	0.096 mg/L	LC-MS/MS	xxx, 2018b

Component of residue definition: Metabolite X12255349					
Matrix type	**Method No.**	**Method Type**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Water (Ecotoxicology)	160126	Primary	0.0000090 mg/L	LC-MS/MS	Hicks, S., 2016

Component of residue definition: Metabolite X12335723					
Matrix type	**Method No.**	**Method Type**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Sediment, Water (Ecotoxicology)	180564	Primary	0.015 mg/L (water) 0.0069 mg/kg (sediment)	LC-MS/MS	Leak, T., 2018

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

5.3.2 Description of analytical methods for the determination of residues of Fenpicoxamid (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) and the EFSA Conclusion (EFSA Journal 2018;16(1):5146) 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	Fenpicoxamid	0.01 mg/kg	Reg (EU) 2019/50
Plant, high acid content		0.01 mg/kg	Reg (EU) 2019/50
Plant, high protein/high starch content (dry commodities)		0.01 mg/kg Wheat 0.6 mg/kg	Reg (EU) 2019/50

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high oil content		0.01 mg/kg	Reg (EU) 2019/50
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Reg (EU) 2019/50
Muscle	X12326349 expressed as fenpicoxamid	0.01 mg/kg	Reg (EU) 2019/50
Milk		0.01 mg/kg	Reg (EU) 2019/50
Eggs		0.01 mg/kg	Reg (EU) 2019/50
Fat		0.01 mg/kg	Reg (EU) 2019/50
Liver, kidney		0.01 mg/kg 0.02 mg/kg (bovine kidney; sheep liver and kidney)	Reg (EU) 2019/50
Soil (Ecotoxicology)	Fenpicoxamid and X642188	0.05 mg/kg	Common Limit EFSA Journal 2018;16(1):5146 $NOEC_{corr}$ = 3.97 mg a.s./kg dsw, *F. candida* $NOEC_{corr}$ = 2.8 mg X642188/kg dsw, *E. fetida*
Drinking water (Human toxicology)	Fenpicoxamid and X642188	0.1 µg/L 0.05 µg/L	Common Limit, Directive 2006/118/EC EFSA Journal 2018;16(1):5146
Surface water (Ecotoxicology)	Fenpicoxamid and X642188	0.05 µg/L NOEC = 0.37 µg a.s./L, *P. promelas* EC_{50} = 0.79 µg X642188/L, *D. magna*	EFSA Journal 2018;16(1):5146 Goudie, O. 2018, Study No. 180562
Air	Fenpicoxamid	~~15 µg/m³~~ LOQ = 0.5 µg for XDE-777 equivalent to 1.39 µg/m³ of ambient air and warm and humid air	EFSA Journal 2018;16(1):5146 AOEL: 0.05 mg/kg bw/d
~~Body tissues (meat or liver)~~	~~Fenpicoxamid~~	~~0.1 mg/kg~~	~~Common Limit, SANCO/825/00 rev. 8.1, Reg (EU) 283/2013, EFSA Journal 2018;16(1):5146~~
Body fluids (urine or blood)	Fenpicoxamid	0.05 mg/L	Common Limit, SANCO/825/00 rev. 8.1, Reg (EU) 283/2013, EFSA Journal 2018;16(1):5146

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 Fenpicoxamid in plant matrices is given in the following tables. These studies have already been evaluated during the EU approval process of the active substance (EFSA 2018).

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

Component of residue definition: Fenpicoxamid					
Matrix type	**Method Type**	**Method No.**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
High water content, high acid content, high oil content, high protein/high starch content (dry)	Primary/ Confirmatory	120615	0.01 mg/kg	LC-MS/MS	Watson, G., 2012, EU agreed
	ILV		0.01 mg/kg	LC-MS/MS	Chambers, J., Jarrett, H., 2013, EU agreed
	Primary/ Confirmatory (Multi-residue)	120998	0.01 mg/kg	LC-MS/MS	Lindner, M., Giesau, A., 2013, EU agreed
	ILV (Multi-residue)		0.01 mg/kg	LC-MS/MS	Amic, S., 2013, EU agreed

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Li, Q., Dixit, V., 2013, EU agreed Senciuc, M., 2021

Extraction efficiency for the primary method (Watson, G., 2012) was evaluated by comparing residue levels determined using the manual extraction procedure outlined in the method (acetonitrile/water, 90/10, v/v) to residue levels determined using the accelerated solvent extraction (ASE) procedure outlined in the wheat nature of residue (NOR) study (Ma, M., Jackson, A.U., 2013). Incurred radiolabeled samples, obtained from the wheat NOR study, were used for the quantitation of fenpicoxamid in both extraction procedures. Comparable extraction efficiency was demonstrated for any fenpicoxamid residue levels above the LOQ (Li, Q., Dixit, V., 2013).

In a more recent study, extraction efficiencies for the primary method (Watson, G., 2012) and the multi-residue method (Linder, M., Giesau, A., 2013) were evaluated by comparing residue levels determined using the extraction procedures outlined in the two analytical methods (Watson: acetonitrile/water (90/10, v/v); Linder: acetonitrile/water (1/1, v/v)) to residue levels determined using the ASE extraction procedure outlined in the wheat nature of residue (NOR) study (Ma, M., Jackson, A.U., 2013). Incurred samples from banana, barley grain, and oilseed rape matrices were used for quantitation of fenpicoxamid in all three extraction procedures. Satisfactory extraction efficiency was demonstrated for both analytical methods in determining fenpicoxamid residue levels (Senciuc, M., 2021).

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 Fenpicoxamid in animal matrices is given in the following tables. These studies have already been evaluated during the EU approval process of the active substance (EFSA 2018).

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

Component of residue definition: X12326349 expressed as fenpicoxamid					
Matrix type	**Method Type**	**Method No.**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Milk, eggs, muscle, fat, kidney, liver	Primary/ Confirmatory	130712	0.01 mg/kg	LC-MS/MS	Garcia-Alix, M., 2014, EU agreed
	ILV		0.01 mg/kg	LC-MS/MS	Lindner M., Grewe, D., 2014, EU agreed

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Garcia-Alix, M., 2014, EU agreed Extraction solvent used in the analytical method is identical to that used in the animal (ruminant) metabolism study (xxx, xxx., 2013): acetonitrile/water/phosphoric acid (75/25/0.1, v/v/v)

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 Fenpicoxamid in soil is given in the following tables. This study has already been evaluated during the EU approval process of the active substance (EFSA 2018).

Table 5.3-6: Validated methods for soil

Component of residue definition: Fenpicoxamid and X642188				
Method type	**Method No.**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Primary/ Confirmatory	131045	0.05 mg/kg	LC-MS/MS	Lindner, M.; Giesau A., 2014, EU agreed

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 Fenpicoxamid in surface and drinking water is given in the following tables. This study has already been evaluated during the EU approval process of the active substance (EFSA 2018).

Table 5.3-7: Validated methods for water

Component of residue definition: Fenpicoxamid and X642188					
Matrix Type	**Method Type**	**Method No.**	**Method LOQ**	**Method Principle**	**Author(s), year / missing / EU agreed**
Drinking water, Surface water	Primary/ Confirmatory	131046	0.05 µg/L	LC-MS/MS	Austin, R., Turner, R., 2014, EU agreed
	ILV		0.05 µg/L	LC-MS/MS	Lindner, M., Giesau, A., 2014b, 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 Fenpicoxamid in air is given in the following tables. This study has already been evaluated during the EU approval process of the active substance (EFSA 2018).

Table 5.3-8: Validated methods for air

Component of residue definition: Fenpicoxamid				
Method type	**Method No.**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Primary/ Confirmatory	120681	0.5 µg (1.39 µg/m^3)	LC-MS/MS	Bacher, R., 2012, 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 Fenpicoxamid in body fluids and tissues is given in the following table. This study has already been evaluated during the EU approval

process of the active substance (EFSA 2018).

Table 5.3-9: Methods for body fluids

Component of residue definition: Fenpicoxamid				
Method type	**Method No.**	**Method LOQ**	**Method Principle**	**Author(s), year / missing**
Primary/ Confirmatory	120682	0.05 mg/L	LC-MS/MS	Göcer, M., 2012, EU agreed

Table 5.3-10: Methods for body tissues

Component of residue definition: Fenpicoxamid				
Method type	**Method No.**	**Method LOQ**	**Method Principle**	**Author(s), year / missing**
Primary/ Confirmatory	130712	0.01 mg/kg	LC-MS/MS	Garcia-Alix, M., 2014, EU agreed

5.3.2.8 Other studies/ information

Not Required.

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 5.1.1/1	Jones, J. Evenson, M.	2017	Analytical Method and Validation for the Determination of Fenpicoxamid in GF-3308 Formulation DAS Report No.: DAS-AM-G-161106 Dow AgroSciences LLC GLP/GEP (Y/N): Y Published (Y/N): Y	N	DAS/Corteva Agriscience
KCP 5.1.1/2	Jacobson, P.	2018	Method Validation for the Determination of Degradants as Impurities (X12314005, X12019520, X12393285, X12335723) in GF-3308 DAS Report No.: DAS-AM-G-180924 Dow AgroSciences LLC GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCP 10.2.1/1	Bergfield, A.	2016	GF-3308: Growth Inhibition Test with the Unicellular Green Alga, Pseudokirchneriella subcapitata DAS# 160103 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.1/2	xxx	2016a	GF-3308: Acute Toxicity to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Flow-Through Test Conditions DAS# 160101 xxx GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS/Corteva Agriscience
KCP 10.2.1/3	Goudie, O.	2016b	GF-3308: Acute Toxicity to the Cladoceran, Daphnia magna, Determined Under Static Renewal Test Conditions DAS# 160102 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.1/4	Goudie, O.J.	2018	X1642188 (a metabolite of XDE-777): Acute Toxicity Test to Cladoceran, Daphnia magna, Determined Under Flow-Through Test Conditions DAS# 180562	N	DAS/Corteva Agriscience

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
			ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No		
KCP 10.2.1/5	Goudie, O.J	2020	GF-3307: A 48-Hour Static-Renewal Acute Toxicity Test with the Cladoceran (Daphnia magna) DAS Report No. 191366 Eurofins EAG Agroscience, LLC, Easton, Maryland, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.1/6	Goudie, O.J.	2021	GF-2925: A Static-Renewal Acute Toxicity Test with the Cladoceran (Daphnia magna) DAS Report No. 202284 Eurofins EAG Agroscience, LLC, Easton, Maryland, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.1/7	Hadsell, R. L., Hoover, E.	2014, revised 2018	GF-3307: Acute Toxicity to the Cladoceran, Daphnia magna, Determined Under Static-Renewal Test Conditions DAS Report No.140489 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.1/8	xxx	2018a	X12019520 (a metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Static-Renewal Test Conditions DAS# 180560 xxx GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS/Corteva Agriscience
KCP 10.2.1/9	xxx	2018b	X12446477 (metabolite of XDE-777): Acute Toxicity to the Rainbow Trout, Oncorhynchus mykiss, Determined Under Static-Renewal Test Conditions DAS# 180561 xxx GLP/GEP (Y/N): Yes Published (Y/N): No	Y	DAS/Corteva Agriscience
KCP 10.2.2/1	Beasley, J.	2018	X1642188 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, Chironomus riparius, Using Spiked Sediment DAS# 180563 ABC Laboratories, Inc., Columbia, Missouri, USA	N	DAS/Corteva Agriscience

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			GLP/GEP (Y/N): Yes Published (Y/N): No		
KCP 10.2.2/2	Dinehart, S.	2019	X642188 (a metabolite of XDE-777): A Prolonged Sediment Toxicity Test with *Lumbriculus variegatus* Using Spiked Sediment DAS# 180639 Eurofins EAG Agroscience, LLC, Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.2/3	Leak, T.	2018	X12335723 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, Chironomus riparius, Using Spiked Sediment DAS# 180564 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.3/2	Hicks, S.	2016	GF-3308: Population Effects Study in an Indoor Aquatic Microcosm with Daphnia magna DAS# 160126 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.2.3/3	Hicks, S.	2017	XDE-777: Population Effects Study in an Indoor Aquatic Microcosm with Daphnia magna DAS# 160125 ABC Laboratories, Inc., Columbia, Missouri, USA GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.3.1.2/1	Verge. E.	2020	GF-3308 - Honey Bee (*Apis mellifera* L.) 22 Day Larval Toxicity Test (Repeated Exposure) DAS# 190305 Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 10.3.1.2/2	Verge. E.	2017	GF-3308 - Assessment of Effects on the Adult Honey Bee, *Apis mellifera* L., in a 10 Day Chronic Feeding Test under Laboratory Conditions DAS# 160522 Eurofins Agroscience Services EcoChem / Eurofins Agroscience Services Ecotox GmbH GLP/GEP (Y/N): Yes	N	DAS/Corteva Agriscience

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
			Published (Y/N): No		
KCP 10.3.1.5/1	Kleinhenz, M.	2017	GF-3308 (XDE-777): Brood Development of the Honey Bee (Apis mellifera L.) in a Semi-Field Tunnel Study in Phacelia tanacetifolia in Germany 2016 DAS# 160515 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany GLP: Yes Published: No	N	DAS/Corteva Agriscience
KCP 5.3.2.2/05	Senciuc, M.	2021	Cross-Validation – Comparing Amounts of Fenpicoxamid Extracted from Samples of Barley Grain, Oil Seed Rapeseed and Banana with Incurred Residues using 3 Different Solvent Systems Lab Study No S20-01536; Sponsor Study No. 200456 EAG Laboratories GmbH, Ulm, Germany GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCA 6.3.1/01	White, T.	2016	Determination of Residues of XDE-777 And Pyraclostrobin, After Two Applications of GF-3309 To Spring And Winter Wheat, At 5 Sites In Northern Europe And 5 Sites In Southern Europe, 2015 Report No. S15-02628, DAS Study ID 150650 Eurofins AgroScience Services, Wilson, Derbyshire DE73 1AG, UK GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCA 6.3.1/02	Eversfield, S.	2016	Determination of Residues of XDE-777 And Pyraclostrobin After Two Applications of GF-3312 And After Two Applications of GF-2925 In Winter Wheat And Spring Wheat At 4 Sites In Northern Europe And 4 Sites In Southern Europe In 2014 Report No. S14-01569, DAS Study ID 140648 Eurofins Agroscience Services, Wilson, Derbyshire, DE73 8AG, UK GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCA 6.3.1/03	Eversfield, S.	2016	Determination of Residues of XDE-777 and Prothioconazole after Two Applications of GF-3307 and after Two Applications of GF-3310 in Winter Wheat and Spring Wheat at 4 sites in Northern Europe and 4 sites in Southern Europe in 2014, Report No. S14-01568, DAS Study ID 140649, Eurofins Agroscience Services Ltd GLP, Unpublished	N	DAS/Corteva Agriscience

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
KCA 6.3.1/04	White, T.	2016	Determination of Residues of XDE-777 and Prothioconazole after Two Applications of GF-3307 to Spring and Winter Wheat, at 5 sites in Northern Europe and 5 sites in Southern Europe, 2015, Report No. S15-02629, DAS Study ID 150649, Eurofins Agroscience Services Ltd GLP, Unpublished	N	DAS/Corteva Agriscience
KCA 6.3.1/05	White, T.	2017	Ddetermination of Residues of Fenpicoxamid (XDE-777) after Two Application of Gf-3308 to Spring And Winter Wheat, at 4 Sites in Northern Europe and 4 sites in Southern Europe, 2016. Report No: S16-03318, DAS Study ID 160393 Eurofins Agroscience Services Ltd GLP, Unpublished	N	DAS/Corteva Agriscience

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 4.1.1 (a)/1	Hamilton T	2013	Analytical Method and Validation for the Determination of Active Ingredient in XDE-777 Technical by Liquid Chromatography The Dow Chemical Company DAS Report No.: ML AL-2013-012856 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCA 4.1.1 /2	Kerbleski HK Hamilton TD Birk KH Zhang L	2013	Analytical Method and Validation for the Determination of Active Ingredient and Impurities in XDE-777 Technical by Liquid Chromatography The Dow Chemical Company DAS Report No.: ML AL-2013-005479 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCA 4.1.1 /3	Crispin TA Hamilton TD	2013	Analytical Method and Validation for the Determination of Residual Solvents and Process Impurities in XDE-777 Technical by Gas Chromatography The Dow Chemical Company	N	DAS/Corteva Agriscience

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**
			DAS Report No.: ML AL-2013-005805 GLP/GEP (Y/N): Y Published (Y/N): N		
KCP 5.2.2/01	Watson, G.	2012	XDE-777 and its Metabolite X642188 – Validation of the Method for the Determination of Residues of XDE-777 and its Metabolite X642188 in Crops by LC-MS/MS Eurofins Agroscience Services Ltd DAS Report No.: 120615 GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 5.2.2/02 (KCA 6.5.3/2)	Eversfield, S.	~~2017~~ 2015	Data generation method for Determination of Residues of XDE-777 in Grain and Processed Products after Two Applications of GF-2925 in Winter Wheat at 2 sites in Northern Europe and 2 sites in Southern Europe in 2014 Eurofins Agroscience Services Ltd DAS Report No.: 140696 GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 5.2.2/03 (KCA 6.4.2/01)	Rawle, N.W.	2013	Data generation method for XDE-777 Livestock Feeding Study: Magnitude of Residue in Milk, Muscle, Liver, Kidney and Fat of Lactating Dairy Cattle CEM Analytical Services Ltd. DAS Report No.: 130949 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS/Corteva Agriscience
KCP 5.2.2/04	Li, Q., Hasting, M., Slinkard, E.W.	2015	Method Validation Study for the Determination of XDE-777 and Its Metabolites in Soil by Liquid Chromatography with Tandem Mass Spectrometry Dow AgroSciences LLC, Indianapolis, Indiana, USA DAS Report No.: 141042 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCP 5.3.2.2/01	Chambers, J., Jarrett H.	2013	Independent Laboratory Validation: XDE-777 and X641288 Residue Determination in Crops (Revision) Battelle UK Ltd DAS Report No.: 120951 GLP/GEP (Y/N): Yes Published (Y/N): No	N	DAS/Corteva Agriscience
KCP 5.3.2.2/02	Lindner M Giesau A	2013	Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of XDE-777 and Its Metabolite X642188 in Matrices of Plant and Animal Origin	N	DAS/Corteva Agriscience

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
			Eurofins Agroscience Services Ltd DAS Report No.: 120998 GLP/GEP (Y/N): Y Published (Y/N): N		
KCP 5.3.2.2/03	Amic S	2013	Independent Laboratory Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of XDE-777 and Its Relevant Metabolite X642188 in Matrices of Plant and Animal Origin Eurofins Agroscience Services Chem SAS DAS Report No.: 130114 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCP 5.3.2.2/04	Li Q Dixit V	2013	Evaluation of the Extraction Efficiency in Analytical Method - Determination of XDE-777 and Its X642188 Metabolite in Agricultural Commodities Using Liquid Chromatography with Tandem Mass Spectrometry Detection Dow AgroSciences LLC DAS Report No.: 121023 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCA 6.2.1/1	Ma, M Jackson, U	2013	A NATURE OF THE RESIDUE STUDY WITH [^{14}C]-XR-777 APPLIED TO WHEAT Dow AgroSciences LLC; Research for Hire DAS Report No.: 110334 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCP 5.3.2.3/01	Garcia-Alix M	2014	Method Validation for the Determination of XDE-777 and Its Metabolite (X12326349) in Animal Matrices CEM Analytical Services DAS Report No.: 131027 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCP 5.3.2.3/02	Lindner M Grewe D	2014	Independent Laboratory Validation of an Analytical Method for the Determination of XDE-777 and its Metabolite X12326349 in Matrices of Animal Origin Eurofins Agrosciences Services DAS Report No.: 130712 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCA 6.2.3	xxx	2013	A NATURE OF THE RESIDUE STUDY IN THE RUMINANT WITH [14C]-XR-777	Y	DAS/Corteva

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**
	Adelfinskaya, Y		Dow AgroSciences LLC Southwest Bio-Labs, Inc. DAS Report No.: 110766 GLP/GEP (Y/N): Y Published (Y/N): N		Agriscience
KCP 5.3.2.4/01	Lindner M Giesau A	2014	Validation of an Analytical Method for the Determination of Residues of XDE-777 and its Metabolite X642188 in Soil and Sediment Eurofins Agrosciences Services DAS Report No.: 131045 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCP 5.3.2.5/01	Austin R Turner R	2014	Method Validation Study for the Determination of Residues of XDE-777 and Its Metabolite X642188 in Water by LC-MS/MS Battelle UK Ltd. DAS Report No.: 131046 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCP 5.3.2.5/02	Lindner M Giesau A	2014b	Independent Laboratory Validation of an Analytical Method for the Determination of XDE-777 and its Metabolite X642188 in Water Eurofins Agrosciences Services DAS Report No.: 130711 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCP 5.3.2.6/01	Bacher R	2012	The Development and Validation of a Method for the Analysis of XDE-777 in Air PTRL Europe GmbH DAS Report No.: 120681 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCP 5.3.2.7/01	Göcer M	2012	Development and Validation of an Analytical Method for the Determination of XDE-777 in Body Fluid(s) PTRL Europe GmbH DAS Report No.: 120682 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCP 5.1.1/1	Speak T	2012	Analytical Method for the Determination of XDE-777 in GF-2925 Dow AgroSciences (CZ) Ltd	N	DAS/Corteva

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
			DAS Report No.: DAS-AM-G-12-19 GLP/GEP (Y/N): Y Published (Y/N): N		Agriscience
KCA 6.3.1/06	Oxspring S	2013	Determination of residues of XDE-777 after two applications of GF-2807 in winter wheat and spring wheat at 6 sites in Northern Europe and 6 sites in Southern Europe 2011 Eurofins Agroscience Services Ltd DAS Report No.: 110414 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCA 6.3.1/07	Eversfield, S	2013	Determination of residues of XDE-777 after two applications of GF-2925 in winter wheat, spring wheat and durum wheat at 6 sites in Northern Europe and 6 sites in Southern Europe IN 2012 Eurofins Agroscience Services Ltd DAS Report No.: 120434 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCA 6.3.1/08	Eversfield, S	2015	Determination of residues of XDE-777 after two applications of gf-2925 in winter wheat and spring wheat at 4 sites in Northern Europe and 4 sites in Southern Europe in 2014 Eurofins Agroscience Services Ltd DAS Report No.: 140650 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCA 6.5.3/1	Tandy, R	2014	Determination of Residue of XDE-777 in grain and processed products after two applications of GF-2925 in winter wheat on 2 sites in Northern Europe and 2 sites in Southern Europe in 2012 Eurofins Agroscience Services Ltd DAS Report No.: 120435 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience
KCA 6.5.3/2	Eversfield, S	2015	Determination of Residues of XDE-777 in Grain and Processed Products after Two Applications of GF-2925 in Winter Wheat at 2 sites in Northern Europe and 2 sites in Southern Europe in 2014 Eurofins Agroscience Services Ltd DAS Report No.: 140696 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS/Corteva Agriscience

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for GF-3308

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

A 2.1.1.1 Analytical method 1

A 2.1.1.1.1.1 Method validation

Comments of zRMS:	The validation of the analytical method for the determination of XDE-777 in freshwater algal nutrient medium (FWAM) using LC-MS/MS was successfully performed following the EU guideline SANCO/3029/99 rev.4, except numer of replicates recoveries. The number of replicate recoveries (n=2 or n=3) assessed at each fortification level was less than described in the guideline (n=5) The mean recovery of each fortification level and the overall mean recovery value was between 70 – 120% with RSD < 20%. This is acceptable according to SANTE/2020/12830, Rev.1. LOQ = 0.985 mg GF-3308/L The validation parameters are acceptable. The method is considered fit for purpose.

Method Identifier No.:	160103 Amendment 1
Performing Laboratory:	ABC Laboratories, Inc. (now EAG Laboratories) Columbia, Missouri, USA
Reference:	KCP 10.2.1/1
Report:	Bergfield, A.; 2016; GF-3308: Growth Inhibition Test with the Unicellular Green Alga, *Pseudokirchneriella subcapitata*; ABC Laboratories, Inc. (now EAG Laboratories), Columbia, Missouri, USA; Lab Study No. 83496; DAS Study No. 160103 ; 09 June 2016; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	Yes The number of replicate recoveries (n=2 or n=3) assessed at each fortification level was less than described in the guideline (n=5)
GLP:	Yes
Acceptability:	Yes
Method Alterations:	None

MATERIALS AND METHODS

Method Principle
Residues of GF-3308, based upon the analysis of XDE-777, were determined from samples of freshwater algal nutrient medium (FWAM) by diluting with 0.2% formic acid in acetonitrile, rinsing the transfer device with 0.2% formic acid in acetonitrile and adding the rinse to the sample, centrifuging for 10 minutes at 3,400 rpm, transferring the supernatant to another culture tube. Further dilutions were performed using formic acid:ACN:water (0.1:50:50) to dilute within the range of the calibration curve, if necessary. The final sample was analysed for GF-3308, based upon the analysis of XDE-777, by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

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

Table A 1: Recovery results from method validation of GF-3308, based upon the analysis of XDE-777, (*m/z* 615.0/239.2) using the analytical method

Matrix	Analyte	Fortification level (mg GF-3308/L)	Mean Recovery (%)	RSD (%)	n	Comments
FWAM	XDE-777	0.985	112	NA	2	
FWAM	XDE-777	2.25	105	5	3	
FWAM	XDE-777	45.9	103	4	3	
FWAM	XDE-777	65.6	108	NA	2	

Table A 2: Characteristics for the analytical method used for validation of GF-3308, based upon the analysis of XDE-777, residues in FWAM

	GF-3308, based upon the analysis of XDE-777
Specificity	*m/z* 615.0/239.2 *m/z* 615.0/515.4 blank value <30% LOQ
Calibration (type, number of data points)	Linear regression analysis with 1/x weighting r≥0.995 5 data points
Calibration range	Concentration range of 0.0100-0.160 ng a.i./mL Sample equivalent range of 0.20-3.3 mg GF-3308/L (method check), 0.41-6.5 mg GF-3308/L (0-hour time point of the definitive test), 0.020-0.33 mg GF-3308/L (24-hour time point of the definitive test), and 0.00041-0.0065 mg GF-3308/L (72-hour time point of the definitive test)
Limit of determination/quantification	LOQ = 0.985 mg GF-3308/L

CONCLUSION
The method was considered acceptable for the determination of GF-3308, based upon the analysis of XDE-777, in FWAM due to acceptable precision and accuracy demonstrated within this study.

A 2.1.1.2 Analytical method 2

A 2.1.1.2.1.1 Method validation

Comments of zRMS:	The validation of the analytical method for the determination of XDE-777 in samples of freshwater using LC-MS/MS was successfully performed following the EU guideline SANCO/3029/99 rev.4, except numer of replicates recoveries. The number of replicate recoveries (N = 4) assessed at each fortifica-tion level was less than described in the guideline (N = 5). The mean recovery of each fortification level and the overall mean recovery value was between 70 – 110% with RSD < 20%. LOQ = 0.0140 mg GF-3308/L, equivalent to 0.070 ng XDE-777/mL The validation parameters are acceptable. The method is considered fit for purpose.

Method Identifier No.:	160103 Amendment 1
Performing Laboratory:	ABC Laboratories, Inc. (now EAG Laboratories) Columbia, Missouri, USA
Reference:	KCP 10.2.1/2
Report:	xxx; 2016; GF-3308: Acute Toxicity to the Rainbow Trout, *Oncorhynchus mykiss*, Determined Under Flow-Through Test Conditions; xxx; Lab Study No. 83494; DAS Study No. 160101 ; 08 July 2016; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4

Guideline Deviations:	Yes 1.The number of replicate recoveries (N = 4) assessed at each fortification level was less than described in the guideline (N = 5)
GLP:	Yes
Acceptability:	Yes
Method Alterations:	160101 Amendment 1 was based on 160103 Amendment 1. The original method was performed in freshwater algal nutrient medium (FWAM) instead of freshwater as used in this study. The original method included centrifugation, rinsing the culture tube, and adding the resulting rinse to the sample, none of which occurred in this study. The original method had MQLs of 0.20, 0.41, 0.020, and 0.00041 mg GF-3308/L and this study had an MQL of 0.0020 mg GF-3308/L. The original method used fortification levels of 0.985, 2.25, 45.9, and 65.6 mg GF-3308/L while this study used fortification levels of 0.0140 and 0.299 mg GF-3308/L.

MATERIALS AND METHODS

Method Principle

Residues of GF-3308, based upon the analysis of XDE-777, were determined from samples of freshwater by diluting with 0.2% formic acid in acetonitrile (ACN). Further dilutions were conducted, if necessary to dilute within the range of the calibration curve, using formic acid:ACN:water (0.1:50:50). The final sample was analysed for GF-3308, based upon the analysis of XDE-777, by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

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

Table A 3: Recovery results from method validation of GF-3308, based upon the analysis of XDE-777, (*m/z* 615.0/239.2) using the analytical method

Matrix	Analyte	Fortification level (mg GF-3308/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	XDE-777	0.0140	107	0	4	
Freshwater	XDE-777	0.299	104	5	4	

Table A 4: Characteristics for the analytical method used for validation of GF-3308, based upon the analysis of XDE-777, residues in freshwater

	GF-3308, based upon the analysis of XDE-777
Specificity	*m/z* 615.0/239.2 *m/z* 615.0/515.4 blank value <30% LOQ
Calibration (type, number of data points)	Linear regression analysis with 1/x weighting r≥0.995 5 data points
Calibration range	Concentration range of 0.0100-0.160 ng/mL Sample equivalent range of 0.0020-0.033 mg GF-3308/L
Limit of determination/quantification	LOQ = 0.0140 mg GF-3308/L, equivalent to 0.070 ng XDE-777/mL

CONCLUSION

The method was considered acceptable for the determination of GF-3308, based upon the analysis of XDE-777, in freshwater due to acceptable precision and accuracy demonstrated within this study.

A 2.1.1.3 Analytical method 3

A 2.1.1.3.1.1 Method validation

Comments of zRMS:	The analytical method of Goudie (2016) for the determination of XDE-777 in samples of freshwater by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4 (11/07/00), except numer of replicates recoveries. The number of replicate recoveries (N = 4) assessed at the highest fortification level was less than described in the guideline (N = 5). LOQ = 0.0279 mg GF-3308/L, equivalent to 0.066 ng XDE-777/mL The mean recovery of each fortification level and the overall mean recovery value was 70 – 120% with an RSD < 20%. This is acceptable according to SANTE/2020/12830, Rev.1. The validation parameters are acceptable. The method is considered fit for purpose.

Method Identifier No.:	160103 Amendment 1
Performing Laboratory:	ABC Laboratories, Inc. (now EAG Laboratories) Columbia, Missouri, USA
Reference:	KCP 10.2.1/3
Report:	Goudie, O.; 2016; GF-3308: Acute Toxicity to the Cladoceran, *Daphnia magna*, Determined Under Static-Renewal Test Conditions; ABC Laboratories, Inc. (now EAG Laboratories), Columbia, Missouri, USA; Lab Study No. 83495; DAS Study No. 160102 ; 01 December 2016; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	Yes The number of replicate recoveries (N = 4) assessed at the highest fortification level was less than described in the guideline (N = 5)
GLP:	Yes
Acceptability:	Yes
Method Alterations:	160102 Amendment 1 was based on 160103 Amendment 1. The original method was performed in freshwater algal nutrient medium (FWAM) instead of freshwater as used in this study. The original method included centrifugation, rinsing the culture tube, and adding the resulting rinse to the sample, none of which occurred in this study. The original method had MQLs of 0.20, 0.41, 0.020, and 0.00041 mg GF-3308/L and this study had an MQL of 0.0042 mg GF-3308/L. The original method used fortification levels of 0.985, 2.25, 45.9, and 65.6 mg GF-3308/L while this study used fortification levels of 0.0279 and 0.572 mg GF-3308/L.

MATERIALS AND METHODS

Method Principle
Residues of GF-3308, based upon the analysis of XDE-777, were determined from samples of freshwater by diluting with 0.2% formic acid in acetonitrile (ACN). Further dilutions were performed using formic acid:ACN:water (0.1:50:50) to dilute within the range of the calibration curve, if necessary. The final sample was analysed for GF-3308, based upon the analysis of XDE-777, by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

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

Table A 5: Recovery results from method validation of GF-3308, based upon the analysis of XDE-777, (*m/z* 615.0/239.2) using the analytical method

Matrix	Analyte	Fortification level (mg GF-3308/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	XDE-777	0.0279	113	8	6	
Freshwater	XDE-777	0.572	109	5	4	

Table A 6: Characteristics for the analytical method used for validation of GF-3308, based upon the analysis of XDE-777, residues in freshwater

	GF-3308, based upon the analysis of XDE-777
Specificity	*m/z* 615.0/239.2 *m/z* 615.0/515.4 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 5 data points
Calibration range	Concentration range of 0.0100 to 0.160 ng/mL Sample equivalent range of 0.0042-0.067 mg GF-3308/L
Limit of determination/quantification	LOQ = 0.0279 mg GF-3308/L, equivalent to 0.066 ng XDE-777/mL

CONCLUSION
The method was considered acceptable for the determination of GF-3308, based upon the analysis of XDE-777, in freshwater due to acceptable precision and accuracy demonstrated within this study.

A 2.1.1.4 Analytical method 4

A 2.1.1.4.1.1 Method validation

Comments of zRMS:	The analytical method of Goudie (2018) for the determination of X642188 (a metabolite of XDE-777) in samples of moderately hard freshwater by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ = 0.02 µg/L. The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is considered fit for purpose.

Method Identifier No.:	180563 Amendment 1
Performing Laboratory:	ABC Laboratories, Inc. (now EAG, Inc.) Columbia, Missouri, USA
Reference:	KCP 10.2.1/4
Report:	Goudie, O; 2018; X642188 (a metabolite of XDE-777): Acute Toxicity to the Cladoceran, Daphnia magna, Determined Under Flow-Through Test Conditions; ABC Laboratories, Inc. (now EAG, Inc.), Columbia, Missouri, USA; Lab Study No. 87148; DAS Study No. 180562 ; 30 August 2018; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes

Method Alterations: 180562 Protocol was based on 180563 Amendment 1, except that the matrix in 180562 Protocol was freshwater and the applicable matrix in 180563 Amendment 1 was freshwater (overlying water).

MATERIALS AND METHODS

Method Principle
Residues of X642188 were determined from samples of moderately hard freshwater by diluting with 0.2% formic acid in acetonitrile (ACN) and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. The final sample is analysed for X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

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

Table A 7: Recovery results from method validation of freshwater (*m/z* 515.00/124.00) using the analytical method

Matrix	Analyte	Fortification level (µg X642188/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	X642188	0.020	106	9	10	5 QC samples from definitive test analyses, ranging from 90 to 115%
Freshwater	X642188	30	99	5	10	5 QC samples from definitive test analyses, ranging from 93 to 107%

Table A 8: Characteristics for the analytical method used for validation of X642188 residues in freshwater

	X642188
Specificity	*m/z* 515.000/124.00 *m/z* 515.000/152.00 *m/z* 515.000/239.00 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.994 6 data points
Calibration range	Concentration range of 0.0050 – 0.16 ng/mL freshwater. Sample equivalent range of 0.010 – 0.32 mg X642188/L in freshwater
Limit of determination/quantification	LOQ = 0.02 µg/L

CONCLUSION
This method was successfully validated for the determination of X642188 in freshwater.

A 2.1.1.5 Analytical method 5

A 2.1.1.5.1.1 Method validation

Comments of zRMS:	The analytical method of Goudie, O.J., Schneider, S.Z., Zhang, L, and. Martin, K.H. (2020) for the determination of fenpicoxamid in samples of freshwater by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ=15.0 µg GF-3307/L (0.705 µg fenpicoxamid/L) The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%.

	The validation parameters are acceptable. The method is considered fit for purpose.

Method Identifier No.:	191366
Performing Laboratory:	Eurofins EAG Agroscience, LLC, Easton, Maryland, USA
Reference:	KCP 10.2.1/5
Report:	Goudie, O.J., Schneider, S.Z., Zhang, L, and. Martin, K.H.; 2020; GF-3307: A 48-Hour Static-Renewal Acute Toxicity Test with the Cladoceran (*Daphnia magna*); Eurofins EAG Agroscience, LLC, 8598 Commerce Drive, Easton, MD 21601, USA; Lab Study No. 379A-305; DAS Study No. 191366 ; 20 February 2020; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	None

MATERIALS AND METHODS

Method Principle

Residues of GF-3307, analyzed for fenpicoxamid and prothioconazole, are determined from samples of freshwater by diluting the samples into calibration curve range using 50:50: 0.1 (v/v/v) acetonitrile:freshwater:formic acid. The final sample is analysed for fenpicoxamid and prothioconazole by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

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

Table A 9: Recovery results from matrix fortification samples of GF-3307 analyzed for fenpicoxamid (*m/z* 615.200/239.000) using the analytical method

Matrix	Analyte	Fortification level (µg GF-3307/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	fenpicoxamid	15.0	94.5	1.71	5	5 QC samples from definitive test analyses, ranging from 92.6 to 97.5%
Freshwater	fenpicoxamid	520	99.6	9.27	5	5 QC samples from definitive test analyses, ranging from 93.7 to 116%

Table A 10: Recovery results from matrix fortification samples of GF-3307 analyzed for prothioconazole (m/z 334.100/326.000) using the analytical method

Matrix	Analyte	Fortification level (µg GF-3307/L)	Mean Recovery (%)	RSD (%)	n	Comments
freshwater	prothioconazole	15.0	96.9	4.62	5	5 QC samples from definitive test analyses, ranging from 90.9 to 103%
freshwater	prothioconazole	520	102	18.2	5	5 QC samples from definitive test analyses, ranging from 90.0 to 134%

Table A 11: Characteristics for the analytical method used for determination of GF-3307, analyzed

for fenpicoxamid and prothioconazole, residues in freshwater

	fenpicoxamid	prothioconazole
Specificity	*m/z* 615.200/239.000 blank value <30% LOQ	m/z 334.100/326.000 blank value <30% LOQ
Calibration (type, number of data points)	Linear regression analysis with 1/x weighting r≥0.998 5 data points	Linear regression analysis with 1/x weighting r≥0.999 5 data points
Calibration range	Concentration range of 0.240 – 4.00 µg a.i./L Sample equivalent range of 0.511-85.1 ug GF-3307/L	Concentration range of 0.240 – 4.00 µg a.i./L Sample equivalent range of 2.47 – 41.2 ug GF-3307/L
Limit of determination/quantification	LOQ=15.0 µg GF-3307/L (~~7.05~~ 0.705 µg fenpicoxamid/L) LOD = 4.50 µg GF-3307/L (~~2.12~~ 0.212 µg fenpicoxamid/L)	LOQ=15.0 µg GF-3307/L (1.46 µg prothioconazole/L) LOD = 4.50 µg GF-3307/L (0.437 µg prothioconazole/L)

CONCLUSION
The method was considered acceptable for the determination of GF-3307, analyzed for fenpicoxamid and prothioconazole, in freshwater.

A 2.1.1.6 Analytical method 6

A 2.1.1.6.1.1 Method validation

Comments of zRMS:	The analytical method of Goudie, O.J., Schneider, S.Z., Sneckenberger, G., and Zhang, L. (2021) for the determination of fenpicoxamid in samples of freshwater by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ=0.160 µg GF 2925/L (19.7 ng a.i./L) The mean recovery of each fortification level and the overall mean recovery value was 70 – 120% with an RSD < 20%. This is acceptable according to SANTE/2020/12830, Rev.1. The validation parameters are acceptable. The method is considered fit for purpose.

Method Identifier No.:	202284 Appendix 6
Performing Laboratory:	Eurofins EAG Agroscience, LLC Easton, Maryland, U.S.A.
Reference:	KCP 10.2.1/6
Report:	Goudie, O.J., Schneider, S.Z., Sneckenberger, G., and Zhang, L.; 2021; GF-2925: A Static-Renewal Acute Toxicity Test with the Cladoceran (*Daphnia magna*); Eurofins EAG Agroscience, LLC, 8598 Commerce Drive, Easton, MD 21601, USA; Lab Study No. 379A-343; DAS Study No. 202284 ; 05 March 2021; Unpublished
Guideline(s):	SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	None

MATERIALS AND METHODS

Method Principle
Residues of GF-2925 (analysed for active ingredient fenpicoxamid) are determined from samples of freshwater. The samples were diluted initially with 0.2% formic acid in acetonitrile to achieve a solvent

composition of 50 : 50 : 0.1 (v/v/v) acetonitrile : freshwater : formic acid. Additional dilutions were performed, as necessary to bring all samples into the range of the calibration curve, using 50 : 50 : 0.1 (v/v/v) acetonitrile : freshwater : formic acid. The final samples are analysed for fenpicoxamid by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

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

Table A 12: Method validation results for fenpicoxamid (*m/z* 615.200/239.000) using the analytical method

Matrix	Analyte	Fortification level (ng a.i./L)	Mean Recovery (%)	RSD (%)	n	Comments
freshwater	fenpicoxamid	19.7	111	8.8	5	
freshwater	fenpicoxamid	6150	108	14	5	

Table A 13: Characteristics for the analytical method used for analysis of GF-2925 (analysed for active ingredient fenpicoxamid) residues in freshwater

	GF-2925 (analysed for fenpicoxamid)
Specificity	*m/z* 615.2/239.0 (Q) *m/z* 615.2/515.1 (C) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.999 6 data points
Calibration range	Concentration range of 3.00-40.0 ng a.i./L (equivalent to 0.024-0.33 µg GF-2925/L)
Limit of determination/quantification	LOD=0.0480 µg GF-2925/L (5.90 ng a.i./L) LOQ=0.160 µg GF-2925/L (19.7 ng a.i./L)

CONCLUSION

The method was considered acceptable for the determination of GF-2925 (analysed for active ingredient fenpicoxamid) in freshwater because the precision of all matrix fortification samples and mean of the high-level matrix fortification samples and overall mean met acceptance criteria. The mean of the low-level matrix fortification samples slightly exceeded the acceptance criteria of 110% (111%).

A 2.1.1.7 Analytical method 7

A 2.1.1.7.1.1 Method validation

Comments of zRMS:	The analytical method of Hadsell, R. (2014) for the determination of fenpicoxamid in samples of freshwater by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ = 0.009 mg GF-3307/L, equivalent to 0.0217 ng a.i./mL. The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is considered fit for purpose.

Method Identifier No.: 140489 Amendment 1

Performing Laboratory: ABC Laboratories, Inc. (now EAG, Inc.)
Columbia, Missouri, USA

Reference: KCP 10.2.1/7

Report:	Hadsell, R.; 2014; GF-3307: Acute Toxicity to the Cladoceran, *Daphnia magna*, Determined Under Static-Renewal Test Conditions; ABC Laboratories, Inc. (now EAG, Inc.), Columbia, Missouri, USA; Lab Study No. 81070; DAS Study No. 140489 ; 28 August 2014, Revised 2018; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	N/A

MATERIALS AND METHODS

Method Principle
Residues of GF-3307, based on analysis of XDE-777, were determined from samples of freshwater by diluting with 0.2% formic acid in acetonitrile and, if necessary, further diluting with 0.1:50:50 acid: acetonitrile:water . The final sample was analysed for XDE-777 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

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

Table A 14: Recovery results from method validation of GF-3307, based on analysis of XDE-777, (*m/z* 615.0/239.2) using the analytical method

Matrix	Analyte	Fortification level (mg GF-3307/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	XDE-777	0.00900	98	4	4	
Freshwater	XDE-777	0.560	100	3	4	

Table A 15: Characteristics for the analytical method used for validation of GF-3307, based on analysis of XDE-777, residues in freshwater

	GF-3307, based on analysis of XDE-777
Specificity	*m/z* 615.0/239.2 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 7 data points
Calibration range	Concentration range of 0.0200-0.750 ng/ XDE-777mL Sample equivalent range of 0.00833-0.313 mg GF-3307/L
Limit of determination/quantification	LOQ = 0.009 mg GF-3307/L, equivalent to 0.0217 ng a.i./mL

CONCLUSION
The method was considered acceptable for the determination of GF-3307 based on XDE-777 in freshwater.

A 2.1.1.8 Analytical method 8

A 2.1.1.8.1.1 Method validation

Comments of zRMS:	The analytical method of xxx (2018) for the determination of X12019520 (a metabolite of XDE-777) in samples of moderately hard freshwater by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4.

	LOQ = 4.9 mg/L. The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The number of replicate recoveries (N = 4) assessed at the lowest fortification level was less than described in the guideline (N = 5). The validation parameters are acceptable. The method is suitable for fit for purpose.

Method Identifier No.:	180560 Protocol
Performing Laboratory:	xxx
Reference:	KCP 10.2.1/8
Report:	xxx.; 2018; X12019520 (a metabolite of XDE-777): Acute Tox-icity to the Rainbow Trout, Oncorhynchus mykiss, Determined Un-der Static-Renewal Test Conditions; xxx; Lab Study No. 87146; DAS Study No. 180560 ; 07 August 2018; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	180560 Protocol was based on 160128 Amendment 2.

MATERIALS AND METHODS

Method Principle

Residues of X12019520 (a metabolite of XDE-777) were determined from samples of moderately hard freshwater by diluting with 0.2% formic acid in acetonitrile, and, if necessary, further diluted with 0.1:50:50 formic acid:acetonitrile:water. The final sample was analysed for X12019520 by liquid chromatography system with tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

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

Table A 16: Recovery results from method validation of X12019520 (*m/z* 189.00/143.00) using the analytical method

Matrix	Analyte	Fortification level (mg X12019520/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	X12019520	4.9	106	7	4	
Freshwater	X12019520	14	110	5	9	

Table A 17: Characteristics for the analytical method used for validation of X12019520 residues in freshwater

	X12019520
Specificity	*m/z* 189.00/143.00 *m/z* 189.00/128.00 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.999 6 data points
Calibration range	Concentration range of 0.010-0.52 ng/mL Sample equivalent range of 0.80-42 mg X12019520/L
Limit of determination/quantification	LOQ = 4.9 mg/L

CONCLUSION
This method was successfully validated for the determination of X12019520 in freshwater.

A 2.1.1.9 Analytical method 9

A 2.1.1.9.1.1 Method validation

Comments of zRMS:	The analytical method of xxx (2018) for the determination of X12019520 (a metabolite of XDE-777) in samples of moderately hard freshwater by HPLC-UV has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ = 0.096 mg/L The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The number of replicate recoveries (N = 4) assessed at the lowest fortification level was less than described in the guideline (N = 5). The validation parameters are acceptable. The method is suitable for fit for purpose.

Method Identifier No.:	180561 Protocol
Performing Laboratory:	ABC Laboratories, Inc. (now EAG, Inc.) Columbia, Missouri, USA
Reference:	KCP 10.2.1/9
Report:	xxx 2018; X12446477 (a metabolite of XDE-777): Acute Tox-icity to the Rainbow Trout, Oncorhynchus mykiss, Determined Un-der Static-Renewal Test Conditions; xxx; Lab Study No. 87147; DAS Study No. 180561 ; 18 July 2018; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	180561 Protocol was based on 140485 Amendment 1.

MATERIALS AND METHODS

Method Principle
Residues of X12446477 (a metabolite of XDE-777) were determined from samples of moderately hard freshwater by diluting, if necessary, with HPLC water. The final sample was analysed for X12446477 by high performance liquid chromatography with ultraviolet detection (HPLC-UV).

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

Table A 18: Recovery results from method validation of X12446477 using the analytical method

Matrix	Analyte	Fortification level (mg X12446477/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	X12446477	0.096	101	1	4	
Freshwater	X12446477	17	106	1	9	

Table A 19: Characteristics for the analytical method used for validation of X12446477 residues in freshwater

	X12446477
Specificity	blank value <30% MQL
Calibration (type, number of data points)	linear regression analysis without weighting r≥0.999 6 data points
Calibration range	Concentration range of 0.050-1.6 mg/L
Limit of determination/quantification	LOQ = 0.096 mg/L

CONCLUSION
This method was successfully validated for the determination of X12446477 in freshwater.

A 2.1.1.10 Analytical method 10

A 2.1.1.10.1.1 Method validation

Comments of zRMS:	The analytical method of Beasley, J. (2018) for the determination of X642188 (a metabolite of XDE-777) in samples of sediment, freshwater and porewater by LC-MS/MS has been validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. The lowest fortification level for X642188 in porewater was 0.000020 mg/L, however precision and accuracy were unacceptable. LOQ = 0.000020 mg/L (overlaying water) LOQ = 14 mg/L (porewater) LOQ = 0.046 mg/kg (sediment) The mean recovery of each fortification level and the overall mean recovery value was 70 – 120% with an RSD < 20% (without freshwater (pore water) at 0.000020 mg/L level). The validation parameters are acceptable. The method is suitable for fit for purpose.

Method Identifier No.:	180563 Amendment 1
Performing Laboratory:	ABC Laboratories, Inc. (now EAG, Inc.) Columbia, Missouri, USA
Reference:	KCP 10.2.2/1
Report:	Beasley, J.; 2018; X642188 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, Chironomus riparius, Using Spiked Sediment; ABC Laboratories, Inc. (now EAG, Inc.), Columbia, Missouri, USA; Lab Study No. 87149; DAS Study No. 180563; 30-Aug-2018; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	Yes, method recoveries for X642188 were outside the acceptable range of 70-110%, and RSD values exceeded 20% at the 0.000020 mg/L concentration level in pore water. Although the method was not sufficiently demonstrated in pore water at the 0.000020 mg/L level, the analytical methods used to support this study were otherwise acceptable and authenticate the values driving the study endpoints. The overall scope and purpose of this study is unaffected by this guideline deviation.
GLP:	Yes

Acceptability: Yes
Method Alterations: None

MATERIALS AND METHODS

Method Principle

Residues of X642188 were determined from samples of sediment by centrifuging the sample to remove pore water (retained for subsequent analysis), then diluting with 0.1:50:50 formic acid:ACN:water, followed by shaking and centrifugation, and transferring the liquid layer to a Falcon tube. The shaking and centrifugation process was repeated two additional times with the resulting transferred liquid layers to the 50-mL Falcon tube, then the liquid was extracted by diluting with 0.1:50:50 formic acid:ACN:water, and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. Residues of X642188 were determined from samples of moderately hard freshwater (pore water) by centrifuging to utilize the supernatant, then diluting the supernatant with 0.2% formic acid in acetonitrile (ACN) and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. Residues of X642188 were determined from samples of moderately hard freshwater (overlaying water) by diluting with 0.2% formic acid in acetonitrile (ACN) and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. The final sample was analysed for X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration in sediment and freshwater (overlaying water), and at the 14 mg X642188/L in overlying and pore water were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). Mean recovery values at 0.000020 mg X642188/L in overlying water were higher than 110%, but the precision of the assay (%RSD) was < 20%, therefore were considered acceptable. Mean recovery values at 0.000020 mg X642188/L in freshwater (pore water) were higher than 110% and the precision of the assay (%RSD) was greater than 20%. Increased low spike (0.000020 mg X642188/L) recoveries in pore water may have been the result of matrix enhancement. The results obtained are summarised in the following tables.

Table A 20: Recovery results from method validation of X642188 (*m/z* 515.000/124.000) in sediment using the analytical method

Matrix	Analyte	Fortification level (mg X642188/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sediment	X642188	0.046	86	12	10	5 method validation samples + 5 QC samples from definitive test analyses, ranging from 70 to 121%
Sediment	X642188	16	89	11	10	5 method validation samples + 5 QC samples from definitive test analyses, ranging from 71 to 115%

Table A 21: Recovery results from method validation of X642188 (*m/z* 515.000/124.000) in freshwater (pore water) using the analytical method

Matrix	Analyte	Fortification level (mg X642188/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater (pore water)	X642188	0.000020	122	47	10	5 method validation samples + 5 QC samples from definitive test analyses, ranging from 83 to 263%
Freshwater (pore water)	X642188	14	98	11	10	5 method validation samples + 5 QC samples from definitive test analyses, ranging from 75 to 111%

Table A 22: Recovery results from method validation of X642188 (*m/z* 515.000/124.000) in freshwater (overlaying water) using the analytical method

Matrix	Analyte	Fortification level (mg X642188/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater (overlaying water)	X642188	0.000020	114	8	5	5 QC samples from definitive test analyses, ranging from 99 to 121%
Freshwater (overlaying water)	X642188	14	99	15	5	5 QC samples from definitive test analyses, ranging from 77 to 115%

Table A 23: Characteristics for the analytical method used for validation of X642188 residues in sediment and freshwater (pore and overlaying water)

	X642188
Specificity	*m/z* 515.000/124.000 *m/z* 515.000/152.000 *m/z* 515.000/239.000 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.997 6 data points
Calibration range	Concentration range of 0.0050 – 0.16 ng/mL in sediment and freshwater (pore and overlaying water). Sample equivalent range of 0.0038 – 0.12 mg X642188/kg in sediment and 0.000010 – 0.0032 mg X642188/L in freshwater (pore and overlaying water)
Limit of determination/quantification	LOQ = 0.000020 mg/L (overlaying water) LOQ = 14 mg/L (porewater) LOQ = 0.046 mg/kg (sediment)

CONCLUSION

This method was successfully validated for the determination of X642188 in sediment, freshwater and porewater (at the 14 mg/L concentration level). Although the method was unable to be validated in porewater at the 0.000020 mg/L level due to unacceptable precision and accuracy, the overall analytical supporting data has been demonstrated to be effective for supporting the purpose of this study.

A 2.1.1.11 Analytical method 11

A 2.1.1.11.1.1 Method validation

Comments of zRMS:	The analytical method of Dinehart, S. (2019) for the determination of X642188 (a metabolite

	of XDE-777) in samples of sediment and freshwater by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ=0.046 mg/kg in sediment LOQ=0.00033 mg/L in water The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is suitable for fit for purpose.

Method Identifier No.:	180563 Amendment 1
Performing Laboratory:	Eurofins EAG Agroscience, LLC Columbia, Missouri
Reference:	KCP 10.2.2/2
Report:	Dinehart, S.; 2019; X642188 (a metabolite of XDE-777): A Prolonged Sediment Toxicity Test with Lumbriculus variegatus Using Spiked Sediment; Eurofins EAG Agroscience, LLC, Columbia, Missouri; Lab Study No. 87169; DAS Study No. 180639; 23 October 2019; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	180639 Amendment No. 2 was based on 180563 Amendment 1 with no modification.

MATERIALS AND METHODS

Method Principle

Residues of X642188 were determined from samples of sediment by centrifuging the sample to remove pore water (retained for subsequent analysis), then diluting with 0.1:50:50 formic acid:ACN:water, followed by shaking and centrifugation, and transferring the liquid layer to a Falcon tube. The shaking and centrifugation process was repeated two additional times with the resulting liquid layers transferred to the 50-mL Falcon tube, then the liquid was extracted by diluting with 0.1:50:50 formic acid:ACN:water, and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. Residues of X642188 were determined from samples of moderately hard freshwater (pore water) by centrifuging to utilize the supernatant, then diluting the supernatant with 0.2% formic acid in acetonitrile (ACN) and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. Residues of X642188 were determined from samples of moderately hard freshwater (overlaying water) by diluting with 0.2% formic acid in acetonitrile (ACN) and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. The final sample was analysed for X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

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

Table A 24: Recovery results from method validation of X642188 (*m/z* 515.000/124.000) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sediment	X642188	0.046	85	4	5	5 QC samples from definitive test analyses, ranging from 81 to 89%
Sediment	X642188	98	91	3	5	5 QC samples from definitive test analyses,

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
						ranging from 86 to 94%

Table A 25: Recovery results from method validation of X642188 (*m/z* 515.000/124.000) using the analytical method

Matrix	Analyte	Fortification level (mg/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater (overlying water)	X642188	0.00033	97	7	5	5 QC samples from definitive test analyses, ranging from 87 to 105%
Freshwater (overlying water)	X642188	96	106	3	5	5 QC samples from definitive test analyses, ranging from 103 to 111%

Table A 26: Recovery results from method validation of X642188 (*m/z* 515.000/124.000) using the analytical method

Matrix	Analyte	Fortification level (mg/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater (pore water)	X642188	0.00033	95	5	5	5 QC samples from definitive test analyses, ranging from 89 to 100%
Freshwater (pore water)	X642188	96	102	4	5	5 QC samples from definitive test analyses, ranging from 97 to 107%

Table A 27: Characteristics for the analytical method used for validation of X642188 residues in sediment and freshwater

	X642188
Specificity	*m/z* 515.000/124.000 *m/z* 515.000/152.000 blank value <LOD
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.996 6 data points
Calibration range	Concentration range of 0.0050 – 0.16 ng/mL in sediment and freshwater (pore and overlaying water). Sample equivalent range of 0.0038 – 0.123 mg/kg in sediment and 0.00010 – 0.0032 mg/L in freshwater (pore and overlaying water)
Limit of quantification	LOQ=0.046 mg/kg in sediment LOQ=0.00033 mg/L in water

CONCLUSION
This method was successfully validated for the determination of X642188 in sediment and freshwater.

A 2.1.1.12 Analytical method 12

A 2.1.1.12.1.1 Method validation

Comments of zRMS:	The analytical method of Leak, T. (2018) for the determination of X12335723 (a metabolite of XDE-777) in samples of overlying water, pore water, and sediment.by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ = 0.015 mg/L (water) LOQ = 0.0069 mg/kg (sediment) The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%.

	The validation parameters are acceptable. The method is suitable for fit for purpose.

Method Identifier No.:	180564 Amendment 1
Performing Laboratory:	ABC Laboratories, Inc. (now EAG, Inc.) Columbia, Missouri, USA
Reference:	KCP 10.2.2/3
Report:	Leak, T.; 2018; X12335723 (a metabolite of XDE-777): Chronic Toxicity in Whole Sediment to Freshwater Midge, Chironomus ri-parius, Using Spiked Sediment; ABC Laboratories, Inc. (now EAG, Inc.), Columbia, Missouri, USA; Lab Study No. 87150; DAS Study No. 180564 ; 31 August 2018; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	None

MATERIALS AND METHODS

Method Principle

Residues of X12335723 were determined from samples of sediment by centrifuging the sample to remove pore water (retained for subsequent analysis), then diluting with 0.1:50:50 formic acid:acetonitrile (ACN):water, followed by shaking and centrifugation, and transferring the liquid layer to a Falcon tube. The shaking and centrifugation process was repeated two additional times with the resulting transferred liquid layers to the 50-mL Falcon tube, then the liquid was extracted by diluting with 0.1:50:50 formic acid:ACN:water, and, if necessary, further diluting with 0.1:25:75 formic acid:ACN:water. Residues of X12335723 were determined from samples of moderately hard freshwater (pore water) by centrifuging to utilize the supernatant, then diluting the supernatant with 0.2% formic acid in acetonitrile (ACN) and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. Residues of X12335723 were determined from samples of moderately hard freshwater (overlying water) by diluting with 0.2% formic acid in acetonitrile (ACN) and, if necessary, further diluting with 0.1:50:50 formic acid:ACN:water. The final sample is analysed for X12335723 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

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

Table A 28: Recovery results from method validation of X12335723 (*m/z* 357.300/257.000) in sediment using the analytical method

Matrix	Analyte	Fortification level (mg X12335723/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Sediment	X12335723	0.0069	95	13	10	5 method validation samples + 5 QC samples from definitive test analyses, ranging from 77 to 117%
Sediment	X12335723	17	92	9	10	5 method validation samples + 5 QC samples from definitive test analyses, ranging from 72 to 103%

Table A 29: Recovery results from method validation of X12335723 (m/z 357.300/257.000) in freshwater (pore water) using the analytical method

Matrix	Analyte	Fortification level (mg X12335723/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater (pore water)	X12335723	0.015	103	2	10	5 method validation samples + 5 QC samples from definitive test analyses, ranging from 99 to 106%
Freshwater (pore water)	X12335723	14	110	7	10	5 method validation samples + 5 QC samples from definitive test analyses, ranging from 92 to 118%

Table A 30: Recovery results from method validation of X12335723 (m/z 357.300/257.000) in freshwater (overlying water) using the analytical method

Matrix	Analyte	Fortification level (mg X12335723/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater (overlying water)	X12335723	0.015	102	2	5	5 QC samples from definitive test analyses, ranging from 100 to 106%
Freshwater (overlying water)	X12335723	14	110	5	5	5 QC samples from definitive test analyses, ranging from 101 to 115%

Table A 31: Characteristics for the analytical method used for validation of X12335723 residues in sediment and freshwater (pore and overlying water)

	X12335723
Specificity	*m/z* 357.300/257.000 *m/z* 357.300/239.000 *m/z* 357.300/211.000 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting / r≥0.998 6 data points
Calibration range	Concentration range of 0.0050 – 0.16 ng/mL in sediment and freshwater (pore and overlying water). Sample equivalent range of 0.0038 – 0.12 mg X12335723/kg in sediment and 0.0040 – 0.13 mg X12335723/L in freshwater (pore and overlying water)
Limit of determination/quantification	LOQ = 0.015 mg/L (water) LOQ = 0.0069 mg/kg (sediment)

CONCLUSION
This method was successfully validated for the determination of X12335723 in overlying water, pore water, and sediment.

A 2.1.1.13 Analytical method 13

A 2.1.1.13.1.1 Method validation

Comments of zRMS:	The analytical method of Hicks, S (2016) for the determination of XDE-777, and X642188 and X12255349 (XDE 777 metabolites) in samples of natural surface water (freshwater) by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ = 0.000050 mg/L for XDE-777

	LOQ = 0.0000040 mg/L for X642188 LOQ = 0.0000090 mg/L for X12255349 The mean recovery of each fortification level and the overall mean recovery value was 70 – 120% with an RSD < 20%. This is acceptable according to SANTE/2020/12830, Rev.1. The validation parameters are acceptable. The method is suitable for fit for purpose.

Method Identifier No.:	160128 Amendment 2
Performing Laboratory:	ABC Laboratories, Inc. (now EAG Laboratories) Columbia, Missouri, USA
Reference:	KCP 10.2.3/2
Report:	Hicks, S.; 2016; GF-3308: Population Effects Study in an Indoor Aquatic Microcosm with *Daphnia magna*; ABC Laboratories, Inc. (now EAG Laboratories), Columbia, Missouri, USA; Lab Study No. 83492; DAS Study No. 160126 ; 07 December 2016; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	Yes. The number of replicate recoveries (N = 1 or 2) assessed at some fortification levels was less than described in the guideline (N = 5)
GLP:	Yes
Acceptability:	Yes
Method Alterations:	160126 Amendment 1 was based on 160128 Amendment 2. The original method used fortification levels of 0.0500 and 120 µg XDE-777/L, 0.0040 and 30 µg X642188/L, and 0.0090 and 30 µg X12255349/L while this study used fortification levels of 0.000050, 0.00020, and 0.030 mg XDE-777/L, 0.0000040, 0.000016, and 0.030 mg X642188/L, and 0.0000090, 0.000046, and 0.030 mg X12255349/L.

MATERIALS AND METHODS

Method Principle

Residues of GF-3308, based on analysis of XDE-777, and X642188 and X12255349 (XDE-777 metabolites) were determined from samples of natural surface water (freshwater) by diluting with 0.2% formic acid in acetonitrile (ACN), centrifuging at 3,600 rpm for 10 minutes, and further diluting within the range of the calibration curve, as needed, with 0.1:50:50 formic acid:ACN:water. The final sample was analysed for XDE-777, X642188, and X12255349 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values of all XDE-777 and the 0.030 mg X642188/L fortification concentrations were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). Mean recovery values at 0.000016 mg X642188/L and all X12255349 fortification concentrations were higher than 110% but were still considered acceptable since the precision of the assay (%RSD) was less than 20%. Mean recovery values at 0.0000040 mg X642188/L fortification concentration were higher than 110% and the (%RSD) was greater than 20%. The results obtained are summarised in the following tables.

Table A 32: Recovery results from method validation of XDE-777 (*m/z* 615.0/239.2) using the analytical method

Matrix	Analyte	Fortification level (mg XDE-777/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	XDE-777	0.000050	100	7	5	5 QC samples from definitive test analyses, ranging from 93 to 112%
Freshwater	XDE-777	0.00020	102	10	2	2 QC samples from definitive test analyses,

Matrix	Analyte	Fortification level (mg XDE-777/L)	Mean Recovery (%)	RSD (%)	n	Comments
						ranging from 95 to 109%
Freshwater	XDE-777	0.030	99	9	5	5 QC samples from definitive test analyses, ranging from 84 to 105%

Table A 33: Recovery results from method validation of X642188 (m/z 515.1/239.0) using the analytical method

Matrix	Analyte	Fortification level (mg X642188/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	X642188	0.0000040	122	26	5	5 QC samples from definitive test analyses, ranging from 88 to 175%
Freshwater	X642188	0.000016	113	16	2	2 QC samples from definitive test analyses, ranging from 100 to 125%
Freshwater	X642188	0.030	107	7	5	5 QC samples from definitive test analyses, ranging from 100 to 117%

Table A 34: Recovery results from method validation of X12255349 (*m/z* 515.2/239.0) using the analytical method

Matrix	Analyte	Fortification level (mg X12255349/L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	X12255349	0.0000090	111	11	6	6 QC samples from definitive test analyses, ranging from 98 to 133%
Freshwater	X12255349	0.000046	111	NA	1	1 QC sample from definitive test analyses, at 111%
Freshwater	X12255349	0.030	111	12	6	6 QC samples from definitive test analyses, ranging from 90 to 127%

Table A 35: Characteristics for the analytical method used for validation of XDE-777 and X642188 residues in freshwater

	XDE-777	X642188	X12255349
Specificity	*m/z* 615.0/239.2 blank value <30% MQL	*m/z* 515.1/239.0 blank value <30% MQL	*m/z* 515.2/239.0 blank value <30% MQL
Calibration (type, number of data points)	linear regression analysis with 1/x weighting Representative y = 1,540,860x – 1,281.859 r≥0.995 6 data points	linear regression analysis with 1/x weighting Representative y = 1,161,291x + 353.8701 r≥0.995 6 data points	linear regression analysis with 1/x weighting Representative y = 636,955.4x + 235.2649 r≥0.99 6 data points
Calibration range	Concentration range of 0.0100-0.500 ng/mL Sample equivalent range of 0.0000200-0.00100 mg XDE-777/L	Concentration range of 0.00079-0.052 ng/mL Sample equivalent range of 0.0000016-0.000104 mg X642188/L	Concentration range of 0.0021-0.10 ng/mL Sample equivalent range of 0.0000042-0.00020 mg X12255349/L
Limit of determination/quantification	LOQ = 0.000050 mg/L	LOQ = 0.0000040 mg/L	LOQ = 0.0000090 mg/L

CONCLUSION
The method was considered acceptable for the determination of GF-3308, based on analysis of XDE-777, and X642188 and X12255349 (XDE-777 metabolites) in natural surface water (freshwater) based on acceptable precision and accuracy demonstrated within this study.

A 2.1.1.14 Analytical method 14
A 2.1.1.14.1.1 Method validation

Comments of zRMS:	The analytical method of Hicks, S (2016) for the determination of fenpicoxamid and its metabolites in samples of natural surface water (freshwater) by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. Method validation results are presented for XDE-777 only. LOQ = 0.0500 µg/L for XDE-777 The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is suitable for fit for purpose.

Method Identifier No.:	160125
Performing Laboratory:	ABC Laboratories, Inc. (now EAG Laboratories) Columbia, Missouri, USA
Reference:	KCP 10.2.3/3
Report:	Hicks, S.; 2016; XDE-777: Population Effects Study in an Indoor Aquatic Microcosm with *Daphnia magna*; ABC Laboratories, Inc. (now EAG Laboratories), Columbia, Missouri, USA; Lab Study No. 83491; DAS Study No. 160125; 14 August 2017; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	No

MATERIALS AND METHODS

Method Principle
Residues of fenpicoxamid and its metabolites were determined from samples of natural surface water (freshwater) by diluting with 0.2% formic acid in acetonitrile (ACN), centrifuging at 3,600 rpm for 10 minutes, and further diluting within the range of the calibration curve, as needed, with 0.1:50:50 formic acid:ACN:water. The final sample was analysed for fenpicoxamid and its metabolites by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION
For fenpicoxamid, all mean recoveries for all fortification levels were within the 70 - 110% range and all RSD values were ≤ 20%. For all metabolites, data from this study was not used to derive any ecotox risk assessment conclusions, so method validation results are negligible and not presented here. The results obtained for fenpicoxamid are summarised in the following table.

Table A 36: Recovery results from method validation of fenpicoxamid (*m/z* 615.0/239.2) using the analytical method

Matrix	Analyte	Fortification level (µg a.i./L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	fenpicoxamid	0.0500	102	9	11	
Freshwater	fenpicoxamid	120	102	10	11	

Table A 37: Characteristics for the analytical method used for validation of fenpicoxamid residues in freshwater

	Fenpicoxamid
Specificity	*m/z* 615.0/239.2 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 6 data points
Calibration range	Concentration range of 0.0100-0.500 ng/mL
Limit of determination/quantification	LOQ = 0.0500 µg/L

CONCLUSION
The method was considered acceptable for the determination of fenpicoxamid in natural surface water (freshwater) based on acceptable precision and accuracy demonstrated within this study.

A 2.1.1.15 Analytical method 15

A 2.1.1.15.1.1 Method validation

Comments of zRMS:	The analytical method of Vergé, E (2019) for the determination of fenpicoxamid in samples of larval diet and deionised water by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ=0.0306 mg a.i./kg, equivalent to 0.60 mg T.P./kg for larval honey bee diet samples LOQ=0.306 mg a.i./L, equivalent to 6.00 mg T.P./L for deionised water samples The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is suitable for fit for purpose.

Method Identifier No.:	190305
Performing Laboratory:	Eurofins Agroscience Services EcoChem GmbH Niefern-Öschelbronn, Germany
Reference:	KCP 10.3.1.2/1
Report:	Vergé, E.; 2019; GF-3308: Honey Bee (*Apis mellifera* L.) 22 Day Larval Toxicity Test (Repeated Exposure); Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany; Lab Study No. S19-00184; DAS Study No. 190305; 07 May 2020; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	No

MATERIALS AND METHODS

Method Principle
Concentrations of GF-3308, based on fenpicoxamid analysis, are determined from larval diet samples by extraction with acetonitrile/water (1:1, v/v). Samples are shaken, centrifuged, and, if necessary, diluted with acetonitrile/water (1:1, v/v) + 0.1 % formic and/or matrix blank extract. The final sample is analysed for fenpicoxamid by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

Concentrations of GF-3308, based on fenpicoxamid analysis, are determined from deionised water samples by extraction with acetonitrile/water (1:1, v/v) + 0.1 % formic. The final sample is analysed for fenpicoxamid by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

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

Table A 38: Recovery results from method validation of GF-3308, based on fenpicoxamid (*m/z* 615/239) analysis, using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Larval honey bee diet	Fenpicoxamid	0.60 mg T.P./kg, equivalent to 0.0306 mg a.i./kg	93	10	7	
		650 mg T.P./kg, equivalent to 33.2 mg a.i./kg	94	5	7	

Table A 39: Recovery results from method validation of GF-3308, based on fenpicoxamid (*m/z* 615/239) analysis, using the analytical method

Matrix	Analyte	Fortification level (mg/L)	Mean Recovery (%)	RSD (%)	n	Comments
Deionised Water	Fenpicoxamid	6.00 mg T.P./L, equivalent to 0.306 mg a.i./L	101	3	5	
		7150 mg T.P./L, equivalent to 365 mg a.i./L	95	3	5	

Table A 40: Characteristics for the analytical method used for validation of GF-3308 residues, based on fenpicoxamid analysis, in larval diet

	Fenpicoxamid
Specificity	*m/z* 615/239 (Q) *m/z* 615/515 (C) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 ≥ 8 data points
Calibration range	Concentration range of 0.1-10 ng/mL
Limit of determination/quantification	LOQ=0.0306 mg a.i./kg, equivalent to 0.60 mg T.P./kg

Table A 41: Characteristics for the analytical method used for validation of GF-3308 residues, based on fenpicoxamid analysis, in deionised water

	Fenpicoxamid
Specificity	*m/z* 615/239 (Q) *m/z* 615/515 (C) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 ≥ 8 data points
Calibration range	Concentration range of 0.25 ng/mL-10 ng/mL
Limit of determination/quantification	LOQ=0.306 mg a.i./L, equivalent to 6.00 mg T.P./L

CONCLUSION

This method was successfully validated for the determination of GF-3308, based on fenpicoxamid analysis, in larval diet and deionised water.

A 2.1.1.16 Analytical method 16
A 2.1.1.16.1.1 Method validation

Comments of zRMS:	An analytical method for the determination of XDE-777 in feeding solution was validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000. Specimen analysis was performed by dilution of feeding solution samples, direct injection and quantification by HPLC-MS/MS detection. The limit of quantification (LOQ) of the analytical method was 25.0 mg/L of test item (1.20 mg/L of XDE-777). The mean recoveries at each fortification level were in the range between 70% and 110% with relative standard deviations below 20%. The validation parameters are acceptable. The method is suitable for fit for purpose.

Method Identifier No.:	160522
Performing Laboratory:	Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, 75223 Niefern-Öschelbronn, Germany
Reference:	KCP 10.3.1.2/2
Report:	Vergé, E.; 2017; GF-3308 - Assessment of Effects on the Adult Honey Bee, *Apis mellifera* L., in a 10 Day Chronic Feeding Test under Laboratory Conditions, Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, D-75223 Niefern-Öschelbronn, Germany; Lab Study No. S16-02528; DAS Study No. 160522 ; 08 March 2017; Unpublished
Guideline(s):	Yes, SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	No

MATERIALS AND METHODS

Method Principle
Concentrations of GF-3308, based on fenpicoxamid analysis, are determined from samples of 50 % (w/v) aqueous sucrose solution by dilution with acetonitrile/water (1:1, v/v) + 0.1 % formic acid. The final sample is analysed for fenpicoxamid by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

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

Table A 42: Recovery results from method validation of GF-3308, based on fenpicoxamid (*m/z* 615/ 239) analysis, using the analytical method

Matrix	Analyte	Fortification level (mg T.P./L)	Mean Recovery (%)	RSD (%)	n	Comments
50 % (w/v) aqueous sucrose solution	fenpicoxamid	25 mg T.P./L, equivalent to 1.20 mg a.i./L	96	8	5	107, 100, 95, 89, 91
		Approx. 7300 mg T.P./L, equivalent to	107	4	5	112, 106, 109, 101, 109

Matrix	Analyte	Fortification level (mg T.P./L)	Mean Recovery (%)	RSD (%)	n	Comments
		350 mg a.i./L*				

*Actual fortification levels were 7300, 7180, 7140, 7640, and 7640 mg T.P./L

Table A 43: Characteristics for the analytical method used for validation of GF-3308 residues, based on fenpicoxamid analysis, in 50% (w/v) aqueous sucrose solution

	Fenpicoxamid
Specificity	*m/z* 615/239 (Q) *m/z* 615/515 (C) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.999 7 data points
Calibration range	Concentration range of 1.0 - 80 ng/mL , resp. 60 ng/mL for feeding solution samples
Limit of determination/quantification	LOQ= 1.20 mg a.i./L, equivalent to 25 mg T.P./L

CONCLUSION
This method was successfully validated for the determination of GF-3308, based on fenpicoxamid analysis, in 50% (w/v) aqueous sucrose solution.

A 2.1.1.17 Analytical method 17
A 2.1.1.17.1.1 Method validation

Comments of zRMS:	The analytical method of Kleinhenz, M (2017) for the determination of fenpicoxamid in samples of pollen and nectar by LC-MS/MS has been successfully validated in accordance with the EU guidance document SANCO/3029/99 rev. 4. LOQ = 0.01 mg/kg The mean recovery of each fortification level and the overall mean recovery value was 70 – 110% with an RSD < 20%. The validation parameters are acceptable. The method is suitable for fit for purpose.

Method Identifier No.:	160515
Performing Laboratory:	Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH Niefern-Öschelbronn, D-75223, Germany
Reference:	KCP 10.3.1.5/1
Report:	Kleinhenz, M.; 2017; GF-3308 (XDE-777): Brood Development of the Honey Bee (*Apis mellifera* L.) in a Semi-Field Tunnel Study in *Phacelia tanacetifolia* in Germany 2016; Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn, Germany; Lab Study No. S16-02036; DAS Study No. 160515 ; 30 March 2017; Unpublished
Guideline(s):	SANCO/3029/99 rev.4
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	None

MATERIALS AND METHODS

Method Principle

Residues of GF-3308, based on fenpicoxamid analysis, are determined from samples of pollen and nectar by extraction with acetonitrile/water (90:10, v/v). An aliquot of the extract is diluted with acetonitrile/water (90:10, v/v) and acidified with formic acid. The final sample extract is analysed for residues of fenpicoxamid by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

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

Table A 44: Recovery results from method validation of fenpicoxamid (*m/z* 615/515) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Pollen	fenpicoxamid	0.01	101	8	6	
Pollen	fenpicoxamid	0.1	101	2	6	
Pollen	fenpicoxamid	40	100	1	6	
Nectar	fenpicoxamid	0.01	99	4	6	
Nectar	fenpicoxamid	0. 1	98	3	6	

Table A 45: Characteristics for the analytical method used for validation of fenpicoxamid residues in pollen and nectar

	Fenpicoxamid
Specificity	*m/z* 615/515 (Q) *m/z* 615/239 (C) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 8 data points
Calibration range	Concentration range of 0.03 – 20 ng/mL, equivalent to 0.003 – 2 mg/kg
Limit of determination/quantification	LOQ = 0.01 mg/kg

CONCLUSION

This method was successfully validated for the determination of fenpicoxamid in pollen and nectar.

zRMS comments:
The following studies 150650, 140648, 140649, 150649, 160393 (KCA 6.3.1/01 – KCA 6.3.1/05) were performed using the same analytical method. The crop method used to analyze for fenpicoxamid residues in wheat studies 150650, 140648, 140649, 150649, 160393 was the EU agreed Method No. 120615 (Watson, G., 2012).
It has been checked that the LOQ level was tested in each of the study.

Conclusion:
The method should be considered as validated for the determination of XDE-777 in wheat (whole plant, grain, straw) with LOQ of 0.01 mg/kg. The residue trials 150650, 140648, 140649, 150649, 160393 (KCA 6.3.1/01 – KCA 6.3.1/05) can be taken into account for XDE-777.

A 2.1.1.18 Analytical method 18

A 2.1.1.18.1.1 Method validation

Comments of zRMS:	The analytical method (reported in Eurofins study no. S12-01537 / Dow AgroSciences study code 120615) used for the residues trials S15-02628 for the determination of fenpicoxamid (XDE-777) and its X642188 metabolite in spring and winter wheat (grains, straw and whole plants) using Liquid Chromatography Mass Spectrometry (LC-MS/MS) were validated with the following LOQ: Fenpicoxamid: 0.01 mg/kg in grain, straw and whole plants. X642188: 0.01 mg/kg in grain, straw and whole plants. The trials can be taken into account.

Data Point:	KCA 6.3.1/01
Report author:	White, T
Report year:	2016
Report title:	Determination of residues of XDE-777 and pyraclostrobin, after two applications of GF-3309 to spring and winter wheat, at 5 sites in Northern Europe and 5 sites in Southern Europe, 2015
Report No.:	150650
Testing Facility Report No.:	S15-02628
Method(s) used:	S12-01537 / Dow AgroSciences study number 120615
Guidelines followed in study:	SANCO/3029/99 rev.4
Deviation from current test guidelines:	No
Analytical Performing Laboratory:	Eurofins Agroscience Services Chem Ltd Wilson, Derbyshire, UK
GLP/Officially recognised testing facilities:	Yes
Acceptability/Reliability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of GF-3309, based on the analysis of XDE-777, X642188 and pyraclostrobin, were determined from wheat samples (whole plant, grain, and straw). XDE-777 and X642188 residues were extracted with acetonitrile/water (90/10, v/v) by homogenisation and shaking. Following extract dilution with acetonitrile/water/formic acid (90/10/0.1, v/v/v), the final sample was analysed for XDE-777 and X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS). Pyraclostrobin analysis is not summarized here.

RESULTS AND DISCUSSION

For XDE-777, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%) with few exceptions. Mean recovery value at 0.01 mg/kg was higher than 110% but is still considered acceptable since the precision of the assay (%RSD) was less than 20%. RSD value at 5 mg/kg was higher than 20% but still considered acceptable due to only minor deviation (20.9%).
For X642188, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%).
The results obtained are summarised in the following tables.

Table A 46: Recovery results from method validation of XDE-777 (m/z 615/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	XDE-777	0.01	98	12.9	5	79, 92, 106, 106, 109

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	XDE-777	5.0	81	20.9	5	100, 98, 64, 72, 70
Wheat, Grain	XDE-777	0.01	101	2.1	5	99, 103, 98, 101, 102
Wheat, Grain	XDE-777	0.1	95	4.2	5	100, 90, 95, 97, 92
Wheat, Straw	XDE-777	0.01	112	14.9	6	118, 124, 84, 114, 101, 129
Wheat, Straw	XDE-777	20	103	3.7	7	102, 105, 102, 107, 96, 107, 103

Table A 47: Recovery results from method validation of X642188 (m/z 515/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	X642188	0.01	98	11.3	5	82, 92, 110, 103, 104
Wheat, Whole plant	X642188	0.50	102	7.0	5	100, 96, 101, 98, 114
Wheat, Grain	X642188	0.01	101	1.1	5	102, 101, 100, 101, 99
Wheat, Grain	X642188	0.10	101	4.4	5	106, 94, 102, 102, 99
Wheat, Straw	X642188	0.01	109	11.7	6	102, 117, 116, 119, 114, 86
Wheat, Straw	X642188	0.1	102	-	1*	102
Wheat, Straw	X642188	1.0	102	4.6	5	104, 105, 103, 94, 105

* While only one fortification was done at 10x LOQ (0.1 mg/kg), five fortifications were done at the 100x LOQ (1.0 mg/kg) to encompass the maximum concentration of observed residues. This is in compliance with SANCO/3029/99 rev.4.

Table A 48: Characteristics for the analytical method used for validation of XDE-777 and X684188 residues in wheat (whole plant, grain and straw)

	XDE-777	X684188
Specificity	m/z 615/239 Quantification m/z 615/515 Confirmation blank value <30% LOQ	m/z 515/239 Quantification m/z 515/124 Confirmation blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 7 data points	linear regression analysis with 1/x weighting r≥0.99 7 data points
Calibration range	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)
Limit of quantitation	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg

CONCLUSION
This method was successfully validated for the determination of XDE-777 and X684188 in wheat (whole plant, grain, and straw).

A 2.1.1.19 Analytical method 19

A 2.1.1.19.1.1 Method validation

Comments of zRMS:	The analytical method (reported in Eurofins study no. S12-01537 / Dow AgroSciences study code 120615) used for the residues trials S14-01569 for the determination of fenpicoxamid (XDE-777) and its X642188 metabolite in spring and winter wheat (grains, straw and whole plants) using Liquid Chromatography Mass Spectrometry (LC-MS/MS) were validated with

	the following LOQ: Fenpicoxamid: 0.01 mg/kg in grain, straw and whole plants. X642188: 0.01 mg/kg in grain, straw and whole plants. The trials can be taken into account.

Data Point:	KCA 6.3.1/02
Report author:	Eversfield, S
Report year:	2017, Amended Report
Report title:	Determination of Residue of XDE-777 and Pyraclostrobin after Two Applications of GF-3312 and after Two Applications of GF-2925 in Winter Wheat and Spring Wheat at 4 sites in Northern Europe and 4 sites in Southern Europe in 2014
Report No.:	140648
Testing Facility Report No.:	S14-01569
Method(s) used:	S12-01537 / Dow AgroSciences study number 120615
Guidelines followed in study:	SANCO/3029/99 rev.4
Deviation from current test guidelines:	Yes, a minimum of 5 recoveries per fortification level was not achieved for each analyte.
Analytical Performing Laboratory:	Eurofins Agroscience Services Chem GmbH Hamburg, Germany
GLP/Officially recognised testing facilities:	Yes/Behörde für Gesundheit und Verbraucherschutz (BGV)
Acceptability/Reliability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of GF-3312 and GF-2925, based on the analysis of XDE-777, X642188, and pyraclostrobin, were determined from wheat samples (whole plant, grain, and straw). XDE-777 and X642188 residues were extracted with acetonitrile/water (90/10, v/v) by homogenisation and shaking. Following extract dilution with acetonitrile/water/formic acid (90/10/0.1, v/v/v), the final sample was analysed for XDE-777 and X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).
Pyraclostrobin analysis is not summarized here.

RESULTS AND DISCUSSION

A reduced method verification set was run slightly prior to field sample analysis (see Tables A49-A50). Procedural recoveries were run concurrently with field samples (see Tables A51-A52)
For XDE-777, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%).
For X642188, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%).
The results obtained are summarised in the following tables.

Table A 49: Recovery results from method verification of XDE-777 (m/z 615/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	XDE-777	0.01	93	5.4	3	96, 88, 92
Wheat, Whole plant	XDE-777	0.1	95	4.0	3	97, 98, 91

Table A 50: Recovery results from method verification of X642188 (m/z 515/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	X642188	0.01	106	4.9	3	100, 110, 107
Wheat, Whole plant	X642188	0.1	92	3.1	3	94, 94, 89

Table A 51: Recovery results from method validation (procedural recoveries) of XDE-777 (m/z 615/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	XDE-777	0.01	96	15	6	110,108,104, 98, 78,77
Wheat, Whole plant	XDE-777	0.1	101	7.5	6	103, 89, 110,108, 98, 99
Wheat, Whole plant	XDE-777	20	96	-	2	101, 91
Wheat, Grain	XDE-777	0.01	90	14	6	88, 107, 104, 87, 75, 80
Wheat, Grain	XDE-777	0.1	102	4.7	6	109, 96, 101, 100, 101, 107
Wheat, Straw	XDE-777	0.01	105	9.2	6	120, 106, 99, 100, 111, 93
Wheat, Straw	XDE-777	0.1	106	4.9	6	106, 104, 105, 104, 116, 101
Wheat, Straw	XDE-777	0.8	108	6.7	3	100, 114, 110
Wheat, Straw	XDE-777	20	109	4.7	3	113, 110, 103

Table A 52: Recovery results from method validation (procedural recoveries) of X642188 (m/z 515/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	X642188	0.01	100	5.9	6	103, 93, 107, 104, 94, 96
Wheat, Whole plant	X642188	0.1	104	4.7	6	105, 95, 108, 108, 102, 103
Wheat, Whole plant	X642188	20	97	-	2	98, 95
Wheat, Grain	X642188	0.01	100	8.1	6	101, 105, 105, 110, 90, 91
Wheat, Grain	X642188	0.1	106	5.7	6	109, 94, 107, 108, 109, 110
Wheat, Straw	X642188	0.01	107	6.0	6	119, 100, 105, 105, 109, 105
Wheat, Straw	X642188	0.1	109	5.1	6	116, 100, 108, 106, 113, 108
Wheat, Straw	X642188	0.8	104	13	3	88, 113, 110

Table A 53: Characteristics for the analytical method used for validation of XDE-777 and X642188 residues in wheat (whole plant, grain and straw)

	XDE-777	X642188
Specificity	m/z 615/239 Quantification m/z 615/515 Confirmation blank value <30% LOQ	m/z 515/239 Quantification m/z 515/124 Confirmation blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 min 6 data points	linear regression analysis with 1/x weighting r≥0.99 min 6 data points
Calibration range	Concentration range of 0.0075-0.375 ng/mL(equivalent sample concentration 0.003- 0.15 mg/kg)	Concentration range of 0.0075-0.375 ng/mL(equivalent sample concentration 0.003- 0.15 mg/kg)
Limit of quantitation	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg

CONCLUSION
This method was considered acceptable for the determination of XDE-777 and X684188 in wheat (whole plant, grain, and straw) based on acceptable precision and accuracy demonstrated within this study.

A 2.1.1.20 Analytical method 20

A 2.1.1.20.1.1 Method validation

Comments of zRMS:	The analytical method (reported in Eurofins study no. S12-01537 / Dow AgroSciences study code 120615) used for the residues trials S15-02629 for the determination of fenpicoxamid (XDE-777) and its X642188 metabolite in spring and winter wheat (grains, straw and whole plants) using Liquid Chromatography Mass Spectrometry (LC-MS/MS) were validated with the following LOQ: Fenpicoxamid: 0.01 mg/kg in grain, straw and whole plants. X642188: 0.01 mg/kg in grain, straw and whole plants. The trials can be taken into account.

Data Point:	KCA 6.3.1/04
Report author:	White, T
Report year:	2016
Report title:	Determination of residues of XDE-777 and prothioconazole, after two applications of GF-3307 to spring and winter wheat, at 5 sites in Northern Europe and 5 sites in Southern Europe, 2015
Report No.:	150649
Testing Facility Report No.:	S15-02629
Method(s) used:	S12-01537 / Dow AgroSciences study number 120615 Bayer Method No. 00598
Guidelines followed in study:	SANCO/3029/99 rev.4
Deviation from current test guidelines:	No
Analytical Performing Laboratory:	Eurofins Agroscience Services Chem Ltd Wilson, Derbyshire, UK
GLP/Officially recognised testing facilities:	Yes/Department of Health (U.K.)
Acceptability/Reliability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of GF-3307, based on the analysis of XDE-777, X642188, and prothioconazole-desthio, were determined from wheat samples.
XDE-777 and X642188 residues were extracted from samples of wheat (whole plant, grain, and straw) with acetonitrile/water (90/10, v/v) by homogenisation and shaking. Following extract dilution with acetonitrile/water/formic acid (90/10/0.1, v/v/v), the final sample was analysed for XDE-777 and X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).
Prothioconazole-desthio residues were extracted from samples of wheat (grain and straw) with acetonitrile/water (80/20, v/v) by homogenisation. Before extraction, a cysteine hydrochloride solution (250 mg/mL) was added for stabilization. Following extract dilution with acetonitrile/water (50/50, v/v) + 20 g/L cysteine HCl, the final sample was analysed for prothioconazole-desthio by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

For XDE-777, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%) with one exception. Mean recovery value at 0.01 mg/kg in straw was higher than 110% but is still considered acceptable since the precision of the assay (%RSD) was less than 20%.
For X642188, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%) with few exceptions. Mean recovery value at 0.01 mg/kg in grain and 5.0 mg/kg in straw was higher than 110% but is still considered acceptable since the precision of the assay (%RSD) was less than 20%.
For prothioconazole-desthio, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%).
The results obtained are summarised in the following tables.

Table A 54: Recovery results from method validation of XDE-777 (m/z 615/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	XDE-777	0.01	102	4.5	5	105, 95, 101, 101, 107
Wheat, Whole plant	XDE-777	5.0	92	2.1	5	89, 93, 93, 92, 94
Wheat, Grain	XDE-777	0.01	107	7.0	5	119, 102, 109, 102, 102
Wheat, Grain	XDE-777	0.1	101	2.2	5	101, 100, 104, 100, 98
Wheat, Straw	XDE-777	0.01	112	2.7	5	115, 113, 109, 109, 113
Wheat, Straw	XDE-777	20	109	3.1	5	113, 112, 106, 107, 106

Table A 55: Recovery results from method validation of X642188 (m/z 515/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	X642188	0.01	94	4.4	5	97, 96, 98, 88, 92
Wheat, Whole plant	X642188	0.5	93	4.1	5	86, 95, 94, 95, 94
Wheat, Grain	X642188	0.01	112	8.5	5	124, 102, 110, 120, 105
Wheat, Grain	X642188	0.1	107	6.1	5	107, 104, 108, 116, 98
Wheat, Straw	X642188	0.01	109	2.1	5	108, 108, 112, 106, 110
Wheat, Straw	X642188	5.0	111	5.4	5	106, 103, 114, 114, 117

Table A 56: Recovery results from method validation of prothioconazole-desthio (m/z 312/70) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Grain	Prothiconazole-desthio	0.01	103	9.5	5	97, 110, 97, 117, 95
Wheat, Grain	Prothiconazole-desthio	0.10	97	5.7	5	100, 92, 101, 90, 102
Wheat, Straw	Prothiconazole-desthio	0.05	100	7.6	6	97, 92, 102, 96, 100, 114
Wheat, Straw	Prothiconazole-desthio	0.50	-	-	1	108
Wheat, Straw	Prothiconazole-desthio	10	103	5.2	5	103, 94, 107, 105, 107

Table A 57: Characteristics for the analytical method used for validation of XDE-777 and X642188 residues in wheat (whole plant, grain and straw)

	XDE-777	X642188
Specificity	m/z 615/239 Quantification m/z 615/515 Confirmation blank value <30% LOQ	m/z 515/239 Quantification m/z 515/124 Confirmation blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 8 data points	linear regression analysis with 1/x weighting r≥0.99 8 data points
Calibration range	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)
Limit of quantitation	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg

Table A 58: Characteristics for the analytical method used for validation of prothioconazole-desthio residues in wheat (grain and straw)

	Prothioconazole-desthio
Specificity	m/z 312/70 Quantification m/z 312/125 Confirmation blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 6 data points
Calibration range	Grain: Concentration range of 0.025-2.5 ng/mL(equivalent sample concentration 0.003- 0.27 mg/kg) Straw: Concentration range of 0.075-5.0 ng/mL(equivalent sample concentration 0.016- 1.1 mg/kg)
Limit of quantitation	LOQ = 0.01 mg/kg (wheat grain) LOQ = 0.05 mg/kg (wheat straw)

CONCLUSION

This method was successfully validated for the determination of XDE-777 and X684188 in wheat (whole plant, grain, and straw) and for prothioconazole-desthio in wheat (grain and straw).

A 2.1.1.21 Analytical method 21

A 2.1.1.21.1.1 Method validation

Comments of zRMS:	The analytical method (reported in Eurofins study no. S12-01537 / Dow AgroSciences study code 120615) used for the residues trials S16-03318 for the determination of fenpicoxamid (XDE-777) and its X642188 metabolite in spring and winter wheat (grains, straw and whole plants) using Liquid Chromatography Mass Spectrometry (LC-MS/MS) were validated with the following LOQ: Fenpicoxamid: 0.01 mg/kg in grain, straw and whole plants. X642188: 0.01 mg/kg in grain, straw and whole plants. The trials can be taken into account.

Data Point:	KCA 6.3.1/05
Report author:	White, T
Report year:	2017
Report title:	Determination of residues of fenpicoxamid (XDE-777) after two application of GF-3308 to spring and winter wheat, at 4 sites in Northern Europe and 4 sites in Southern Europe, 2016
Report No.:	160393
Testing Facility Report No.:	S16-03318
Method(s) used:	S12-01537 / Dow AgroSciences study number 120615
Guidelines followed in study:	SANCO/3029/99 rev.4
Deviation from current test guidelines:	No
Analytical Performing Laboratory:	Eurofins Agroscience Services Chem Ltd Wilson, Derbyshire, UK
GLP/Officially recognised testing facilities:	Yes/Department of Health (U.K.)
Acceptability/Reliability:	Yes

MATERIALS AND METHODS

Method Principle
Residues of GF-3308, based on the analysis of XDE-777 and its metabolite X642188, were extracted from samples of wheat (whole plant, grain, and straw) with acetonitrile/water (90/10, v/v) by homogenisation and shaking. Following extract dilution with acetonitrile/water/formic acid (90/10/0.1, v/v/v), the final sample was analysed for XDE-777 and X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION
For XDE-777, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%).
For X642188, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%) with one exception. Mean recovery value at 0.5 mg/kg in whole plant was higher than 110% but is still considered acceptable since the precision of the assay (%RSD) was less than 20%.
The results obtained are summarised in the following tables.

Table A 59: Recovery results from method validation of XDE-777 (m/z 615/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	XDE-777	0.01	107	6.9	5	100, 104, 102, 118, 111 SD=7.4%

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	XDE-777	5.0	110	8.8	5	104, 104, 100, 119, 121 SD=9.7%
Wheat, Whole plant	XDE-777	10	105	1.5	3	103, 105, 106 SD=1.5%
Wheat, Grain	XDE-777	0.01	100	6.3	5	93, 99, 106, 106, 94 SD=6.3%
Wheat, Grain	XDE-777	0.1	106	1.2	5	107, 105, 107, 104, 106 SD=1.3%
Wheat, Straw	XDE-777	0.01	72	16.2	5	72, 78, 82, 74, 52 SD=11.6%
Wheat, Straw	XDE-777	20	99	2.8	5	100, 95, 101, 98, 102 SD=2.8%

Table A 60: Recovery results from method validation of X642188 (m/z 515/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	X642188	0.01	103	2.2	5	105, 100, 101, 103, 105 SD=2.3%
Wheat, Whole plant	X642188	0.5	112	3.2	5	108, 114, 108, 116, 113 SD=3.6%
Wheat, Grain	X642188	0.01	96	4.2	5	94, 94, 103, 97, 93 SD=4.1%
Wheat, Grain	X642188	0.10	100	1.4	5	101, 101, 101, 99, 98 SD=1.4%
Wheat, Straw	X642188	0.01	75	5.4	5	79, 77, 76, 72, 69 SD=4.0%
Wheat, Straw	X642188	1.0	97	3.4	5	101, 94, 100, 94, 96 SD=3.3%

Table A 61: Characteristics for the analytical method used for validation of XDE-777 and X642188 residues in wheat (whole plant, grain and straw)

	XDE-777	X642188
Specificity	m/z 615/239 Quantification m/z 615/515 Confirmation blank value <30% LOQ	m/z 515/239 Quantification m/z 515/124 Confirmation blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 7 data points	linear regression analysis with 1/x weighting r≥0.99 7 data points
Calibration range	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)
Limit of quantitation	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg

CONCLUSION

This method was successfully validated for the determination of XDE-777 and X684188 in wheat (whole plant, grain and straw).

A 2.1.1.22 Analytical method 22

Wheat samples (whole plant, grain and straw) from DAS Study 110414 and DAS Study 120434 were analysed for the same analytes (XDE-777 and X684188) at the same laboratory (Eurofins UK) around the same time (approximately July – October 2012) using the same analytical method (DAS # 120615) and LOQ (0.01 mg/kg). As such, method validation data can be considered together, further solidifying the conclusion that the method was successfully validated for the determination of XDE-777 and X684188 in wheat samples.

A 2.1.1.22.1.1 Method validation #1

Comments of zRMS:	The study has already been evaluated in DAR for XDE-777, Volume 3 - B.5 (UK, 2017).

Data Point:	KCA 6.3.1/06
Report author:	Oxspring, S
Report year:	2013
Report title:	Determination of residues of XDE-777 after two applications of GF-2807 in winter wheat and spring wheat at 6 sites in Northern Europe and 6 sites in Southern Europe 2011
Report No.:	110414
Testing Facility Report No.:	S11-01041
Method(s) used:	S12-01537 / Dow AgroSciences study number 120615
Guidelines followed in study:	SANCO/3029/99 rev.4
Deviation from current test guidelines:	Yes, a minimum of 5 recoveries per fortification level was not achieved for each analyte
Analytical Performing Laboratory:	Eurofins Agroscience Services Chem Ltd Wilson, Derbyshire, UK
GLP/Officially recognised testing facilities:	Yes
Acceptability/Reliability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of GF-2807, based on the analysis of XDE-777 and its metabolite X642188, were extracted from samples of wheat (whole plant, grain, and straw) with acetonitrile/water (90/10, v/v) by homogenisation and shaking. Following extract dilution with acetonitrile/water/formic acid (90/10/0.1, v/v/v), the final sample was analysed for XDE-777 and X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

For XDE-777, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%).
For X642188, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%).
The results obtained are summarised in the following tables.

Table A 62: Recovery results from method validation of XDE-777 (m/z 615/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	XDE-777	0.01	94	4.1	8	91, 90, 95, 91, 93, 102, 96, 95
Wheat, Whole	XDE-777	5.0	97	2.6	6	95, 93, 98, 96, 100, 98

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
plant						
Wheat, Whole plant	XDE-777	20	94	-	2	93, 94
Wheat, Grain	XDE-777	0.01	91	2.7	4	94, 92, 89, 89
Wheat, Grain	XDE-777	0.1	98	2.7	4	100, 99, 97, 94
Wheat, Straw	XDE-777	0.01	99	5.8	4	94, 95, 106, 102
Wheat, Straw	XDE-777	5.0	105	-	2	106, 103
Wheat, Straw	XDE-777	20	105	-	2	105, 104

Table A 63: Recovery results from method validation of X642188 (m/z 515/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	X642188	0.01	92	14.3	8	83, 84, 78, 79, 108, 112, 99, 95
Wheat, Whole plant	X642188	5.0	103	6.7	6	100, 95, 109, 113, 100, 99
Wheat, Whole plant	X642188	20	95	2.2	2	96, 93
Wheat, Grain	X642188	0.01	94	3.4	4	91, 91, 97, 96
Wheat, Grain	X642188	0.1	101	1.9	4	101, 98, 102, 102
Wheat, Straw	X642188	0.01	93	6.3	4	87, 89, 99, 97
Wheat, Straw	X642188	5.0	101	-	2	103, 98
Wheat, Straw	X642188	20	106	-	2	109, 103

Table A 64: Characteristics for the analytical method used for validation of XDE-777 and X642188 residues in wheat (whole plant, grain and straw)

	XDE-777	X642188
Specificity	m/z 615/239 Quantification m/z 615/515 Confirmation blank value <30% LOQ	m/z 515/239 Quantification m/z 515/124 Confirmation blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 7 data points	linear regression analysis with 1/x weighting r≥0.99 7 data points
Calibration range	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)
Limit of quantitation	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg

CONCLUSION
This method was considered acceptable for the determination of XDE-777 and X684188 in wheat (whole plant, grain, and straw) based on acceptable precision and accuracy demonstrated within this study.

A 2.1.1.22.1.2 Method validation #2

Comments of zRMS:	The study has already been evaluated in DAR for XDE-777, Volume 3 - B.5 (UK, 2017).

Data Point:	KCA 6.3.1/07
Report author:	Eversfield, S
Report year:	2013
Report title:	Determination of residues of XDE-777 after two applications of GF-2925 in winter wheat, spring wheat and durum wheat at 6 sites in Northern Europe and 6 sites in Southern Europe
Report No.:	120434
Testing Facility Report No.:	S12-01351
Method(s) used:	S12-01537 / Dow AgroSciences study number 120615
Guidelines followed in study:	SANCO/3029/99 rev.4
Deviation from current test guidelines:	Yes, a minimum of 5 recoveries per fortification level was not achieved for each analyte.
Analytical Performing Laboratory:	Eurofins Agroscience Services Chem Ltd Wilson, Derbyshire, UK
GLP/Officially recognised testing facilities:	Yes/Department of Health (U.K.)
Acceptability/Reliability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of GF-2925, based on the analysis of XDE-777 and its metabolite X642188, were extracted from samples of wheat (whole plant, grain and straw) with acetonitrile/water (90/10, v/v) by homogenisation and shaking. Following extract dilution with acetonitrile/water/formic acid (90/10/0.1, v/v/v), the final sample was analysed for XDE-777 and X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

For XDE-777, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%).
For X642188, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%) with one exception. Mean recovery value at 5.0 mg/kg for grain was higher than 110% but was still considered acceptable since the precision of the assay was very good.
The results obtained are summarised in the following tables.

Table A 65: Recovery results from method validation of XDE-777 (m/z 615/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	XDE-777	0.01	96	5.8	8	101, 89, 97, 96, 99, 104, 89, 90
Wheat, Whole plant	XDE-777	5.0	95	4.6	8	94, 89, 96, 102, 99, 98, 90, 95
Wheat, Grain	XDE-777	0.01	97	2.1	10	99, 98, 96, 96, 101, 94, 97, 96, 99, 98
Wheat, Grain	XDE-777	0.5	99	2.6	8	99, 99, 101, 99, 98, 95, 99, 104
Wheat, Grain	XDE-777	5.0	101	-	2	99, 103
Wheat, Straw	XDE-777	0.01	101	7.8	6	107, 110, 94, 90, 104, 98
Wheat, Straw	XDE-777	20	98	3.7	6	96, 99, 95, 95, 104, 101

Table A 66: Recovery results from method validation of X642188 (m/z 515/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	X642188	0.01	91	7.1	8	104, 94, 93, 86, 95, 92, 86, 81
Wheat, Whole plant	X642188	5.0	95	6.1	8	100, 93, 98, 103, 92, 85, 91, 97
Wheat, Grain	X642188	0.01	95	9.3	10	113, 105, 89, 88, 100, 95, 88, 89, 86, 96
Wheat, Grain	X642188	0.5	96	3.3	8	95, 99, 94, 95, 94, 90, 97, 100
Wheat, Grain	X642188	5.0	111	-	2	110, 112
Wheat, Straw	X642188	0.01	97	11.3	6	106, 97, 87, 83, 98, 112
Wheat, Straw	X642188	20	96	10.1	6	107, 104, 84, 85, 101, 97

Table A 67: Characteristics for the analytical method used for validation of XDE-777 and X642188 residues in wheat (whole plant, grain and straw)

	XDE-777	X642188
Specificity	m/z 615/239 Quantification m/z 615/515 Confirmation blank value <30% LOQ	m/z 515/239 Quantification m/z 515/124 Confirmation blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 7 data points	linear regression analysis with 1/x weighting r≥0.99 7 data points
Calibration range	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)
Limit of quantitation	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg

CONCLUSION
This method was considered acceptable for the determination of XDE-777 and X684188 in wheat (whole plant, grain, and straw) based on acceptable precision and accuracy demonstrated within this study.

A 2.1.1.23 Analytical method 23*

*When assessing method acceptability, it is important to note the commonalities between DAS Study 140650 (See A 2.1.1.23), DAS Study 140649 (See A 2.1.1.24), and DAS Study 120435 (See A 2.1.1.25). In all three studies, wheat grain samples were analysed for XDE-777 and X684188 using the same analytical method (DAS Study 120435) and LOQ (0.01 mg/kg) at the same laboratory (Eurofins UK) around the same time (approximately June – December 2014). In DAS 140650 (See A 2.1.1.23) and DAS Study 140649 (See A 2.1.1.24), wheat straw and whole plant samples were also analysed for XDE-777 and X684188. This emphasizes the conclusion that the method was successfully validated for the determination of XDE-777 and X684188 in wheat samples.

A 2.1.1.23.1.1 Method validation

Comments of zRMS:	The study has already been evaluated in DAR for XDE-777, Volume 3 - B.5 (UK, 2017).

Data Point: KCA 6.3.1/08
Report author: Eversfield, S
Report year: 2015

Report title:	Determination of residues of XDE-777 after two applications of GF-2925 in winter wheat and spring wheat at 4 sites in Northern Europe and 4 sites in Southern Europe in 2014
Report No.:	140650
Testing Facility Report No.:	S14-01414
Method(s) used:	S12-01537 / Dow AgroSciences study number 120615
Guidelines followed in study:	SANCO/3029/99 rev.4
Deviation from current test guidelines:	Yes, a minimum of 5 recoveries per fortification level was not achieved for each analyte.
Analytical Performing Laboratory:	Eurofins Agroscience Services Chem Ltd Wilson, Derbyshire, UK
GLP/Officially recognised testing facilities:	Yes/Department of Health (U.K.)
Acceptability/Reliability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of GF-2925, based on the analysis of XDE-777 and its metabolite X642188, were extracted from samples of wheat (whole plant, grain, and straw) with acetonitrile/water (90/10, v/v) by homogenisation and shaking. Following extract dilution with acetonitrile/water/formic acid (90/10/0.1, v/v/v), the final sample was analysed for XDE-777 and X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

For XDE-777, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%).
For X642188, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%).
The results obtained are summarised in the following tables.

Table A 68: Recovery results from method validation of XDE-777 (m/z 615/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	XDE-777	0.01	89	-	2	87, 90
Wheat, Whole plant	XDE-777	0.1	101	3	3	100, 104, 98
Wheat, Whole plant	XDE-777	5.0	90	-	1	90
Wheat, Grain	XDE-777	0.01	102	10.8	4	95, 91, 107, 115
Wheat, Grain	XDE-777	0.1	102	3.5	4	102, 97, 105, 104
Wheat, Straw	XDE-777	0.01	102	7.0	7	108, 103, 96, 91, 98, 109, 109
Wheat, Straw	XDE-777	0.05	102	6.7	3	110, 97, 100
Wheat, Straw	XDE-777	1.0	91	5.6	3	90, 87, 97
Wheat, Straw	XDE-777	20	87	-	2	88, 86

Table A 69: Recovery results from method validation of X642188 (m/z 515/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat, Whole plant	X642188	0.01	91	-	2	95, 86
Wheat, Whole plant	X642188	0.1	91	5.6	3	89, 87, 96
Wheat, Whole plant	X642188	5.0	90	-	1	90
Wheat, Grain	X642188	0.01	100	6.4	4	99, 91, 104, 105
Wheat, Grain	X642188	0.1	84	11.8	4	94, 90, 73, 78
Wheat, Straw	X642188	0.01	102	6.8	4	110, 106, 98, 95
Wheat, Straw	X642188	1.0	95	7.7	4	103, 100, 88, 90

Table A 70: Characteristics for the analytical method used for validation of XDE-777 and X642188 residues in wheat (whole plant, grain and straw)

	XDE-777	X642188
Specificity	m/z 615/239 Quantification m/z 615/515 Confirmation blank value <30% LOQ	m/z 515/239 Quantification m/z 515/124 Confirmation blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 7 data points	linear regression analysis with 1/x weighting r≥0.99 7 data points
Calibration range	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)
Limit of quantitation	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg

CONCLUSION
This method was considered acceptable for the determination of XDE-777 and X684188 in wheat (whole plant, grain, and straw) based on acceptable precision and accuracy demonstrated within this study.

A 2.1.1.24 Analytical method 24*

*When assessing method acceptability, it is important to note the commonalities between DAS Study 140650 (See A 2.1.1.23), DAS Study 140649 (See A 2.1.1.24), and DAS Study 120435 (See A 2.1.1.25). In all three studies, wheat grain samples were analysed for XDE-777 and X684188 using the same analytical method (DAS Study 120435) and LOQ (0.01 mg/kg) at the same laboratory (Eurofins UK) around the same time (approximately June – December 2014). In DAS 140650 (See A 2.1.1.23) and DAS Study 140649 (See A 2.1.1.24), wheat straw and whole plant samples were also analysed for XDE-777 and X684188. This emphasizes the conclusion that the method was successfully validated for the determination of XDE-777 and X684188 in wheat samples.

A 2.1.1.24.1.1 Method validation

Comments of zRMS:	The analytical method (reported in Eurofins study no. S12-01537 / Dow AgroSciences study code 120615) used for the residues trials S14-01568 for the determination of fenpicoxamid (XDE-777) and its X642188 metabolite in wheat (grains, straw and whole plants) using Liquid Chromatography Mass Spectrometry (LC-MS/MS) were validated with the following LOQ: Fenpicoxamid: 0.01 mg/kg in grain, straw and whole plants.

	X642188: 0.01 mg/kg in grain, straw and whole plants. The trials can be taken into account.

Data Point:	KCA 6.3.1/03
Report author:	Eversfield, S
Report year:	2016
Report title:	Determination of Residues of XDE-777 and Prothioconazole After Two Applications of GF-3307 and After Two Applications of GF-3310 in Winter Wheat and Spring Wheat at 4 Sites in Northern Europe and 4 Sites in Southern Europe in 2014
Report No.:	140649
Testing Facility Report No.:	S14-01568
Method(s) used:	S12-01537 / Dow AgroSciences study number 120615 Bayer Method No. 00598
Guidelines followed in study:	SANCO/3029/99 rev.4
Deviation from current test guidelines:	Yes, a minimum of 5 recoveries per fortification level was not achieved for each analyte
Analytical Performing Laboratory:	Eurofins Agroscience Services Chem Ltd Wilson, Derbyshire, UK
GLP/Officially recognised testing facilities:	Yes/Department of Health (U.K.)
Acceptability/Reliability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of GF-3307 and GF-3310, based on the analysis of XDE-777, X642188 and prothioconazole-desthio, were determined from wheat samples.
XDE-777 and X642188 residues were extracted from samples of wheat (whole plant, grain, and straw) with acetonitrile/water (90/10, v/v) by homogenisation and shaking. Following extract dilution with acetonitrile/water/formic acid (90/10/0.1, v/v/v), the final sample was analysed for XDE-777 and X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).
Prothioconazole-desthio residues were extracted from samples of wheat (grain and straw) with acetonitrile/water (80/20, v/v) by homogenisation. Before extraction, a cysteine hydrochloride solution (250 mg/mL) was added for stabilization. Following extract dilution with acetonitrile/water (50/50, v/v) + 20 g/L cysteine HCl, the final sample was analysed for prothioconazole-desthio by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

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

Table A 71: Recovery results from method validation of XDE-777 (*m/z* 615/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Whole Plant	XDE-777	0.01	101.5	6.5	4	105, 109, 96, 96
Whole Plant	XDE-777	1.0	90.0	8.5	6	91, 85, 87, 80, 101, 96
Whole Plant	XDE-777	5.0	104.0	-	2	102, 106
Grain	XDE-777	0.01	109.2	1.8	6	112, 106, 109, 109, 109, 110

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	XDE-777	0.1	107.5	3.4	4	109, 112, 105, 104
Straw	XDE-777	0.01	111.0	2.9	6	112, 114, 107, 108, 110, 115
Straw	XDE-777	40.0	104.5	-	2	104, 105

Table A 72: Recovery results from method validation of X642188 (*m/z* 515/239) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Whole Plant	X642188	0.01	101.3	15.1	4	114, 115, 87, 89
Whole Plant	X642188	1.0	86.8	5.8	4	86, 80, 90, 91
Grain	X642188	0.01	100.3	4.3	4	101, 97, 97, 106
Grain	X642188	0.1	101.5	3.6	4	97, 100, 104, 105
Straw	X642188	0.01	96.3	13.9	8	101, 90, 83, 103, 109, 72, 102, 110
Straw	X642188	5.0	101.0	8.5	6	104, 100, 107, 112, 88, 95

Table A 73: Recovery results from method validation of prothioconazole-desthio (*m/z* 312/70) using the analytical method

Matrix	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	Prothioconazole-desthio	0.01	107.2	2.0	5	104, 110, 107, 107, 108
Grain	Prothioconazole-desthio	0.1	105.4	2.0	5	102, 105, 107, 107, 106
Straw	Prothioconazole-desthio	0.05	101.2	6.8	9	113, 111, 99, 98, 100, 101, 100, 99, 90
Straw	Prothioconazole-desthio	5.0	103.3	5.8	7	105, 114, 107, 102, 100, 99, 96
Straw	Prothioconazole-desthio	10.0	105.0	1.0	3	105, 106, 104

Table A 74: Characteristics for the analytical method used for validation of XDE-777 and X642188 in wheat (whole plant, grain, and straw)

	XDE-777	X642188
Specificity	*m/z* 615/239 (Q) *m/z* 615/515 (C) blank value <30% LOQ	*m/z* 515/239 (Q) *m/z* 515/124 (C) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 8 data points	linear regression analysis with 1/x weighting r≥0.995 8 data points
Calibration range	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)
Limit of determination/quantification	LOQ = 0.01 mg/kg	LOQ = 0.01 mg/kg

Table A 75: Characteristics for the analytical method used for validation of prothioconazole-desthio residues in wheat (grain and straw)

	Prothioconazole-desthio
Specificity	*m/z* 312/70 (Q) *m/z* 312/125 (C) blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.995 7 data points
Calibration range	Grain: Concentration range of 0.025-5 ng/mL(equivalent sample concentration 0.003- 0.6 mg/kg) Straw: Concentration range of 0.075-10.0 ng/mL(equivalent sample concentration 0.016-2.1 mg/kg)
Limit of determination/quantification	LOQ = 0.01 mg/kg (wheat grain) LOQ = 0.05 mg/kg (wheat straw)

CONCLUSION
This method was considered acceptable for the determination of XDE-777 and X684188 in wheat (whole plant, grain, and straw) and for prothioconazole-desthio in wheat (grain and straw) based on acceptable precision and accuracy demonstrated within this study.

A 2.1.1.25 Analytical method 25*

*When assessing method acceptability, it is important to note the commonalities between DAS Study 140650 (See A 2.1.1.23), DAS Study 140649 (See A 2.1.1.24), and DAS Study 120435 (See A 2.1.1.25). In all three studies, wheat grain samples were analysed for XDE-777 and X684188 using the same analytical method (DAS Study 120435) and LOQ (0.01 mg/kg) at the same laboratory (Eurofins UK) around the same time (approximately June – December 2014). In DAS 140650 (See A 2.1.1.23) and DAS Study 140649 (See A 2.1.1.24), wheat straw and whole plant samples were also analysed for XDE-777 and X684188. This emphasizes the conclusion that the method was successfully validated for the determination of XDE-777 and X684188 in wheat samples.

A 2.1.1.25.1.1 Method validation

Comments of zRMS:	The study has already been evaluated in DAR for XDE-777, Volume 3 - B.5 (UK, 2017).

Data Point:	KCA 6.5.3/1
Report author:	Tandy, R
Report year:	2014
Report title:	Determination of Residue of XDE-777 in grain and processed products after two applications of GF-2925 in winter wheat on 2 sites in Northern Europe and 2 sites in Southern Europe in 2012
Report No.:	120435
Testing Facility Report No.:	S12-01369
Method(s) used:	S12-01537 / Dow AgroSciences study number 120615
Guidelines followed in study:	SANCO/3029/99 rev.4
Deviation from current test guidelines:	Yes, a minimum of 5 recoveries per fortification level was not achieved for each analyte.
Analytical Performing Laboratory:	Eurofins Agroscience Services Chem Ltd Wilson, Derbyshire, UK
GLP/Officially recognised testing facilities:	Yes/Department of Health (U.K.)
Acceptability/Reliability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of GF-2925, based on the analysis of XDE-777 and its metabolite X642188, were extracted from samples of wheat (grain and processed fractions) with acetonitrile/water (90/10, v/v) by homogenisation and shaking. Following extract dilution with acetonitrile/water/formic acid (90/10/0.1, v/v/v), the final sample was analysed for XDE-777 and X642188 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

For XDE-777, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%).
For X642188, mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%) with a few exceptions. Mean values at 0.1 mg/kg for cleaned grain, wholemeal flour, and dried starch were higher than 110% but were still considered acceptable since the precision of the assay was very good.
The results obtained are summarised in the following tables.

Table A 76: Recovery results from method validation of XDE-777 (m/z 615/239) using the analytical method

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	XDE-777	0.01	99	-	2	97, 101
Grain	XDE-777	0.1	101	-	2	102, 99
Grain Not Cleaned	XDE-777	0.01	96	-	2	97, 94
Grain Not Cleaned	XDE-777	0.1	99	-	2	98, 99
Cleaned Grain	XDE-777	0.01	84	4.5	4	83, 80, 89, 85
Cleaned Grain	XDE-777	0.1	94	3.6	4	93, 90, 95, 98
Grain after Conditioning	XDE-777	0.01	107	-	2	109, 105
Grain after Conditioning	XDE-777	0.1	109	-	2	109, 109
Shorts	XDE-777	0.01	96	-	2	94, 101
Shorts	XDE-777	0.1	104	-	2	103, 104
Fine Bran	XDE-777	0.01	104	-	2	102, 105
Fine Bran	XDE-777	0.1	107	-	2	113, 101
Coarse Bran	XDE-777	0.01	108	-	2	111, 104
Coarse Bran	XDE-777	0.1	108	-	2	107, 108
Total Bran	XDE-777	0.01	104	-	2	106, 102
Total Bran	XDE-777	0.1	101	-	2	101, 101
Middlings	XDE-777	0.01	98	-	2	96, 100
Middlings	XDE-777	0.1	99	-	2	96, 101
Refined Flour (Type 550)	XDE-777	0.01	108	-	2	106, 109
Refined Flour (Type 550)	XDE-777	0.1	108	-	2	107, 108
White Bread	XDE-777	0.01	91	-	2	91, 91
White Bread	XDE-777	0.1	93	-	2	93, 93

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wholemeal Flour	XDE-777	0.01	98	-	2	97, 99
Wholemeal Flour	XDE-777	0.1	97	-	2	96, 98
Wholemeal Bread	XDE-777	0.01	79	-	2	78, 79
Wholemeal Bread	XDE-777	0.1	96	-	2	95, 97
Dried Starch	XDE-777	0.01	90	19.4	4	64, 99, 96, 101
Dried Starch	XDE-777	0.1	104	4.7	4	101, 99, 110, 105
Dried Gluten	XDE-777	0.01	93	-	2	96, 89
Dried Gluten	XDE-777	0.1	100	-	2	103, 97
Gluten Feed Meal	XDE-777	0.01	104	-	2	105, 103
Gluten Feed Meal	XDE-777	0.1	97	-	2	94, 100
Wheat Germ	XDE-777	0.01	102	-	2	102, 102
Wheat Germ	XDE-777	0.1	99	-	2	100, 98

Table A 77: Recovery results from method validation of X642188 (m/z 515/239) using the analytical method

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	X642188	0.01	108	-	2	110, 106
Grain	X642188	0.1	106	-	2	115, 96
Grain Not Cleaned	X642188	0.01	90	-	2	87, 93
Grain Not Cleaned	X642188	0.1	89	-	2	86, 92
Cleaned Grain	X642188	0.01	98	-	2	100, 96
Cleaned Grain	X642188	0.1	114	-	2	111, 116
Grain after Conditioning	X642188	0.01	77	-	2	71, 82
Grain after Conditioning	X642188	0.1	73	-	2	68, 78
Shorts	X642188	0.01	84	4.8	4	84, 87, 86, 78
Shorts	X642188	0.1	91	1.9	4	93, 90, 89, 91
Fine Bran	X642188	0.01	82	-	2	83, 81
Fine Bran	X642188	0.1	91	-	2	89, 93
Coarse Bran	X642188	0.01	102	-	2	96, 107
Coarse Bran	X642188	0.1	97	-	2	98, 95
Total Bran	X642188	0.01	92	-	2	98, 86
Total Bran	X642188	0.1	97	-	2	96, 97
Middlings	X642188	0.01	90	-	2	88, 92
Middlings	X642188	0.1	97	-	2	95, 99
Refined Flour (Type 550)	X642188	0.01	94	-	2	99, 89

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Refined Flour (Type 550)	X642188	0.1	108	-	2	108, 108
White Bread	X642188	0.01	97	-	2	100, 94
White Bread	X642188	0.1	99	-	2	102, 95
Wholemeal Flour	X642188	0.01	110	-	2	103, 117
Wholemeal Flour	X642188	0.1	111	-	2	109, 112
Wholemeal Bread	X642188	0.01	90	-	2	91, 89
Wholemeal Bread	X642188	0.1	98	-	2	98, 98
Dried Starch	X642188	0.01	100	-	2	94, 105
Dried Starch	X642188	0.1	115	-	2	119, 110
Dried Gluten	X642188	0.01	109	-	2	111, 106
Dried Gluten	X642188	0.1	106	-	2	110, 101
Gluten Feed Meal	X642188	0.01	101	-	2	100, 101
Gluten Feed Meal	X642188	0.1	106	-	2	105, 106
Wheat Germ	X642188	0.01	92	-	2	91, 92
Wheat Germ	X642188	0.1	100	-	2	99, 100

Table A 78: Characteristics for the analytical method used for validation of XDE-777 and X642188 residues in wheat (processed fractions)

	XDE-777	X642188
Specificity	m/z 615/239 Quantification m/z 615/515 Confirmation blank value <30% LOQ	m/z 515/239 Quantification m/z 515/124 Confirmation blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 min 7 data points	linear regression analysis with 1/x weighting r≥0.99 min 7 data points
Calibration range	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)	Concentration range of 0.0075-1.0 ng/mL(equivalent sample concentration 0.003- 0.40 mg/kg)
Limit of quantitation	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg

CONCLUSION
This method was considered acceptable for the determination of XDE-777 and X684188 in wheat (grain and processed fractions) based on acceptable precision and accuracy demonstrated within this study.

A 2.1.1.26 Analytical method 26

A 2.1.1.26.1.1 Method validation

Comments of zRMS:	The study has already been evaluated in DAR for XDE-777, Volume 3 - B.5 (UK, 2017).

Data Point: KCA 6.5.3/2

Report author:	Eversfield, S
Report year:	~~2017~~ 2015
Report title:	Determination of Residues of XDE-777 in Grain and Processed Products after Two Applications of GF-2925 in Winter Wheat at 2 sites in Northern Europe and 2 sites in Southern Europe in 2014
Report No.:	140696
Testing Facility Report No.:	S14-02186
Method(s) used:	XDE-777/01369
Guidelines followed in study:	SANCO/3029/99 rev.4
Deviation from current test guidelines:	Yes, a minimum of 5 recoveries per fortification level was not achieved for each analyte.
Analytical Performing Laboratory:	Eurofins Agroscience Services Chem Ltd Wilson, Derbyshire, UK
GLP/Officially recognised testing facilities:	Yes/Department of Health (U.K.)
Acceptability/Reliability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of GF-2925, based on the analysis of XDE-777 and its metabolites (X642188, X12335723, X12019520, X12314005 and X12264475), were extracted from samples of wheat (grain and processed fractions) with acetonitrile/water/H_3PO_4 (90/10/0.1, v/v/v) by homogenisation and shaking.
For XDE-777, X642188, X12019520 and X12314005: initial extracts were diluted with acetonitrile/water/H_3PO_4 (10/90/0.1, v/v/v) and the final sample was analysed for XDE-777, X642188, X12019520 and X12314005 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).
For X12264475 and X12335723: following pH adjustment, extract concentration and re-dissolution of the initial extract, extracts were liquid-liquid partitioned with ethyl acetate. An aliquot of the aqueous layer was analysed for X12264475 and X12335723 by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

A reduced method verification set was run slightly prior to field sample analysis (see Tables A79-A84). For XDE-777, X642188, X12335723, X12019520, X12314005 and X12264475 mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%). Procedural recoveries were run concurrently with field samples (see Tables A85-A90). For XDE-777, X642188, X12335723, X12019520, X12314005 and X12264475 mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤ 20%) with few exceptions of the RSD. The data was still considered acceptable since the deviation was minor in all cases (RSD <22%).
The results obtained are summarised in the following tables.

Table A 79: Recovery results from method verification of XDE-777 (m/z 615/239) using the analytical method

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	XDE-777	0.01	94	3.8	3	95, 97, 90
Grain	XDE-777	0.1	95	3.4	3	93, 94, 99
Bran	XDE-777	0.01	91	5.4	3	97, 88, 89
Bran	XDE-777	0.1	99	5.2	3	93, 100, 103
Refined White Flour	XDE-777	0.01	108	6.1	3	114, 101, 110

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Refined White Flour	XDE-777	0.1	107	2.3	3	105, 110, 107
Wholemeal Bread	XDE-777	0.01	109	13.4	3	92, 115, 119
Wholemeal Bread	XDE-777	0.1	89	2.8	3	89, 92, 87

Table A 80: Recovery results from method verification of X642188 (m/z 515/239) using the analytical method

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	X642188	0.01	98	6.0	3	102, 100, 91
Grain	X642188	0.1	95	2.6	3	93, 95, 98
Bran	X642188	0.01	93	6.6	3	98, 94, 86
Bran	X642188	0.1	102	8.1	3	93, 109, 105
Refined White Flour	X642188	0.01	91	6.7	3	98, 88, 87
Refined White Flour	X642188	0.1	100	3.2	3	104, 98, 99
Wholemeal Bread	X642188	0.01	108	10.4	3	95, 114, 115
Wholemeal Bread	X642188	0.1	87	2.3	3	87, 89, 85

Table A 81: Recovery results from method verification of X12335723 (m/z 357/257) using the analytical method

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	X12335723	0.01	72	19.6	3	57, 74, 85
Grain	X12335723	0.1	94	5.0	3	99, 90, 92
Bran	X12335723	0.01	94	2.2	3	96, 95, 92
Bran	X12335723	0.1	96	0.6	3	95, 96, 96
Refined White Flour	X12335723	0.01	103	2.6	3	101, 102, 106
Refined White Flour	X12335723	0.1	102	3.9	3	106, 102, 98
Wholemeal Bread	X12335723	0.01	96	2.1	3	96, 98, 94
Wholemeal Bread	X12335723	0.1	99	1.2	3	98, 100, 98

Table A 82: Recovery results from method verification of X12019520 (m/z 189/143) using the analytical method

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	X12019520	0.01	94	4.0	3	96, 97, 90
Grain	X12019520	0.1	98	1.2	3	97, 99, 97
Bran	X12019520	0.01	88	6.3	3	94, 84, 85
Bran	X12019520	0.1	96	8.5	3	89, 105, 94

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Refined White Flour	X12019520	0.01	104	5.9	3	109, 105, 107
Refined White Flour	X12019520	0.1	107	0.5	3	107, 107, 108
Wholemeal Bread	X12019520	0.01	98	11.0	3	86, 103, 106
Wholemeal Bread	X12019520	0.1	85	2.0	3	86, 86, 83

Table A 83: Recovery results from method verification of X12314005 (m/z 277/189) using the analytical method

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	X12314005	0.01	96	6.8	3	96, 102, 89
Grain	X12314005	0.1	93	2.8	3	91, 92, 96
Bran	X12314005	0.01	90	9.2	3	97, 93, 81
Bran	X12314005	0.1	97	7.6	3	91, 105, 94
Refined White Flour	X12314005	0.01	103	4.4	3	108, 99, 102
Refined White Flour	X12314005	0.1	103	2.2	3	104, 100, 104
Wholemeal Bread	X12314005	0.01	101	4.3	3	96, 104, 103
Wholemeal Bread	X12314005	0.1	80	5.2	3	79, 85, 77

Table A 84: Recovery results from method verification of X12264475 (m/z 257/152) using the analytical method

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	X12264475	0.01	96	3.1	3	93, 99, 96
Grain	X12264475	0.1	98	6.0	3	102, 100, 91
Bran	X12264475	0.01	89	1.3	3	90, 88, 90
Bran	X12264475	0.1	87	3.0	3	90, 86, 85
Refined White Flour	X12264475	0.01	91	3.5	3	93, 92, 87
Refined White Flour	X12264475	0.1	90	4.0	3	93, 91, 86
Wholemeal Bread	X12264475	0.01	92	2.7	3	89, 92, 94
Wholemeal Bread	X12264475	0.1	91	2.2	3	91, 93, 89

Table A 85: Recovery results from method validation (procedural recoveries) of XDE-777 (m/z 615/239*) using the analytical method

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	XDE-777	0.01	92	10.1	6	97, 96, 95, 77, 103, 86
Grain	XDE-777	0.1	101	11.0	4	103, 94, 115, 90

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	XDE-777	0.5	100	-	2	90, 95, 105
Shorts	XDE-777	0.01	99	8.5	5	105, 84, 100, 102, 102
Shorts	XDE-777	0.1	96	12.6	5	103, 83, 114, 91, 91
Bran	XDE-777	0.01	106	3.9	4	105, 110, 109, 101
Bran	XDE-777	0.1	99	12.9	4	107, 83, 111, 94
Middlings	XDE-777	0.01	93	9.9	4	95, 81, 91, 103
Middlings	XDE-777	0.1	101	8.5	4	108, 90, 97, 107
Refined Flour (Type 550)	XDE-777	0.01	98	18.5	4	100, 76, 95, 120
Refined Flour (Type 550)	XDE-777	0.1	101	11.0	4	117, 97, 91, 100
White Bread	XDE-777	0.01	95	14.6	4	96, 88, 114, 82
White Bread	XDE-777	0.1	94	9.3	4	94, 92, 106, 85
Wholemeal Flour	XDE-777	0.01	94	9.1	4	101, 85, 102, 89
Wholemeal Flour	XDE-777	0.1	100	3.9	4	99, 98, 96, 105
Wholemeal Bread	XDE-777	0.01	92	16.4	4	91, 71, 104, 102
Wholemeal Bread	XDE-777	0.1	91	18.3	4	93, 71, 111, 87
Dried Starch	XDE-777	0.01	91	6.2	4	87, 87, 99, 91
Dried Starch	XDE-777	0.1	103	8.5	4	105, 91, 112, 102
Dried Gluten	XDE-777	0.01	98	7.9	4	94, 108, 98, 90
Dried Gluten	XDE-777	0.1	97	8.0	4	89, 93, 97, 107
Gluten Feed Meal	XDE-777	0.01	99	3.3	4	95, 97, 102, 101
Gluten Feed Meal	XDE-777	0.1	100	12.6	4	92, 91, 118, 98
Wheat Germ	XDE-777	0.01	92	12.6	4	95, 80, 85, 106
Wheat Germ	XDE-777	0.1	95	8.3	4	88, 89, 96, 105

*SRM m/z 615/515 was used for a single batch of sample analyses

Table A 86: Recovery results from method validation (procedural recoveries) of X642188 (m/z 515/239*) using the analytical method

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	X642188	0.01	96	4.4	6	98, 92, 89, 99, 97, 99
Grain	X642188	0.1	104	14.0	4	121, 93, 91, 112
Grain	X642188	0.5	105	-	2	99, 110
Shorts	X642188	0.01	94	14.2	5	109, 74, 92, 92, 103
Shorts	X642188	0.1	95	12.8	5	111, 88, 85, 85, 104
Bran	X642188	0.01	104	5.9	4	103, 97, 104, 112
Bran	X642188	0.1	97	10.2	4	104, 83, 97, 104
Middlings	X642188	0.01	89	15.4	4	85, 80, 81, 109

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Middlings	X642188	0.1	103	8.2	4	108, 98, 93, 111
Refined Flour (Type 550)	X642188	0.01	91	21.4	4	95, 62, 103, 102
Refined Flour (Type 550)	X642188	0.1	99	14.8	4	111, 81, 93, 111
White Bread	X642188	0.01	92	7.5	4	87, 91, 102, 88
White Bread	X642188	0.1	97	15.6	4	91, 94, 118, 83
Wholemeal Flour	X642188	0.01	96	13.3	4	103, 79, 108, 94
Wholemeal Flour	X642188	0.1	97	10.4	4	88, 90, 99, 110
Wholemeal Bread	X642188	0.01	91	18.5	4	89, 70, 111, 94
Wholemeal Bread	X642188	0.1	90	17.9	4	86, 75, 113, 87
Dried Starch	X642188	0.01	96	8.4	4	90, 89, 106, 99
Dried Starch	X642188	0.1	102	7.2	4	102, 92, 109, 106
Dried Gluten	X642188	0.01	100	7.0	4	91, 99, 108, 101
Dried Gluten	X642188	0.1	100	9.7	4	95, 89, 110, 106
Gluten Feed Meal	X642188	0.01	97	5.3	4	95, 102, 91, 101
Gluten Feed Meal	X642188	0.1	103	11.7	4	89, 98, 110, 116
Wheat Germ	X642188	0.01	94	10.5	4	98, 87, 85, 106
Wheat Germ	X642188	0.1	100	10.0	4	98, 92, 94, 114

*SRM m/z 515/124 was used for a single batch of sample analyses

Table A 87: Recovery results from method validation (procedural recoveries) of X12335723 (m/z 357/257) using the analytical method

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	X12335723	0.01	93	5.9	7	86, 93, 99, 98, 85, 94, 99
Grain	X12335723	0.1	108	3.9	4	105, 112, 110, 103
Grain	X12335723	0.5	85	13.7	3	90, 72, 94
Shorts	X12335723	0.01	90	14.4	4	107, 91, 78, 82
Shorts	X12335723	0.1	83	21.3	4	103, 91, 77, 62
Bran	X12335723	0.01	87	17.1	4	95, 65, 90, 97
Bran	X12335723	0.1	91	12.5	4	90, 75, 99, 99
Middlings	X12335723	0.01	96	7.2	5	102, 87, 95, 104, 93
Middlings	X12335723	0.1	98	12.9	5	119, 87, 92, 92, 98
Refined Flour (Type 550)	X12335723	0.01	91	14.5	4	106, 87, 96, 75
Refined Flour (Type 550)	X12335723	0.1	92	13.2	4	104, 95, 93, 75
White Bread	X12335723	0.01	79	9.0	4	70, 81, 87, 78
White Bread	X12335723	0.1	83	18.1	4	92, 61, 93, 86

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wholemeal Flour	X12335723	0.01	92	5.9	4	96, 94, 94, 84
Wholemeal Flour	X12335723	0.1	94	5.2	4	96, 100, 90, 90
Wholemeal Bread	X12335723	0.01	88	15.9	4	68, 87, 98, 97
Wholemeal Bread	X12335723	0.1	93	11.4	4	81, 92, 107, 93
Dried Starch	X12335723	0.01	96	6.7	4	97, 104, 93, 89
Dried Starch	X12335723	0.1	96	7.6	4	96, 103, 99, 86
Dried Gluten	X12335723	0.01	80	14.5	4	89, 89, 69, 90
Dried Gluten	X12335723	0.1	94	14.1	4	71, 83, 91, 113
Gluten Feed Meal	X12335723	0.01	98	7.2	4	95, 93, 108, 94
Gluten Feed Meal	X12335723	0.1	95	11.4	4	90, 87, 111, 92
Wheat Germ	X12335723	0.01	83	6.4	4	77, 80, 85, 89
Wheat Germ	X12335723	0.1	81	12.1	4	67, 82, 89, 86

Table A 88: Recovery results from method validation (procedural recoveries) of X12019520 (m/z 189/143) using the analytical method

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	X12019520	0.01	100	5.1	6	107, 93, 100, 105, 97, 100
Grain	X12019520	0.1	104	5.8	4	112, 106, 99, 100
Grain	X12019520	0.5	98	-	2	96, 100
Shorts	X12019520	0.01	98	12.8	4	109, 80, 98, 103
Shorts	X12019520	0.1	96	9.2	4	103, 83, 98, 99
Bran	X12019520	0.01	96	4.1	4	92, 94, 101, 97
Bran	X12019520	0.1	96	9.1	4	89, 88, 100, 106
Middlings	X12019520	0.01	89	11.4	4	85, 99, 77, 96
Middlings	X12019520	0.1	98	7.7	4	105, 99, 87, 99
Refined Flour (Type 550)	X12019520	0.01	96	11.8	4	107, 80, 98, 97
Refined Flour (Type 550)	X12019520	0.1	95	17.0	4	97, 72, 99, 110
White Bread	X12019520	0.01	99	3.4	4	96, 103, 99, 96
White Bread	X12019520	0.1	90	1.6	4	88, 91, 91, 90
Wholemeal Flour	X12019520	0.01	98	6.9	4	104, 91, 103, 93
Wholemeal Flour	X12019520	0.1	97	11.6	4	94, 85, 98, 112
Wholemeal Bread	X12019520	0.01	98	9.4	5	108, 87, 96, 107, 92
Wholemeal Bread	X12019520	0.1	89	9.3	5	89, 79, 94, 99, 82

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Dried Starch	X12019520	0.01	97	7.0	4	100, 88, 104, 96
Dried Starch	X12019520	0.1	97	10.2	4	106, 83, 98, 101
Dried Gluten	X12019520	0.01	89	14.5	4	86, 72, 94, 102
Dried Gluten	X12019520	0.1	91	9.1	4	83, 87, 93, 102
Gluten Feed Meal	X12019520	0.01	96	8.1	4	95, 105, 86, 96
Gluten Feed Meal	X12019520	0.1	97	9.4	4	86, 93, 105, 104
Wheat Germ	X12019520	0.01	93	9.5	4	99, 84, 87, 102
Wheat Germ	X12019520	0.1	95	6.1	4	87, 94, 96, 101

Table A 89: Recovery results from method validation (procedural recoveries) of X12314005 (m/z 277/189) using the analytical method

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	X12314005	0.01	95	8.7	6	94, 94, 103, 107, 85, 89
Grain	X12314005	0.1	105	9.6	4	119, 103, 96, 100
Grain	X12314005	0.5	95	-	2	93, 96
Shorts	X12314005	0.01	101	7.9	5	105, 87, 104, 104, 106
Shorts	X12314005	0.1	91	11.9	5	106, 77, 88, 88, 97
Bran	X12314005	0.01	103	7.1	4	96, 97, 106, 111
Bran	X12314005	0.1	96	9.6	4	94, 84, 101, 105
Middlings	X12314005	0.01	92	5.9	4	88, 92, 89, 100
Middlings	X12314005	0.1	99	6.9	4	106, 95, 91, 102
Refined Flour (Type 550)	X12314005	0.01	95	18.1	4	92, 72, 110, 106
Refined Flour (Type 550)	X12314005	0.1	98	20.9	4	110, 68, 103, 112
White Bread	X12314005	0.01	94	9.6	4	93, 89, 107, 87
White Bread	X12314005	0.1	94	9.1	4	91, 90, 107, 89
Wholemeal Flour	X12314005	0.01	96	7.6	4	107, 91, 94, 93
Wholemeal Flour	X12314005	0.1	94	9.3	4	81, 97, 95, 101
Wholemeal Bread	X12314005	0.01	94	13.4	4	100, 75, 102, 97
Wholemeal Bread	X12314005	0.1	90	14.1	4	92, 76, 106, 85
Dried Starch	X12314005	0.01	94	8.0	4	99, 84, 100, 91
Dried Starch	X12314005	0.1	95	13.2	4	106, 77, 97, 100
Dried Gluten	X12314005	0.01	93	3.7	4	90, 96, 96, 90
Dried Gluten	X12314005	0.1	95	9.6	4	90, 88, 93, 108
Gluten Feed Meal	X12314005	0.01	96	8.1	4	95, 93, 89, 107
Gluten Feed Meal	X12314005	0.1	98	10.4	4	86, 92, 105, 107

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Wheat Germ	X12314005	0.01	93	7.8	4	98, 89, 84, 99
Wheat Germ	X12314005	0.1	95	7.3	4	87, 94, 95, 104

Table A 90: Recovery results from method validation (procedural recoveries) of X12264475 (m/z 257/152*) using the analytical method

Matrix (wheat)	Analyte	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n	Comments
Grain	X12264475	0.01	89	13.8	7	88, 79, 101, 79, 78, 88, 110
Grain	X12264475	0.1	104	5.6	4	105, 96, 104, 110
Grain	X12264475	0.5	85	6.1	3	89, 79, 86
Shorts	X12264475	0.01	87	9.6	4	98, 89, 81, 80
Shorts	X12264475	0.1	86	7.9	4	94, 84, 78, 89
Bran	X12264475	0.01	93	7.9	4	87, 94, 103, 88
Bran	X12264475	0.1	87	3.7	4	84, 90, 84, 89
Middlings	X12264475	0.01	91	12.2	5	100, 91, 72, 98, 93
Middlings	X12264475	0.1	96	15.0	5	114, 90, 82, 108, 85
Refined Flour (Type 550)	X12264475	0.01	86	13.4	4	80, 96, 94, 72
Refined Flour (Type 550)	X12264475	0.1	87	9.1	4	87, 85, 98, 79
White Bread	X12264475	0.01	84	21.9	4	67, 110, 82, 77
White Bread	X12264475	0.1	88	2.2	4	87, 85, 89, 89
Wholemeal Flour	X12264475	0.01	81	9.1	4	81, 81, 90, 72
Wholemeal Flour	X12264475	0.1	84	6.7	4	85, 92, 80, 80
Wholemeal Bread	X12264475	0.01	82	12.3	4	69, 81, 93, 86
Wholemeal Bread	X12264475	0.1	94	8.4	4	86, 100, 102, 89
Dried Starch	X12264475	0.01	87	3.6	4	91, 84, 85, 87
Dried Starch	X12264475	0.1	87	17.5	4	96, 100, 85, 66
Dried Gluten	X12264475	0.01	73	8.3	4	69, 71, 70, 82
Dried Gluten	X12264475	0.1	82	11.2	4	79, 71, 93, 83
Gluten Feed Meal	X12264475	0.01	94	12.3	4	106, 80, 90, 101
Gluten Feed Meal	X12264475	0.1	90	14.0	4	81, 77, 101, 100
Wheat Germ	X12264475	0.01	84	7.4	4	83, 89, 87, 75
Wheat Germ	X12264475	0.1	85	5.8	4	79, 84, 86, 91

*SRM m/z 257/124 was used for a single batch of sample analyses

Table A 91: Characteristics for the analytical method used for validation of XDE-777, X642188, and X1234005 residues in wheat (grain and processed fractions)

	XDE-777	X642188	X12314005
Specificity	m/z 615/239 Quantification m/z 615/515 Confirmation blank value <30% LOQ	m/z 515/239 Quantification m/z 515/124 Confirmation blank value <30% LOQ	m/z 277/189 Quantification m/z 277/143 Confirmation blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 min 6 data points	linear regression analysis with 1/x weighting r≥0.99 min 6 data points	linear regression analysis with 1/x weighting r≥0.99 min 6 data points
Calibration range	Concentration range of 0.075-5.0 ng/mL(equivalent sample concentration 0.003- 0.20 mg/kg)	Concentration range of 0.075-5.0 ng/mL(equivalent sample concentration 0.003- 0.20 mg/kg)	Concentration range of 0.075-5.0 ng/mL(equivalent sample concentration 0.003- 0.20 mg/kg)
Limit of quantitation	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg

Table A 92: Characteristics for the analytical method used for validation of X12019520, X12335723, and X12264475 residues in wheat (grain and processed fractions)

	X12019520	X12335723	X12264475
Specificity	m/z 189/143 Quantification m/z 189/128 Confirmation blank value <30% LOQ	m/z 357/257 Quantification m/z 257/124 or m/z 357/152 Confirmation blank value <30% LOQ	m/z 257/152 Quantification m/z 257/124 Confirmation blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting r≥0.99 min 6 data points	linear regression analysis with 1/x weighting r≥0.99 min 6 data points	linear regression analysis with 1/x weighting r≥0.99 min 6 data points
Calibration range	Concentration range of 0.075-5.0 ng/mL(equivalent sample concentration 0.003- 0.20 mg/kg)	Concentration range of 0.15-10 ng/mL(equivalent sample concentration 0.003- 0.20 mg/kg)	Concentration range of 0.15-10 ng/mL(equivalent sample concentration 0.003- 0.20 mg/kg)
Limit of quantitation	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg

CONCLUSION

This method was considered acceptable for the determination of XDE-777 and its metabolites in wheat (grain and processed fractions) based on acceptable precision and accuracy demonstrated within this study.

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

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

A 2.1.2.1.1 Method validation/Extraction efficiency

Comments of zRMS:	The objective of this cross-validation study was to compare residue amounts of fenpicoxamid extracted from samples of barley grain, oil seed rapeseed and banana with incurred residues when extracting with solvent systems as used in method DAS#120615, and QuEChERS method (DAS#120998) and when extracting with solvent systems as were used in metabolism study DAS #110334, in accordance to the technical guideline on the evaluation of extraction efficiency of residue analytical methods, SANTE 2017/10632, rev. 3. For XDE-777, the average residue values from the Method 1 (MOR Method, DAS #120615) and Method 2 (MRM Method, DAS # 120998) are similar to the residue values obtained from the ASE extraction, Method 3 (NOR Method, DAS #110334) for all three matrices. The extraction efficiency results obtained by MOR Method (DAS #120615) and MRM Method (DAS # 120998) were higher than 70% when compared with the results obtained for the method NOR Method (DAS #110334). The average of % extracted ranged from 107-118%. The %RSDs were calculated to be less than 20%. This study has proven the satisfactory extraction efficiency of the extraction used in the analytical methods (MOR Method/ DAS #120615, MRM Method/DAS # 120998) for the quantitative determination of residues of XDE-777 when compared with the NOR Method/DAS #110334 for fenpicoxamid (XDE-777) in banana, barley grain and oilseed rape seed matrices. The study is acceptable.

Method Identifier No.:	120615, 120988, 110334
Performing Laboratory:	EAG Laboratories GmbH Ulm, Germany
Reference:	KCP 5.3.2.2/05
Report:	Senciuc, M.; 2021; Summary of Cross-Validation - Comparing Amounts of Fenpicoxamid Extracted from Samples of Barley Grain, Oil Seed Rapeseed and Banana with Incurred Residues using 3 Different Solvent Systems; EAG Laboratories GmbH; Ulm, Germany; Lab Study No. Study No. S20-01536; DAS Study No. 200456; 28 January 2021; Unpublished
Guideline(s):	Yes, OPPTS 860.1340, SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4, SANTE 2017/10632 rev.3, Dir98-02
Guideline Deviations:	No
GLP:	Yes
Acceptability:	Yes
Method Alterations:	None

STUDY SUMMARY

This study was conducted to evaluate the extraction efficiency of Dow AgroSciences residue analytical method DAS#120615 "XDE-777 and its Metabolite X642188 – Validation of the Method for the Determination of Residues of XDE-777 and its Metabolite X642188 in Crops by LC-MS/MS" and Dow AgroSciences residue analytical method DAS#120998, "Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of XDE-777 and Its Metabolite X642188 in Matrices of Plant and Animal Origin" with respect to NOR Study DAS# 110334 "A Nature of the Residue Study with [14C]-XR-777 Applied to Wheat". This method is applicable for the quantitative

determination of residues Fenpicoxamid (XDE-777), in agricultural commodities (wet crops, dry crops, and oily crops).
Incurred residues are extracted from banana fruit, barley grain and oilseed rape seeds using acetonitrile/water, 90/10 v/v (analytical method 120998) and acetonitrile/water, 50/50 v/v followed by cleaned up using PSA/magnesium sulfate (analytical method 120998). Extracted residue levels are determined by LC-MS/MS. The method limit of quantitation (LOQ) is 0.01 mg/kg (ppm). The methods are considered suitable for enforcement purposes based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340, SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1, as well as PMRA Regulatory Directive Dir98-02.
Results obtained by Method 1 (MOR Method, DAS #120615) and Method 2 (MRM Method, DAS # 120998) are similar to the residue values obtained from the ASE extraction, Method 3 (NOR Method, DAS #110334) for all three matrices. The % RSDs were calculated to be less than 20%. The average of % extracted ranged from 107%-118%, if considering that the residue extracted by NOR Method, DAS #110334 is 100%.
The extraction efficiency results obtained by MOR Method (DAS #120615) and MRM Method (DAS # 120998) were higher than 70% when compared with the results obtained for the method NOR Method (DAS #110334).

Extraction efficiency results obtained when compared with NOR Method: DAS #110334	Banana	Barley Grain	Oilseed Rape Seeds
MOR Method: DAS #120615	115%	115%	118%
MRM Method: DAS # 120998	118%	111%	107%

This study has proven the satisfactory extraction efficiency of the extraction used in the analytical methods (MOR Method/ DAS #120615, MRM Method/DAS # 120998) for the quantitative determination of residues of XDE-777 when compared with the NOR Method/DAS #110334 for fenpicoxamid (XDE-777) in banana, barley grain and oilseed rape seed matrices.
Extraction efficiency is acceptable based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340, SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1, as well as PMRA Regulatory Directive Dir98-02.

MATERIALS AND METHODS

Test Item(s)

Test item (Common name):	Fenpicoxamid (XDE-777)
Purity:	98.7%
Description (physical state):	White powder
Lot/batch no.:	SYN-FS08251-080 / TSN 302306

Method Scope

This method is applicable for the quantitative determination of residues Fenpicoxamid (XDE- 777) in agricultural commodities (banana, barley grain, oilseed rapeseed). The method was concurrently validated over the concentration range of 0.01-0.1 mg/kg, except barley grain with a range of 0.01 to 2.0 mg/kg, always with a validated limit of quantitation of 0.01 mg/kg.

Method Principle

Residues of Fenpicoxamid (XDE- 777) are extracted from incurred samples with acetonitrile/water, 90/10 v/v for analytical method 120615 and respectively with acetonitrile/water, 50/50 v/v for analytical method 120998. The final sample is analysed for Fenpicoxamid (XDE- 777), by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC- MS/MS).
Within the nature of residue study, residues of Fenpicoxamid (XDE- 777), are extracted from samples by using acetonitrile containing 0.1% phosphoric acid following by acetonitrile/water/phosphoric acid 50/50/0.1 v/v/v. The final sample is analysed for Fenpicoxamid (XDE- 777), by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

Linearity
For analyte, the linearity of detector response was evaluated using matrix-matched standards, except for banana extracted using the analytical method from DAS study 120615. Calibration curves were calculated by linear regression analysis with 1/x weighting. For analytical method from DAS study 120615 and DAS study 110334, calibration curves resulting from the injection of at least 5 standards over the concentration range of 0.0075-1.0 ng/mL (or the sample equivalent range of 0.003-0.4 mg/kg) demonstrated linearity with correlation coefficients (r) of at least 0.999. For analytical method listed in DAS study 120998, calibration curves resulting from the injection of at least 5 standards over the concentration range of 0.075-5.0 ng/mL (or the sample equivalent range of 0.003-0.20 mg/kg) demonstrated linearity with correlation coefficients (r) of at least 0.999.

Selectivity

Table A 93: Transitions monitored

Fenpicoxamid (XDE- 777)	m/z Q1/Q3 615/239 (quantitative)
Fenpicoxamid (XDE- 777)	m/z Q1/Q3 615/515 (confirmatory)*

* this transition was only monitored, but not reported.

RESULTS AND DISCUSSION

Extraction Efficiency
Extraction efficiency is sufficiently proven because the residue amount obtained for the incurred samples extracted using the method listed in the studies DAS 120615 and DAS 120998 differs by no more than 30% compared to the results obtained with the solvent from the DAS study 110334. The results obtained are summarised in the following tables.

Table A 94: Extraction efficiency data for Fenpicoxamid (XDE-777) (m/z 615/239Q) using analytical method 120615

Matrix	Residue Analytical Method	NOR Method	%NOR Findings	n Method 120615 / NOR 110334
	mean (mg/kg)	mean (mg/kg)	(%)	
Banana	0.0242	0.0210	115%	3/4
Barley Grain	1.017	0.886	115%	4/4
Oilseed Rape Seed	0.0160	0.0135	118%	3/3

Table A 95: Extraction efficiency data for Fenpicoxamid (XDE-777) (m/z 615/239Q) using analytical method 120998

Matrix	Residue Analytical Method	NOR Method	%NOR Findings	n Method 120998 / NOR 110334
	mean (mg/kg)	mean (mg/kg)	(%)	
Banana	0.0246	0.0210	118%	4/4
Barley Grain	0.980	0.886	111%	4/4
Oilseed Rape Seed	0.0144	0.0135	107%	4/3

CONCLUSION
Extraction efficiency is acceptable based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340, SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1, as well as SANTE 2017/10632 rev.3 and PMRA Regulatory Directive Dir98-02.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

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)

No new or additional studies have been submitted.

A 2.1.2.7 Other Studies/Information

No new or additional studies have been submitted.