Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

Prepared according to **assimilated Regulation No 1107/2009**
as it applies in Great Britain

Inpyrfluxam

Volume 3 – B.5 (S-2399 60 g/L EC)

Methods of Analysis

Great Britain

March 2026

Version History

When	What
November 2025	Initial DAR
March 2026	Updates made after ECP

Contents

B.5. Methods of Analysis

B.5.1. Methods used for the generation of pre-authorisation data

B.5.1.1. Analysis of the plant protection product

(a) Methods for the determination of the active substance and/or variant in the plant protection product

Reference:	KCP 5.1.1/01
Report Title:	Validation of analytical HPLC method for determination of active substance content in an emulsifiable concentrate (EC) containing S-2399
Author(s) & Year:	[redacted] 2019
Document No, Authority registration No	Study No.: 24896; Report No.: TPA-0079
Guideline(s):	SANCO 3030/99 rev. 5
Deviations:	No
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Reference items

Inpyrfluxam (pure) batch MM180515G (99.8% w/w)

Inpyrfluxam (technical) batch 13CG0617G (95.5% w/w)

S-2940 batch KM130426G (100.1% w/w)

S-2399 60 g/L EC (V16-7) ('S-2399 60 g/L EC') batch V16-7L1901 (content of inpyrfluxam: 60 g/L)

Blank formulation of S-2399 60 g/L EC batch V18-30F1901

Inpyrfluxam in 'S-2399 60 g/L EC' is determined by HPLC and is conducted in two parts:

1) Method MET/24896-A: Determination of total isomer content (inpyrfluxam and S-2940)

2) Method MET/24896-B: Determination of *R*-isomer ratio and inpyrfluxam (a.i) content

Each method was validated below.

Method MET/24896-A: Total isomer content (inpyrfluxam and S-2940)

Principle of the method

Around 1600 mg of test item (containing around 105 mg of inpyrfluxam) is weighed into a conical flask and 10 mL of internal standard (8 g/L ethyl benzoate in acetonitrile) is added. The sample is sonicated in an ultrasonic bath for 10 minutes. An aliquot of 1 mL is transferred to a conical flask and 20 mL of acetonitrile is added. The sample is mixed well and filtered through a 0.45 µm filter before being injected and assayed by HPLC-UV. The final test sample concentration is 8 mg formulation/mL (0.525 mg active substance/mL) and the samples are prepared in triplicate.

Method conditions

Column	SCAS Sumipax ODS Z-CLUE, 3 µm, 150 x 4.6 mm
Mobile phase	Acetonitrile: 0.1% phosphoric acid solution (65:35, v/v)
Column temperature	40 °C
Injection volume	10 µL
Flow rate	1 mL/min
Detector wavelength	240 nm
Retention time	5 mins

Table B.5.1.1-1: Summary of method validation for the determination of total isomer content (inpyrfluxam and S-2940) in 'S-2399 60 g/L EC'

Analyte	LOQ (mg/kg)	Recovery fortification level (%)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Total isomer content (inpyrfluxam and S-2940)	Not required	90	98.1 – 98.8 (98.4)	First precision test 0.17 @ 6.611% w/w (n=6) Modified Horwitz = 2.02 @ 6.611% w/w therefore, Hr = 0.08 Second precision test 0.11 @ 6.614% w/w (n=6) Modified Horwitz = 2.02 @ 6.614% w/w therefore, Hr = 0.06	0.239 – 0.714 mg/mL (equivalent to 28.2-85.2 g/L in the product, approximately 47 – 142% of the nominal content) (n = 5) y = 1.09x – 0.01 r = 1.000
		100	99.3 – 99.4 (99.3)		
		110	100.0 – 102.0 (100.1)		

Specificity

No interferences were observed at the retention time of inpyrfluxam or the internal standard. Analyte identity was confirmed by retention time match and comparison of UV spectra of the analyte with that of a reference standard.

Linearity

Linearity of detector response was demonstrated using five external standard solutions across the concentration range of 0.239 to 0.714 mg/mL. The correlation coefficient (r) was determined to be 1.000 (slope = 1.09, intercept = -0.01). The linear range is equivalent to an inpyrfluxam concentration of 47 – 142% of the nominal content in the test item.

Precision (repeatability)

Repeatability data was generated from analysis of six individual samples of 'S-2399 60 g/L EC' on two separate occasions. The relative standard deviations (RSD) obtained were within the guideline requirements.

Accuracy (Recovery)

Recovery data was generated from samples of blank formulation fortified at 90, 100 and 110 % of the nominal active substance concentration. Two determinations were made at each level and the mean recovery at each level reported. Recovery values ranged from 98.4 – 100.1% with a mean overall recovery of 99.3%. The mean recoveries obtained for each fortification level were within the guideline requirements.

LOQ

An LOQ is not required for the determination of active substance content in the preparation.

Conclusion

The analytical method is validated in accordance with SANCO/3030/99 rev. 5 for the determination of the total isomer content (inpyrfluxam and S-2940) in the formulation 'S-2399 60 g/L EC' using HPLC-UV.

Method MET/24896-B: R-isomer ratio and inpyrfluxam content

Principle of the method

Around 1600 mg of test item (containing around 105 mg of inpyrfluxam) is weighed into a conical flask and 100 mL of diluting solvent (n-hexane:isopropanol, 95:5 v/v) is added. The sample is sonicated in an ultrasonic bath for 10 minutes. The sample is mixed well and filtered through a 0.45 µm filter before being injected and assayed by HPLC-UV. The final test sample concentration is 16 mg formulation/mL (1.05 mg active substance/mL) and the samples are prepared in triplicate.

The separation of R- (S-2399) and S- (S-2940) isomers is achieved using normal phase liquid chromatography with a chiral column and ultraviolet detection.

Method conditions

Column	Phenomenex Lux Cellulose-1, 3 µm, 250 x 4.6 mm
Mobile phase	n-hexane: isopropanol (95:5 v/v)
Column temperature	40 °C
Injection volume	10 µL
Flow rate	1.25 mL/min

Detector wavelength	254 nm
Retention time:	Inpyrfluxam: 23 mins S-2940: 15.7 mins

Table B.5.1.1-2: Summary of method validation for the determination of *R*-isomer ratio and the content of inpyrfluxam in 'S-2399 60 g/L EC'

Analyte	LOQ (mg/kg)	Recovery fortification level (%)	Recoveries % range (mean)	Repeatability % RSD (n)†	Linearity
Inpyrfluxam*	Not required	90	100.0 – 100.1 (100.1)	First precision test 0.0 @ 98.96% w/w (n=6) Modified Horwitz = 1.34 @ 98.96% w/w therefore, Hr = 0.00 Second precision test 0.0 @ 98.96% w/w (n=6) Modified Horwitz = 1.34 @ 98.96% w/w therefore, Hr = 0.00	0.507 – 1.507 mg/mL (3.17-9.42% w/w equivalent to 49 – 145% of the nominal concentration) (n = 5) y = 15.58x + 105.08 r = 0.996
		100	99.9 – 100.0 (100.0)		
		110	100.0 – 100.0 (100.0)		
S-2940	Not required	90	98.5 – 98.8 (98.7)	-	0.000546 – 0.1091 mg/mL (0.003 – 0.682% w/w S-2940, equivalent to 0.05-10.44% inpyrfluxam) (n = 6) y = 16.18x – 5.32
		100	99.5 – 101.5 (100.5)		

		110	100.1 – 100.6 (100.4)		r = 0.999

*Inpyrfluxam content = total isomer content x *R*-isomer ratio
†Precision was calculated using R-isomer ratio (% area of inpyrfluxam) which isn't acceptable according to SANCO 3030/99 rev. 5. However, the method can still be considered fit for purpose based of the data provided.

Specificity

No interferences were observed at the retention time of interest in the control solutions. Analyte identity was confirmed by retention time match and comparison of UV spectra of the analyte with that of a reference standard. The peaks of inpyrfluxam and S-2940 were well resolved from one another and demonstrated to be optically pure.

Linearity

Linearity of detector response was demonstrated using five external standard solutions across the concentration range of 0.507 to 1.507 mg/mL for inpyrfluxam. The correlation coefficient (r) was determined to be 0.996 (slope = 15.58, intercept = 105.08). The linear range is equivalent to an inpyrfluxam concentration of 49 – 145% w/w in the test item.

Linearity of detector response was demonstrated using six external standard solutions across the concentration range of 0.000546 to 0.1091 mg/mL for S-2940. The correlation coefficient (r) was determined to be 0.999 (slope = 16.18, intercept = -5.32). The linear range is equivalent to a S-2940 concentration of 0.003 – 0.682% w/w corresponding to 0.05 – 10.44% inpyrfluxam.

Precision (repeatability)

Repeatability data was generated from analysis of six individual samples of 'S-2399 60 g/L EC' on two separate occasions. The relative standard deviations (RSD) obtained were within the guideline requirements. Please note, inpyrfluxam content was calculated using the R-isomer ratio. Further information is to be submitted by the applicant.

Accuracy (Recovery)

Recovery data was generated from samples of blank formulation fortified at 90, 100 and 110 % of the nominal test item concentrations of inpyrfluxam and S-2940. Two determinations were made at each level and the mean recovery at each level reported. Recovery values ranged from 100.0 – 100.1% for inpyrfluxam, with a mean overall

recovery of 100.0%. Recovery values ranged from 98.7 – 100.5% for S-2940, with a mean overall recovery of 99.9%. The mean recoveries obtained for each fortification level for both inpyrfluxam and S-2940 were within the guideline requirements.

LOQ

An LOQ is not required for the determination of active substance content in the preparation.

Conclusion

The analytical method is sufficiently validated in accordance with SANCO/3030/99 rev. 5 for the determination of the *R*-isomer ratio and inpyrfluxam content in the formulation 'S-2399 60 g/L EC' using HPLC-UV.

B.5.1.2. Methods of the determination of residues

B.5.1.2.1. Methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies

All relevant methods are reported in Volume 3 CA B 5.1.2 of this DAR.

B.5.1.2.2. Methods in soil, water and any additional matrices used in support of efficacy studies

All relevant methods are reported in Volume 3 CA B 5.1.2 of this DAR.

B.5.1.2.3. Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

All relevant methods are reported in Volume 3 CA B 5.1.2 of this DAR.

B.5.1.2.4. Methods in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies

No methods of analysis to support operator, worker, resident and bystander exposure studies for the product have been submitted.

B.5.1.2.5. Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies

All relevant methods are reported in Volume 3 CA B 5.1.2 of this DAR.

B.5.1.2.6. Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

Table B.5.1.2.6-1: *Summary overview*

Data point	Study	Conclusion	Studies relying on
KCP 5.1.2/01	Fish, Acute Toxicity Test. Report No.: [redacted] 2020 Study No.: TPW-0120	Method is fit for regulatory purposes for the determination of inpyrfluxam in S-2399 60 g/L EC. LOQ= 0.0005 µg/mL in treated mains water. **Acceptable**	KCP 10.2.1/01 Study No.: TPW-0120
KCP 5.1.2/02	Acute Toxicity to *Daphnia magna.* [redacted] 2020 Study No.: TPW-0116	Method is fit for regulatory purposes for the determination of inpyrfluxam in S-2399 60 g/L EC. LOQ= 0.001 µg/mL in Elendt medium **Acceptable**	KCP 10.2.1/02 Study No.: TPW-0116
KCP 5.1.2/03	Inhibition of Growth to the Alga Raphidocelis subcapitata (Formerly known as Pseudokirchn eriella subcapitata). [redacted] 2020 Study No.: TPW-0119	Method is fit for regulatory purposes for the determination of inpyrfluxam in S-2399 60 g/L EC. LOQ= 0.001 µg/mL in OECD medium **Acceptable**	KCP 10.2.1/03 Study No.: TPW-0119

Data point	Study	Conclusion	Studies relying on
KCP 5.1.2/05	S-2399 60 g/L EC: Chronic Oral Toxicity Test (10-Day Feeding) to the Honey Bee (*Apis mellifera* L.) under Laboratory Conditions [redacted] 2021 Study No.: TPW-0135	Method is fit for regulatory purposes for the determination of inpyrfluxam in S-2399 60 g/L EC. LOQ= 4.25 mg a.i./kg in 50% w/v aqueous sucrose solution **Acceptable**	KCP 10.3.1.2/01 Study No.: TPW-0135
KCP 5.1.2/06	S-2399 60 g/L EC: Honey Bee (Apis mellifera L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions [redacted] 2021 Study No.: TPW-0136	Method is fit for regulatory purposes for the determination of inpyrfluxam in S-2399 60 g/L EC. LOQ= 0.654 mg a.i./kg in Larval Diet LOQ= 0.654 mg/L in deionised water **Acceptable**	KCP 10.3.1.3/01 Study No.: TPW-0136
KCP 5.1.2/07	S-2399 60G/L EC: Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test. [redacted] [redacted] 2020 Study No.: TPW-0127	Method is fit for regulatory purposes for the determination of inpyrfluxam in S-2399 60 g/L EC. LOQ= 12.5 g/L in deionised water **Acceptable**	KCP 10.6.2/01 Study No.: TPW-0127

Data point	Study	Conclusion	Studies relying on
			KCP 10.6.2/02 Study No.: TPW-0117

Reference:	KCP 5.1.2/01
Report Title:	Fish, Acute Toxicity Test
Author(s) & Year:	[redacted] 2020
Document No, Authority registration No	Study No.: 3202418 Report No.: TPW-0120
Guideline(s):	Not confirmed
Deviations:	No
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Principle of the method

Each test sample (10 mL) was added to a 20 mL glass vial and 10 mL of acetonitrile (containing 0.2% formic acid) was added. The vial was capped and shaken well. If necessary, the sample was diluted with treated mains water: acetonitrile (1:1, v/v) (containing 0.1% formic acid) to bring the concentrations within the calibration range. The samples were analysed by HPLC-TOF/MS using the conditions outlined below.

Method conditions

Instrumentation AB Sciex TripleTOF5600+ Coupled to Shimadzu SIL-30ACMP Quaternary HPLC system. Analyst TF 1.7.1 data collection software

Column Waters Acquity BEH phenyl, 1.7 µm, 50 x 2.1 mm

Mobile phase A = 0.1% formic acid in water (LC-MS grade)
B = 0.1% formic acid in acetonitrile (LC-MS grade)

Time (minutes)	%A	%B
0.01	95	5
0.50	95	5
2.50	0	100
3.90	0	100
4.00	95	5
5.00	95	5

Column oven temperature 50 °C
Injection volume 25 µL
Flow rate 0.50 mL/min
Run time 5.00 minutes
Ionisation Electrospray (ESI)
Polarity Positive
Mass Range 50-500 Da
Ions Monitored Product of 334.173: 258.070-258.120 Da
Scan Type sMRM
Retention time ~2.3 minutes

Table B.5.1.2.6-2: Summary of method validation for the determination of inpyrfluxam in treated mains water

Analyte	LOQ (µg/mL)	Recovery fortification level (µg/mL)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity	Specificity
Inpyrfluxam	0.0005	0.0005	96.0 – 128 (106) N = 5	12 @ ~0.0005 µg/mL (N=5)	0.00005 – 0.01 µg/mL (n = 7*) y = 13,028,600x + 868.77271 r = 0.99431	Retention time match to reference standard. No significant interfering peaks observed in the blank matrix.
		0.01	97.5 – 107 (102) N = 5	3.6 @ ~0.01 µg/mL (N=5)		
		1.00	101 – 110 (105) N = 5	3.5 @ ~1.00 µg/mL (N=5)		

*eight concentrations were tested but one was removed as it was an outlier.

Matrix effects

The matrix effects have not been addressed

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a matrix blank sample. Analyte identity was confirmed by comparison of the retention time of the analyte in the test sample with that of the reference standard.

Linearity

Linearity was demonstrated by the analysis of eight standards of increasing concentration. One calibration point was removed from the calibration as it was an outlier. The range of standard concentrations used was 0.00005 – 0.01 µg a.i./mL. The concentrations extend over an appropriate range, and the response was linear with a correlation coefficient (r) of 0.99431. If necessary, samples were diluted into calibration range.

The raw calibration data was not provided, which is required according to SANTE 2020/12830 rev.1. The notifier was asked to provide the raw calibration data. The study was completed prior to the implementation of SANTE 2020/12830 rev.1 and raw calibration data was not required under the previous guidance (SANCO 3029/99 rev.4). All of the other requirements have been addressed and it is clear that the calibration response is linear. This omission is considered to be minor and no further consideration is required.

Precision (repeatability)

The precision of the method was assessed via analysis of the accuracy samples. The reported %RSDs were <20%.

Accuracy (recovery)

The accuracy of the method was assessed by analysing five samples fortified with S-2399 60 g/L EC at concentrations of 0.0005, 0.01 and 1.00 µg/mL. The samples were diluted where necessary as outlined in the sample preparation. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 120%.

Procedural recoveries

Procedural recoveries were conducted using four replicate samples at three fortification levels of 0.0005, 0.01, 1.0 µg/mL. The mean recoveries at each of the fortification levels are 107, 103 and 110 respectively. The procedural recoveries are acceptable at all fortification levels.

LOQ

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been demonstrated to be 0.0005 µg/mL.

Stability of standards and extracts

Stability of inpyrfluxam sample extracts does not need to be addressed as the samples were prepared and analysed on the same day.

Inpyrfluxam standards were prepared and analysed on the same day. This was confirmed by the notifier in response to a request for additional information.

Stock solutions stability was not fully addressed.

Conclusion

The analytical method is not acceptably validated according to SANTE/2020/12830 rev. 1 for the determination of the active substance inpyrfluxam in treated mains water as the matrix effects and stock solution stability have not been determined. However, the study was generated prior to the implementation of SANTE 2020/12830 rev.1 and SANCO 3029/99 rev.4 did not require matrix effects and stock solution stability to be addressed. As all other validation requirements have been met (including the minimum validation requirements outlined in SANTE 2020/12830 rev.1), the method is considered to be fit for purpose.

Reference:	KCP 5.1.2/02
Report Title:	Acute Toxicity to *Daphnia magna*
Author(s) & Year:	[redacted] 2020
Document No, Authority registration No	Study No.: 3202417 Report No.: TPW-0116
Guideline(s):	Not confirmed
Deviations:	No
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Principle of the method

Each test sample (10 mL) was added to a 20 mL glass vial and 10 mL of acetonitrile (containing 0.2% formic acid) was added. The vial was capped and shaken well. If necessary, the sample was diluted with Elendt medium: acetonitrile (1:1, v/v) (containing 0.1% formic acid) to bring the concentrations within the calibration range. The samples were analysed by HPLC-TOF/MS using the conditions outlined below.

Method conditions

Instrumentation	AB Sciex TripleTOF5600+ Coupled to Shimadzu SIL-30ACMP Quaternary HPLC system. Analyst TF 1.7.1 data collection software
Column	Waters Acquity BEH phenyl, 1.7 µm, 50 x 2.1 mm

Mobile phase A = 0.1% formic acid in water (LC-MS grade)
B = 0.1% formic acid in acetonitrile (LC-MS grade)

Time (minutes)	%A	%B
0.01	95	5
0.50	95	5
2.50	0	100
3.90	0	100
4.00	95	5
5.00	95	5

Column oven temperature 50 °C
Injection volume 25 µL
Flow rate 0.50 mL/min
Run time 5.00 minutes
Ionisation Electrospray (ESI)
Polarity Positive
Mass Range 50-500 Da
Ions Monitored Product of 334.173: 258.070-258.120 Da
Scan Type sMRM
Retention time ~2.3 minutes

Table B.5.1.2.6-3: Summary of method validation for the determination of inpyrfluxam in Elendt medium

Analyte	LOQ (µg/mL)	Recovery fortification level (µg/mL)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity	Specificity
Inpyrfluxam	0.001	0.001	100 – 108 (104) N = 5	3.2 @ ~0.001 µg/mL (N=5)	0.0001 – 0.02 µg/mL (n = 7*) y = -64,083,100x^2 +10,762,100 + 254.11300 r = 0.99977	Retention time match to reference standard. No significant interfering peaks observed in the blank matrix.
		0.1	98.3 – 103 (101) N = 5	1.9 @ ~0.1 µg/mL (N=5)		
		10.0	97.3 – 103 (100)	2.4 @ ~10.0 µg/mL		

			N = 5	(N=5)		

* *eight concentrations were tested but one was removed as it was an outlier

Matrix effects

The matrix effects have not been addressed.

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a matrix blank sample. Analyte identity was confirmed by comparison of the retention time of the analyte in the test sample with that of the reference standard.

Linearity

Linearity was demonstrated by the analysis of eight standards of increasing concentration. One calibration point was removed from the calibration as it was an outlier. The range of standard concentrations used was 0.0001 – 0.02 µg a.i./mL. The concentrations extend over an appropriate range. A quadratic calibration was used as it provided a better fit than a linear calibration. This is likely due to ion suppression from the matrix. As this has been justified, it is acceptable. The correlation coefficient (r) is 0.99977.

The raw calibration data was not provided, which is required according to SANTE 2020/12830 rev.1. The notifier was asked to provide the raw calibration data. The study was completed prior to the implementation of SANTE 2020/12830 rev.1 and raw calibration data was not required under the previous guidance (SANCO 3029/99 rev.4). All of the other requirements have been addressed and it is clear that the calibration response is linear. This omission is considered to be minor and no further consideration is required.

Precision (repeatability)

The precision of the method was assessed via analysis of the accuracy samples. The reported %RSDs were <20%.

Accuracy (recovery)

The accuracy of the method was assessed by analysing five samples fortified with S-2399 60 g/L EC at concentrations of 0.001, 0.1 and 10.0 µg/mL. The samples were diluted where necessary as outlined in the sample preparation. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 120%.

Procedural recoveries

Procedural recoveries were conducted using two replicate samples at three fortification levels of 0.001, 0.1, 10.0 µg/mL. The mean recoveries at each of the fortification levels are 105.5, 101 and 103.5 respectively. The procedural recoveries are acceptable at all fortification levels.

LOQ

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been demonstrated to be 0.001 µg/mL.

Stability of standards and extracts

Stability of inpyrfluxam in sample extracts were tested after storage at <-10 °C for 7 days. The recoveries of four replicate samples (fortification level 0.1 µg/mL) were determined and compared to the initial fortification level. The recoveries of one sample at 0.001 and 10.0 µg/mL were also determined. All recoveries were within the acceptable range of 70 – 120 %. The stability of inpyrfluxam in sample extracts was sufficiently demonstrated. It is noted the storage period of the final extracts was not confirmed in the study.

Inpyrfluxam standards were prepared and analysed on the same day. This was confirmed by the notifier in response to a request for additional information.

Stock solution stability was not fully addressed.

Conclusion

The analytical method is not acceptably validated according to SANTE/2020/12830 rev. 1 for the determination of the active substance inpyrfluxam in Elendt medium as the matrix effects and stock solution stability have not been determined. However, the study was generated prior to the implementation of SANTE 2020/12830 rev.1 and SANCO 3029/99 rev.4 did not require matrix effects and stock solution stability to be addressed. As all other validation requirements have been met (including the minimum validation requirements outlined in SANTE 2020/12830 rev.1), the method is considered to be fit for purpose.

Reference:	KCP 5.1.2/03
Report Title:	Inhibition of Growth to the Alga Raphidocelis subcapitata (Formerly known as Pseudokirchneriella subcapitata)
Author(s) & Year:	[redacted] 2020
Document No, Authority registration No	Study No.: 3202416 Report No.: TPW-0119
Guideline(s):	Not confirmed

Deviations:	No
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Principle of the method

Each test sample (10 mL) was added to a 20 mL glass vial and 10 mL of acetonitrile (containing 0.2% formic acid) was added. The vial was capped and shaken well. If necessary, the sample was diluted with OECD medium: acetonitrile (1:1, v/v) (containing 0.1% formic acid) to bring the concentrations within the calibration range. The samples were analysed by HPLC-TOF/MS using the conditions outlined below.

Method conditions

Instrumentation AB Sciex TripleTOF5600+ Coupled to Shimadzu SIL-30ACMP Quaternary HPLC system. Analyst TF 1.7.1 data collection software

Column Waters Acquity BEH phenyl, 1.7 µm, 50 x 2.1 mm

Mobile phase A = 0.1% formic acid in water (LC-MS grade)
B = 0.1% formic acid in acetonitrile (LC-MS grade)

Time (minutes)	%A	%B
0.01	95	5
0.50	95	5
2.50	0	100
3.90	0	100
4.00	95	5
5.00	95	5

Column oven temperature 50 °C

Injection volume 25 µL

Flow rate 0.50 mL/min

Run time 5.00 minutes

Ionisation Electrospray (ESI)

Polarity Positive

Mass Range 50-500 Da

Ions Monitored Product of 334.173: 258.070-258.120 Da

Scan Type sMRM

Retention time ~2.3 minutes

Table B.5.1.2.6-4: Summary of method validation for the determination of inpyrfluxam in OECD medium

Analyte	LOQ (µg/mL)	Recovery fortification level (µg/mL)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity	Specificity
Inpyrfluxam	0.001	0.001	105 – 113 (109) N = 5	3.6 @ ~0.001 µg/mL (N=5)	0.0001 – 0.02 µg/mL (n = 8) y = 1,070,720x – 459.6 r = 0.99851	Retention time match to reference standard. No significant interfering peaks observed in the blank matrix.
		0.1	93.3 – 101 (97.8) N = 5	2.9 @ ~0.1 µg/mL (N=5)		
		10.0	94.7 – 102 (98.3) N = 5	3.0 @ ~10.0 µg/mL (N=5)		

Matrix effects

The matrix effects have not been addressed.

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a matrix blank sample. Analyte identity was confirmed by comparison of the retention time of the analyte in the test sample with that of the reference standard.

Linearity

Linearity was demonstrated by the analysis of eight standards of increasing concentration. The range of standard concentrations used was 0.0001 – 0.02 µg/mL. The concentrations extend over an appropriate range, and the response was linear with a correlation coefficient (r) of 0.99851.

The raw calibration data was not provided, which is required according to SANTE 2020/12830 rev.1. The notifier was asked to provide the raw calibration data. The study was completed prior to the implementation of SANTE 2020/12830 rev.1 and raw calibration data was not required under the previous guidance (SANCO 3029/99 rev.4). All of the other requirements have been addressed and it is clear that the calibration response is linear. This omission is considered to be minor and no further consideration is required.

Precision (repeatability)

The precision of the method was assessed via analysis of the accuracy samples. The reported %RSDs were <20%.

Accuracy (recovery)

The accuracy of the method was assessed by analysing five samples fortified with S-2399 60 g/L EC at concentrations of 0.001, 0.1 and 10.0 µg/mL. The samples were diluted where necessary as outlined in the sample preparation. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 120%.

Procedural recoveries

Procedural recoveries were conducted using two replicate samples at three fortification levels of 0.001, 0.1, 10.0 µg/mL. The mean recoveries at each of the fortification levels are 107.5, 108.5 and 115.5 respectively. The procedural recoveries are acceptable at all fortification levels.

LOQ

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been demonstrated to be 0.001 µg/mL.

Stability of standards and extract

Stability of inpyrfluxam sample extracts does not need to be addressed as the samples were prepared and analysed on the same day.

Inpyrfluxam standards were prepared and analysed on the same day. This was confirmed by the notifier in response to a request for additional information.

Stock solution stability was not fully addressed.

Conclusion

The analytical method is not acceptably validated according to SANTE/2020/12830 rev. 1 for the determination of the active substance inpyrfluxam in OECD medium as the matrix

effects and stock solution stability have not been determined. However, the study was generated prior to the implementation of SANTE 2020/12830 rev.1 and SANCO 3029/99 rev.4 did not require matrix effects and stock solution stability to be addressed. As all other validation requirements have been met (including the minimum validation requirements outlined in SANTE 2020/12830 rev.1), the method is considered to be fit for purpose.

Reference:	KCP 5.1.2/05
Report Title:	S-2399 60 g/L EC: Chronic Oral Toxicity Test (10-Day Feeding) to the Honey Bee (*Apis mellifera* L.) under Laboratory Conditions
Author(s) & Year:	[redacted] (2021)
Document No, Authority registration No	Study No.: S20-00800 Report No.: TPW-0135
Guideline(s):	SANCO/3029/99 rev.4
Deviations:	No
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Principle of the method

Samples were thawed to ambient temperature. Sample bottles were weighed with the lid before sample preparation. The whole sample was transferred into a 250 mL glass bottle. The original sample vessel was rinsed with 25 mL of HPLC grade water and the rinse was transferred into the 250 mL glass bottle. The samples were shaken well on a vortex mixer. The original sample vessel was rinsed with 100 mL of acetonitrile and the rinse was transferred into the 250 mL glass bottle. The sample was shaken by hand and vortexed for at least two minutes. The original sample vessel was weighed with the lid again and the exact weight was noted. To each sample, five QuEChERS salt mixtures (4.0 g magnesium sulfate, 1.0 g of sodium chloride, 1.0 g of trisodium citrate and 0.5 g of disodium citrate sesquihydrate = Bekolut Citrat-Kit-01) were added and the samples were shaken well on a horizontal flatbed shaker for at least 15 minutes. An aliquot of about 40 mL of each sample was transferred into a 50 mL centrifuge tube. The samples were centrifuged at about 4000 rpm for two minutes. The final sample extract was diluted by a factor of 10 with acetonitrile/water (1:1, v/v). The samples were further diluted with sucrose solution blank extract to be within the calibration range. The samples were analysed by HPLC-MS/MS.

Method conditions

HPLC system Agilent 1290 Infinity HPLC system

Pre-column UHPLC guard column (AJ0-9000, Phenomenex) with 2.1 mm C18 Cartridge (AJ0-8782, Phenomenex)

Column Thermo Accucore Phenyl-Hexyl, 50 mm x 4.6 mm, 2.6 µm (Part. No. 17926-054630)

Mobile phase A = Deionised water + 0.5% formic acid
B = Methanol

Time (minutes)	%A	%B
0.0	70	30
4.50	10	90
5.00	10	90
5.10	70	30
6.50	70	30

Column oven temperature 40 °C

Injection volume 5 µL

Flow rate 0.8 mL/min

MS system SCIEX API 6500

Ionisation Electrospray ionisation (ESI, TurbolonSpray)

Polarity Positive

Mass transition monitored 334 → 238 (used for quantification)
334 → 258

Scan Type MS/MS, Multiple Reaction Monitoring (MRM)

Retention time ~4.1 minutes

Table B.5.1.2.6-5: Summary of method validation for the determination of inpyrfluxam in 50% w/v aqueous sucrose solution

Analyte	LOQ (mg a.i./kg)	Recovery fortification level (mg a.i./kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity	Specificity
Inpyrfluxam (m/z 334 → 238)	4.25	4.25 (65 mg/kg test item)	91 – 97 (94) N = 5	3 @ ~4.25 mg a.i./kg (N=5)	0.4 – 10 ng/mL (n = 7) y = 253000 + 9890 r = 0.9995	Retention time match to reference standard. No significant interfering peaks observed in
		1302	79 – 102 (88)	11 @ ~1302 mg a.i./kg (N=5)		

		(19900 mg/kg test item)	N = 5			the blank matrix.

Matrix effects

The effects of the matrix (50% w/v aqueous sucrose solution) on the LC-MS/MS response were assessed by comparing the response between matrix-matched standards with solvent standards at identical concentrations. The matrix effects were not significant (<20%). The calibration curve was generated using matrix-matched calibration standards. Therefore, the matrix effects have been satisfactorily addressed.

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a matrix blank sample. Analyte identity was confirmed by comparison of the retention time of the analyte in the test sample with that of the reference standard.

Linearity

Linearity was demonstrated by the analysis of seven matrix-matched standards of increasing concentration. The range of standard concentrations used was 0.4 – 10 ng/mL. The concentrations extend over an appropriate range, and the response was linear with a correlation coefficient (r) of 0.9995. If necessary, samples were diluted to within the calibration range.

Precision (repeatability)

The precision of the method was assessed via analysis of the accuracy samples. The reported %RSDs were <20%.

Accuracy (recovery)

The accuracy of the method was assessed by analysing five samples fortified with S-2399 60 g/L EC at concentrations of 4.25 and 1302 mg a.i./kg. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 120%.

Procedural recoveries

Procedural recoveries were conducted using four replicate samples at two fortification levels of 4.25 and 1302 mg a.i./kg. The mean recoveries at each of the fortification levels

are 83 and 96% respectively. The procedural recoveries are acceptable at all fortification levels.

LOQ

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been demonstrated to be 4.25 mg a.i./kg.

Stability of standards and extracts

The interval from the preparation of the final dilutions to the injection of samples did not exceed 24 hours and therefore no further information is required.

Inpyrfluxam standards were stored between 1 and 10 °C in the dark.

Stock solutions of inpyrfluxam were prepared in methanol. In study TPA-0027, the stability of inpyrfluxam in methanol was demonstrated by comparing the response of a freshly prepared solution to a solution stored for 104 days at 1 - 10 °C. The recovery was 98%. Therefore, the stability of stock solutions has been demonstrated.

Conclusion

The analytical method is not acceptably validated according to SANTE/2020/12830 rev. 1 for the determination of the active substance inpyrfluxam in 50% w/v aqueous sucrose solution as the standard solution stability has not been determined. However, the study was generated prior to the implementation of SANTE 2020/12830 rev.1 and SANCO 3029/99 rev.4 did not require standard solution stability to be addressed. As all other validation requirements have been met (including the minimum validation requirements outlined in SANTE 2020/12830 rev.1), the method is considered to be fit for purpose.

Reference:	KCP 5.1.2/06
Report Title:	S-2399 60 g/L EC: Honey Bee (Apis mellifera L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions
Author(s) & Year:	[redacted] (2021)
Document No, Authority registration No	Study No.: S20-00798 Report No.: TPW-0136
Guideline(s):	SANCO/3029/99 rev.4
Deviations:	No
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Larval diet samples
Samples were thawed to ambient temperature. Sample bottles were weighed with the lid before sample preparation. The whole sample was transferred into a 250 mL glass bottle. The original sample vessel was rinsed with 25 mL of HPLC grade water and the rinse was transferred into the 250 mL glass bottle. The samples were shaken well on a vortex mixer. The original sample vessel was rinsed with 100 mL of acetonitrile and the rinse was transferred into the 250 mL glass bottle. The sample was shaken by hand and vortexed for at least two minutes. The original sample vessel was weighed with the lid again and the exact weight was noted. To each sample, five QuEChERS salt mixtures (4.0 g magnesium sulfate, 1.0 g of sodium chloride, 1.0 g of trisodium citrate and 0.5 g of disodium citrate sesquihydrate = Bekolut Citrat-Kit-01) were added and the samples were shaken well on a horizontal flatbed shaker for at least 15 minutes. An aliquot of about 40 mL of each sample was transferred into a 50 mL centrifuge tube. The samples were centrifuged at about 4000 rpm for two minutes. The final sample extract was diluted by a factor of 10 with acetonitrile/water (1:1, v/v). The samples were further diluted with larval diet blank extract to be within the calibration range. The samples were analysed by HPLC-MS/MS.

Water samples
The water stock solution sample was allowed to come to ambient temperature and homogenised well using a vortex mixer. The samples were diluted by a factor of 10 with acetonitrile/water (1:1, v/v) (1 mL of sample + 9mL of acetonitrile/water (1:1, v/v). The samples were further diluted with acetonitrile/water (1:1, v/v) to be within calibration range.

Method conditions

HPLC system Agilent 1290 Infinity HPLC system

Pre-column UHPLC guard column (AJ0-9000, Phenomenex) with 2.1 mm C18 Cartridge (AJ0-8782, Phenomenex)

Column Thermo Accucore Phenyl-Hexyl, 50 mm x 4.6 mm, 2.6 µm (Part. No. 17926-054630)

Mobile phase A = Deionised water + 0.5% formic acid
B = Methanol

Time (minutes)	%A	%B
0.0	70	30
4.50	10	90
5.00	10	90
5.10	70	30
6.50	70	30

Column oven temperature 40 °C

Injection volume 5 µL

Flow rate 0.8 mL/min

MS system SCIEX API 6500

Ionisation Electrospray ionisation (ESI, TurbolonSpray)

Polarity Positive

Mass transition monitored 334 → 238 (used for quantification)
334 → 258

Scan Type MS/MS, Multiple Reaction Monitoring (MRM)
Retention time ~4.1 minutes

Table B.5.1.2.6-6: Summary of method validation for the determination of inpyrfluxam in larval diet and deionised water

Analyte	Matrix	LOQ	Recovery fortification level	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity	Specificity
Inpyrfluxam (m/z 334 → 238)	Larval Diet	0.654 mg a.i./kg	0.654 mg a.i./kg	98 – 106 (101) N = 5	3 @ ~0.654 mg a.i./kg (N=5)	0.4 – 10 ng/mL (n = 7) y = 595000 + 16300 r = 0.9998	Retention time match to reference standard. No significant interfering peaks observed in the blank matrix.
			200 mg a.i./kg	101 – 104 (102) N = 5	1 @ ~200 mg a.i./kg (N=5)		
	Deionised water	0.654 mg a.i./L	0.654 mg a.i./L	103 – 111 (107) N = 5	3 @ ~0.654 mg a.i./L (N=5)	0.4 – 10 ng/mL (n = 7) y = 858000 + 14900 r = 0.9994	
			2176 mg a.i./L	105 – 113 (110) N = 5	3 @ ~2176 mg a.i./L (N=5)		

Matrix effects

The effects of the matrices (larval diet and deionised water) on the LC-MS/MS response were assessed by comparing the response between matrix-matched standards with solvent standards at identical concentrations. The matrix effects were not significant (<20%). The calibration curve for larval diet was generated using matrix-matched calibration standards. Therefore, the matrix effects have been satisfactorily addressed.

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a matrix blank sample. The chromatogram of a control blank was provided in response to a request for additional information. Analyte identity was confirmed by two ion transitions and comparison of the retention time of the analyte in the test sample with that of the reference standard.

Linearity
Larval Diet

Linearity was demonstrated by the analysis of seven matrix-matched standards of increasing concentration. The range of standard concentrations used was 0.4 – 10 ng/mL. The concentrations extend over an appropriate range, and the response was linear with a correlation coefficient (r) of 0.9998. If necessary, samples were diluted to within calibration range.

Deionised Water

Linearity was demonstrated by the analysis of seven standards of increasing concentration. The range of standard concentrations used was 0.4 – 10 ng/mL. The concentrations extend over an appropriate range, and the response was linear with a correlation coefficient (r) of 0.9994. If necessary, samples were diluted to within calibration range.

Precision (repeatability)

The precision of the method was assessed via analysis of the accuracy samples. The reported %RSDs were <20%.

Accuracy (recovery)
The accuracy of the method was assessed by analysing five samples fortified with S-2399 60 g/L EC. For the larval diet matrix, the fortification levels used were 0.654 and 200 mg a.i./kg. For the deionised water matrix, the fortification levels used were 0.654 and 2176 mg a.i./L Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 120%.

Procedural recoveries

Procedural recoveries were conducted using four replicate samples at two fortification levels for larval diet and two replicates at two fortification levels for deionised water. For both matrices, the fortification levels used were the same as was used for the recovery of the method. The mean procedural recoveries are within the range 70 to 120% and are therefore acceptable except for the LOQ fortification level in deionised water were results were concluded to be invalid due to a preparation error. This is considered acceptable.

LOQ

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been demonstrated to be 0.654 mg a.i./kg in larva diet and 0.654 mg a.i./L in deionised water.

Stability of standards and extracts

The interval from the preparation of the final dilutions to the injection of samples did not exceed 72 hours. As the recoveries were between 70-120%, no further information is

required. The procedural recovery samples were stored under the same conditions with the final extracts and analysed at the same time, as the procedural recoveries are acceptable, the stability of final sample extracts has been demonstrated.

Inpyrfluxam standards were stored between 1 and 10 °C in the dark.

Stock solutions of inpyrfluxam were prepared in methanol. In study TPA-0027, the stability of inpyrfluxam in methanol was demonstrated by comparing the response of a freshly prepared solution to a solution stored for 104 days at 1 - 10 °C. The recovery was 98%. Therefore, the stability of stock solutions has been demonstrated.

Conclusion

The analytical method is not acceptably validated according to SANTE/2020/12830 rev. 1 for the determination of the active substance inpyrfluxam in larva diet and deionised water as the standard solution stability has not been determined. However, the study was generated prior to the implementation of SANTE 2020/12830 rev.1 and SANCO 3029/99 rev.4 did not require standard solution stability to be addressed. As all other validation requirements have been met (including the minimum validation requirements outlined in SANTE 2020/12830 rev.1), the method is considered to be fit for purpose

Reference:	KCP 5.1.2/07
Report Title:	S-2399 60 g/L EC: Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test
Author(s) & Year:	[redacted] and [redacted] 2020
Document No, Authority registration No	Study No.: 141471086 Report No.: TPW-0127
Guideline(s):	SANCO/3029/99 rev.4
Deviations:	No
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Principle of the method

An aliquot of each sample was diluted with solvent mixture (methanol: pure water (50:50, v/v). The samples were analysed by HPLC-UV using the following conditions.

Method conditions

System	Waters Acquity
Pre-column	Kinetex C18 (50 x 2.1 mm; 2.6 µm)
Column	Thermo Accucore Phenyl-Hexyl, 50 mm x 4.6 mm, 2.6 µm (Part. No. 17926-054630)
Mobile phase	40% HPLC water + 0.1% formic acid 60% Methanol
Temperature	30 °C
Injection volume	2 µL
Flow rate	0.3 mL/min
MS system	DA – detector at 191 – 400 nm, monitoring wavelength at 240 nm.
Retention time	~2.5 minutes

Table B.5.1.2.6-7: Summary of method validation for the determination of inpyrfluxam in deionised water

Analyte	LOQ (g/L)	Recovery fortification level (g/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity	Specificity
Inpyrfluxam	12.5	12.5	96 – 104 (99) N = 5	3 @ ~12.5 g/L (N=5)	2.5 – 25.0 mg a.i./L (n = 6) y = 12814 + 3062 r = 0.9996	Retention time match to reference standard. No significant interfering peaks observed in the blank matrix.
		25	99 – 102 (101) N = 5	1 @ ~25 g/L (N=5)		

Matrix effects

The matrix effects have not been addressed.

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of significant (>30% LOQ) interfering peaks in the chromatogram of a matrix blank sample.

Linearity

Linearity was demonstrated by the analysis of six standards of increasing concentration. The range of standard concentrations used was 2.5 – 25 g/L. The concentrations extend over an appropriate range, and the response was linear with a correlation coefficient (r) of 0.9996. Samples were diluted to within calibration range.

Precision (repeatability)

The precision of the method was assessed via analysis of the accuracy samples. The reported %RSDs were <20%.

Accuracy (recovery)

The accuracy of the method was assessed by analysing five samples fortified with S-2399 60 g/L EC. The fortification levels used were 12.5 and 25 g/L. The samples were diluted by a factor of 100. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 70 to 120%.

LOQ

The limit of quantitation (LOQ), defined as the lowest fortification level where acceptable precision and accuracy data were obtained, has been demonstrated to be 12.5 g/L.

Stability of standards and extracts

The sample preparation and analysis happened on the same day. No further consideration is required.

Conclusion

The analytical method is acceptably validated according to SANTE/2020/12830 rev. 1 for the determination of the active substance inpyrfluxam in deionised water as the matrix effects have not been determined. However, the study was generated prior to the implementation of SANTE 2020/12830 rev.1 and SANCO 3029/99 rev.4 did not require matrix effects to be addressed. As all other validation requirements have been met (including the minimum validation requirements outlined in SANTE 2020/12830 rev.1), the method is considered to be fit for purpose.

B.5.1.2.7. Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

No method validation required from the physical and chemical properties tests.

B.5.2. Methods for post-approval control and monitoring purposes

Methods for post-approval control and monitoring purposes are reported in Volume 3 CA B.5.2 of this DAR.

B.5.3. References Relied On

Literature search

A literature review has been carried out for the active substance inpyrfluxam. One literature search was submitted to address all areas of the risk assessment. HSE has assessed the suitability of the mechanics of the literature search in line with EFSA guidance on conducting literature searches (EFSA Journal 2011). The literature review was conducted in accordance with Article 8(5) of Regulation No. 1107/2009 at the time of completion, and was conducted to comply with the EFSA guidance document as published in EFSA Journal 2011; 9(2):2092.

The process of selection of relevant scientific peer-reviewed open literature was based on a single-concept search in the CAS and Dialog platform databases. The time period was limited to studies published July 2013 up to July 2023 using the search criteria of inpyrfluxam, metabolites and mixtures and related CAS numbers, common names, trade names and lab codes.

A stepwise process for selection of relevant scientific peer-reviewed open literature was undertaken:

- A rapid assessment of the summary records references (e.g., titles, abstracts, index terms, keywords) was conducted.
- Summary records which appeared to be relevant went to the next level of evaluation.
- These were further evaluated and categorized into “reliable without restriction”, “reliable with restriction”, “not reliable” and “not assignable”.

The results of the literature review are as follows:

Summary of the review	n	Justification
Total number of summary records retrieved from search	352	Appendix 1
Number of summary records excluded after rapid assessment for relevance (by title/abstract)	349	Appendix 6

Number of summary records of potential/unclear relevance assessed in further detail (by abstract/full-text)	3	Appendices 4 and 5
Number of studies excluded from the risk assessment after detailed assessment of full-text documents (i.e. not relevant)	2	
Number of studies not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	1	
Number of studies included in the dossier as supporting information (reliability criteria 1-2)	1	

In the area of analytical method data no records were considered relevant for the assessment of analytical methods of the active substance inpyrfluxam.

Conclusion

Regarding the literature search undertaken by the applicant, it is considered that the search is acceptable in terms of databases searched and the search criteria applied. No references of relevance to this assessment were identified.

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	Data Protection Claimed Y/N	Justification if Data Protection is claimed	Owner	Previous evaluation
KCP 5.1.1/01	[redacted]	2019	Validation of analytical HPLC method for the determination of active substance content in an emulsifiable concentrate (EC) containing S-2399 Walloon Agricultural Research Centre, Belgium Study No.: 24896 Report No.: TPA-0079 GLP: Yes Published: No	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SUM	N.A.

KCP 5.1.2/01	[redacted]	2020	Acute Toxicity to Daphnia magna Smithers ERS Limited, UK Study No.: 3202418 Report No.: TPW-0120 GLP: Yes Published: No	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SUM	N.A.
KCP 5.1.2/02	[redacted]	2020	Acute Toxicity to Daphnia magna Smithers ERS Limited, UK Study No.: 3202417 Report No.: TPW-0116 GLP: Yes Published: No	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SUM	N.A.
KCP 5.1.2/03	[redacted]	2020	Inhibition of Growth to the Alga Raphidocelis	N	Y	The study is necessary for this regulatory	SUM	N.A.

			subcapitata (Formerly known as Pseudokirchneriella subcapitata) Smithers ERS Limited UK Study No.: 3202416 Report No.: TPW-0119 GLP: Yes Published: No			decision and is eligible for data protection		
KCP 5.1.2/05	[redacted]	2021	S-2399 60 g/L EC: Chronic Oral Toxicity Test (10-Day Feeding) to the Honey Bee *(Apis mellifera* L.) under Laboratory Conditions Trialcamp S.L.U., Spain	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SUM	N.A.

			Study No.: S20-00800 Report No.: TPW-0135 GLP: Yes Published: No					
KCP 5.1.2/06	[redacted]	2021	S-2399 60 g/L EC: Honey Bee (*Apis mellifera* L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions Trialcamp S.L.U., Spain Study No.: S20-00798 Report No.: TPW-0136 GLP: Yes Published: No	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SUM	N.A.

KCP 5.1.2/07	[redacted] and [redacted]	2020	S-2399 60G/L EC: Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test ibacon GmbH, Germany Study No.: 141471086 Report No.: TPW-0127 GLP: Yes Published: No	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SUM	N.A.

Further information

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This publication is available on the HSE website at www.hse.gov.uk/xxx

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