Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

Prepared according to **Regulation (EC) 1107/2009**
as it applies in Great Britain (GB PPP)

Cinmethylin (BAS 684 H)

Volume 3 – B.5 (AS)
Methods of analysis

Great Britain

November 2020

Version History

When	What
November 2020	Initial DAR

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B.5. METHODS OF ANALYSIS

B.5.1. METHODS USED FOR THE GENERATION OF PRE-AUTHORISATION DATA

B.5.1.1. Methods for the analysis of the active substance as manufactured

Report:	KCA 4.1.1/01; Nemitz A. (2015a)
Title:	Determination of Cinmethylin in Technical Grade Active Ingredient (TGAI) by means of GC
Report No.:	2015/1174457
Guideline(s):	n/a
Guideline deviation(s):	n/a
GLP/GEP:	Not required for method description

Report:	KCA 4.1.1/02; Nemitz A. (2015b)
Title:	Validation of the analytical method APL0687/01: Determination of Cinmethylin in Technical Grade Active Ingredient (TGAI) by means of GC
Report No.:	2015/1174458
Guideline(s):	OECD Principles of Good Laboratory Practice, GLP Principles of the German Chemikaliengesetz (Chemicals Act), 2004/10/EC, EC 1107/2009 of the European Parliament, CIPAC 3807 (improved version), SANCO/3030/99 rev. 4 (11 July 2000), EPA 830.1000, EPA 830.1800, ABNT NBR 14029
Guideline deviation(s):	None
GLP/GEP:	Yes

Cinmethylin can be analysed using analytical method APL0687/01) in which the active substance is dissolved in acetonitrile and analysed by high resolution GC-FID and MS. Quantification is carried out using di-ethyl phthalate as internal standard.

Reference items:

Cinmethylin (BASF Reg. No. 900202), batch COD-002038, purity 94.9 % /w (technical), CoA provided, expiry 01/07/19

Cinmethylin (BASF Reg. No. 900202), batch L87-84, purity 99.0 %w/w (pure), CoA provided, expiry 01/08/17

Sample preparation:

Samples containing approximately 50 mg of cinmethylin are weighed to the nearest 0.01 mg into three separate 50 mL volumetric flasks. Acetonitrile is added up to the mark and the solution shaken. Subsequently 2 mL of the mixed sample solution and 2 mL di-ethyl phthalate internal standard solution (ISS) are transferred to a 20 mL volumetric flask and filled up to the calibration mark with acetonitrile before shaking vigorously.

Analytical method (APL0687/01):

1 µL of the sample solution is injected in the high resolution GC-FID system for analysis. The following method and conditions were noted:

Column	Agilent J&W DB-5, 60 m x 0.25 mm x 0.25 µm
Oven temperature	230 °C
Detector	FID
Detector temperature	280 °C
Injector temperature	250 °C
Carrier gas	Helium
Inlet pressure	250 kPa (constant)
Column flow	approx. 1.13 mL/min (starting parameter)
Hydrogen flow	47 mL/min (optimized)
Air flow	450 mL/min (optimized)
Make up flow (helium)	30 mL/min
Split	1/50

Analysis run time	16 min
Injection volumes	1 µL
Retention times	approx. 6.4 min diethyl phthalate (ISS) approx. 10.3 min cinmethylin
Detector	MS
Inlet pressure	36.26 psi (constant)
Column flow	approx. 1.08 mL/min (starting parameter)
Energy	approx. EI 70 eV
Scan	50 – 350 m/z
MS Source temperature	230 °C
MS Quad temperature	150 °C

Table B.5.1.1-1 Validation of analytical method APL0687/01 for the determination of cinmethylin in technical material

LOQ (%w/w)	Recovery fortification level (%w/w)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity	Specificity
Not required for determination of the active substance in the technical material according to SANCO/3030/99 rev. 4. However, the accuracy of the method was assessed by comparing the concentrations of the analytical standards determined from the calibration line against the theoretical concentrations. Recoveries ranged from 99.0 - 100.9 % (mean 99.8 %, %RSD = 0.55).			%RSD = 0.45 at 93.23 %w/w (n=7) Acceptable Modified Horwitz value = 1.35 at 93.23 %w/w	43.536 - 195.913 mg/L (44 - 134 %w/w) n=7 r=0.99997 y = 0.691x – 0.235	Retention time match with analytical standard. GC-MS spectra match the test item.

Identity:

The identity of the active substance cinmethylin was confirmed by accordance of the retention times in combination with the comparison of the MS-spectra of the test item and reference item.

Specificity:

No significant interference was observed between the active substance, the solvent blank and the internal standard.

Linearity:

Linearity was measured using a series of 7 calibration standards in a concentration range of 43.54 - 195.91 mg/L (corresponding to approx. 44 - 196 %w/w). The concentrations extend over an appropriate range when compared to the content of cinmethylin in the technical material, and the correlation coefficient of >0.999 demonstrates an acceptable linear correlation.

Accuracy:

The accuracy of the method was assessed by analysing three sample solutions containing approximately 50, 100 and 150 % of the nominal concentration of cinmethylin. Recoveries were found to be in the range 99.3 - 100.1 % at all fortification levels. As recovery data are not required for the active substance in the technical material according to SANCO/3030/99 rev. 4, these data have not been considered further within this evaluation and are presented for completeness only.

Precision:

The precision of the method was assessed *via* analysis in duplicate of 7 samples from cinmethylin technical material batch COD-002038. The reported %RSD was within the acceptable Horowitz value.

Conclusion:

The analytical method is fully validated according to SANCO/3030/99 rev. 4 for the determination of the active substance cinmethylin in the technical material *via* high resolution GC-FID/MS.

B.5.1.2. Methods for risk assessment

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

Report:	**KCA 4.1.2/1 Ertunc, T., et al. (2017a)**
Title	Validation of analytical method L0308/01 for the determination of BAS 684 H enantiomers in soil and sediment
Guidelines:	SANCO/3029/99 rev. 4 SANCO/825/00 rev. 8.1 (16 November 2010) EPA 850.6100
GLP:	Yes
Deviations	None reported
Previous evaluation:	None

Method L0308/01 was developed and validated for the determination of the enantiomers of cinmethylin (Reg. No. 5925632 and Reg. No. 5925581) in soil and sediment.

Sample preparation:

5 g of soil or sediment sample was extracted twice by shaking first with 10 mL pure acetonitrile followed by 10 mL of a mixture of acetonitrile and pure water (60/40, v/v). Both extracts were combined and residues in the soil or sediment extracts were directly determined by reversed-phase chiral LC-MS/MS.

LC-MS/MS conditions:

Chromatographic system: Waters Acquity LC-System
Analytical column: Daicel Chiralpak IA-3, 150 x 4.6 mm, particle size 3 µm
Target column temperature: 10 °C
Target sample temperature: 15 °C
Injection volume: 25 µL (partial loop with needle overfill; load ahead enabled; loop offline disabled)
Injection procedure: Weak wash with water/acetonitrile (600 µL)
Strong wash with acetonitrile (200 µL)
Mobile phase A: Water/formic acid (1000/1, v/v)
Mobile phase B: Acetonitrile/formic acid (1000/1, v/v)
Flow rate: 800 µL/min
Pressure limit of column: 300 bar (instrument set to 4351 psi)
Gradient (including wash and equilibration):

Time (min)	Phase A (%)	Phase B (%)
Initial	40	60
8.0	30	70
8.1	20	80
15.0	20	80
15.1	40	60
20.0	40	60

Gradient slope: linear gradient with curve initial to 6

Divert valve switching times: 0.0 min: to waste
5.5 min: to MS
8.0 min: to waste
Detection system: AB Sciex API6500 Triple Quadrupole Mass Spectrometer
Ionisation: Turbo Spray (ESI)
Analytes:

	Transitions	Polarity	Expected retention times
Reg. No. 5925581	275 → 105 *	Positive	Approx.. 6.1 min
	275 → 153		
Reg. No. 5925632	275 → 105 *	Positive	Approx.. 6.4 min

275 → 153

* Proposed as quantification transition.

A summary of the method validation data is given in Table B.5.1.2.1-1.

Table B.5.1.2.1-1 Summary of validation data for determination of cinmethylin (enantiomers Reg No. 5925581 and 5925632) in sediment and soil (LUFA 2.3 and Li 10)

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity*
Sediment	**Reg. 5925581 (1S,2R,4R)** *m/z* 275 → 105	0.005	0.005 0.05	95.1 – 101.5 (97.9) 97.5 – 104.7 (100.6)	3.4 (5) 2.7 (5)	0.25 – 10 ng/mL (0.001 – 0.04 mg/kg) n = 6 x 2 r = 0.9993
	Reg. 5925581 (1S,2R,4R) *m/z* 275 → 153	0.005	0.005 0.05	84.0 – 94.3 (89.4) 84.8 – 93.5 (90.8)	4.7 (5) 4.2 (5)	0.25 – 10 ng/mL (0.001 – 0.04 mg/kg) n = 6 x 2 r = 0.9999
	Reg. 5925632 (1R,2S,4S) *m/z* 275 → 105	0.005	0.005 0.05	85.6 – 109.6 (99.5) 91.2 – 104.8 (99.7)	10.1 (5) 5.3 (5)	0.25 – 10 ng/mL (0.001 – 0.04 mg/kg) n = 6 x 2 r = 0.9988
	Reg. 5925632 (1R,2S,4S) *m/z* 275 → 153	0.005	0.005 0.05	92.8 – 98.4 (95.5) 91.2 – 102.4 (96.5)	2.4 (5) 4.7 (5)	0.25 – 10 ng/mL (0.001 – 0.04 mg/kg) n = 6 x 2 r = 0.9996
LUFA 2.3 (soil)	**Reg. 5925581 (1S,2R,4R)** *m/z* 275 → 105	0.005	0.005 0.05	95.1 – 110.3 (102.8) 94.3 – 107.1 (101.4)	5.5 (5) 4.6 (5)	0.25 – 10 ng/mL (0.001 – 0.04 mg/kg) n = 6 x 2 r = 0.9985
	Reg. 5925581 (1S,2R,4R) *m/z* 275 → 153	0.005	0.005 0.05	99.9 – 106.3 (102.7) 92.8 – 107.9 (101.2)	3.0 (5) 5.8 (5)	0.25 – 10 ng/mL (0.001 – 0.04 mg/kg) n = 6 x 2 r = 0.9979
	Reg. 5925632 (1R,2S,4S) *m/z* 275 → 105	0.005	0.005 0.05	98.4 – 104.0 (100.7) 87.2 – 96.0 (93.3)	2.7 (5) 3.7 (5)	0.25 – 10 ng/mL (0.001 – 0.04 mg/kg) n = 6 x 2 r = 0.9992
	Reg. 5925632 (1R,2S,4S) *m/z* 275 → 153	0.005	0.005 0.05	93.6 – 104.8 (99.2) 86.0 – 96.8 (94.4)	4.7 (5) 3.8 (5)	0.25 – 10 ng/mL (0.001 – 0.04 mg/kg) n = 6 x 2 r = 0.9995
Li 10 (soil)	**Reg. 5925581 (1S,2R,4R)** *m/z* 275 → 105	0.005	0.005 0.05	95.9 – 102.3 (99.8) 103.9 – 108.7 (105.2)	2.6 (5) 1.9 (5)	0.25 – 10 ng/mL (0.001 – 0.04 mg/kg) n = 6 x 2 r = 0.9985

	Reg. 5925581 (1S,2R,4R) *m/z* 275 → 153	0.005	0.005 0.05	95.1 – 101.5 (99.0) 102.3 – 108.7 (104.9)	2.4 (5) 2.6 (5)	0.25 – 10 ng/mL (0.001 – 0.04 mg/kg) n = 6 x 2 r = 0.9979
	Reg. 5925632 (1R,2S,4S) *m/z* 275 → 105	0.005	0.005 0.05	96.2 – 100.6 (98.4) 96.4 – 107.2 (102.3)	1.7 (5) 3.5 (5)	0.25 – 10 ng/mL (0.001 – 0.04 mg/kg) n = 6 x 2 r = 0.9992
	Reg. 5925632 (1R,2S,4S) *m/z* 275 → 153	0.005	0.005 0.05	97.6 – 103.2 (101.2) 102.4 – 104.8 (103.9)	2.3 (5) 1.3 (5)	0.25 – 10 ng/mL (0.001 – 0.04 mg/kg) n = 6 x 2 r = 0.9995

*Linearity plots were not provided in the study report for the matrix matched sediment samples, only calibration curves for each analyte and mass transition in acetonitrile/water were presented. These data are acceptable for the soil matrices (LUFA 2.3 and Li 10) as the soil matrix does not have a significant effects however matrix matched samples are required for sediment. The study report includes a table of results used to plot response factor vs. nominal concentration (see pages 51 – 54), these data tables also include the peak area for each nominal concentration and therefore can be used to generate calibration curves. The data show a linear response in the sediment matrix with good correlation, therefore no additional data are required.

Matrix effects:

Matrix effects were assessed preparing matrix-matched standards for each matrix. For soil samples, it was demonstrated that the matrix load in the tested matrix-matched standards had no influence on the detection of Reg. No. 5925581 and Reg. No. 5925632 (response factors ranged from 91.3 to 95.8 %). Hence, quantification of soil samples was completed using solvent based standards.

For sediment samples, it was demonstrated that the matrix load in the tested matrix-matched standards had an impact on the detection of Reg. No. 5925581 and Reg. No. 5925632 (response factors ranged from 65.8 – 75.3 %). Hence, quantification of sediment samples was completed using matrix matched standards.

Linearity:

Acceptable linearity ($r \geq 0.9979$); was observed in the range of 0.25 ng/mL to 10 ng/mL for the two mass transitions of Reg. No. 5925581 and Reg. No. 5925632. Six calibration levels distributed over the concentration range given above were used. Calibration standards for soil analysis were prepared in acetonitrile/water (80/20, v/v), for analysis of sediment samples matrix-matched standards were used covering the same concentration range as given above. The range encompasses the LOQ by at least ± 20 %, for residues around 10 x LOQ the extract was diluted by a factor of 10 to be within the linear range.

Accuracy and Precision:

Soil and sediment were fortified with concentration of cinmethylin at 0.005 mg/kg (LOQ) and 0.05 mg/kg (10 x LOQ). The mean recoveries were in the acceptable range of 70 – 120 %, with %RSD of ≤ 20 % at each level.

Specificity and Confirmation of Analyte Identity:

The LC-MS/MS is considered specific to the analytes therefore additional confirmation of identity is not required. The ion transitions monitored for each analyte are appropriate.

Stability of samples:

Stability was confirmed for Reg. No. 5925581 and Reg. No. 5925632 in stock, fortification, and calibration standard solutions for a duration of 28 days, when stored under refrigerated conditions in the dark. Stock solutions of each analyte were prepared in acetonitrile, whereas fortification and calibration standard solutions were prepared in a mixture of acetonitrile/water (80/20, v/v). Additionally, stability was confirmed for Reg. No. 5925581 and Reg. No. 5925632 in soil and sediment matrix-matched standard solutions for a duration of 14 days, when stored under refrigerated conditions in the dark.

Stability was confirmed for Reg. No. 5925581 and Reg. No. 5925632 in soil and sediment extracts, prepared in acetonitrile/water (80/20, v/v), for a duration of 14 days in LUFA 2.3 soil, 15 days in Li 10 soil and 19 days in sediment, when stored under refrigerated conditions in the dark.

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4.

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

No studies submitted.

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

Several of the toxicological studies submitted in this dossier were conducted in the1980s and 1990s. The available analytical methodologies and validation data supporting these studies as far as was reported in the original study reports, have been assessed for their suitability. Where information is missing or lacking in detail, conclusions on the acceptability of the method have been made in conjunction with toxicology experts.

In addition the toxicological evaluation highlighted additional studies where method validation data could be expected. These have been considered and are discussed at the end of this section.

Report:	**KCA 4.1.2/3; [redacted] (1984a)** **Also submitted as KCA 5.3.1/3**
Title	Five week dietary feeding study of sd95481 technical in dogs CI-420-004
Guidelines:	None
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The method in this study was used for the determination of cinmethylin in 'dog chow' by GC. No further information or validation data are available as the time for retention of archived data has expired. The results presented indicate that the content of cinmethylin in the prepared chow was within ±3% of the target concentrations. The method for analysis is regarded as fit for the purpose of dose verification.

Report:	**KCA 4.1.2/4; [redacted] (1983a)** **Also submitted under KCA 5.3.1/3, KCA 5.3.2/2**
Title	Sub chronic feeding study of sd95481 in the rat. Volume I CI-425-001
Guidelines:	None
GLP:	No
Deviations	N/A
Previous evaluation:	None

Report:	**KCA 4.1.2/5; [redacted] (1983a)** **Also submitted under KCA 5.3.1/3 ,KCA 5.3.2/4**
Title	Sub chronic feeding study of sd95481 in the mouse CI-425-002
Guidelines:	None
GLP:	No
Deviations	N/A
Previous evaluation:	None

The method in these studies was used for the determination of cinmethylin in 'powdered feed'. No further information on the method or validation data are available as the time for retention of archived data has expired.. The results for the determination of cinmethylin the feed samples are presented in Table B.5.1.2.3-1. The results

indicate that the content of cinmethylin in the prepared feed was within ±6% of the target concentrations. The method for analysis is regarded as fit for the purpose of dose verification.

Table B.5.1.2.3-1: Results of the dose verification analysis in certified powdered feed

Matrix	Analyte	Target Dose level (mg/kg)	Cinmethylin content (mg/kg)		Repeatability % RSD (n)
			Range	Mean	
Powdered feed	Cinmethylin	30	28.6 – 31.6	29.9	4.3 (7)
		100	94.0 – 104.0	99.9	3.6 (7)
		300	270 - 310	294	4.5 (7)
		1000	950 - 1014	982	2.2 (7)

Report:	**KCA 4.1.2/6; [redacted] (1987a)** **Also submitted under KCA 5.3.2/5**
Title	13 week dietary feeding study in beagle dogs of cinch herbicide technical CI-425-003
Guidelines:	None
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The method in this study was used for the determination of cinmethylin in 'dog chow'. No further information on the method or validation data are available as the time for retention of archived data has expired. The results for the determination of cinmethylin the chow as tested for homogeneity of the chow and dose verification are presented in Table B.5.1.2.3-2. The results indicate that the content of cinmethylin in the prepared feed was within ±13% of the target concentrations. The method for analysis is regarded as fit for the purpose of dose verification.

Table B.5.1.2.3-2: Results of the dose verification and homogeneity analysis in 'dog chow'

Matrix	Analyte	Target Dose level (mg/kg)	Cinmethylin content (mg/kg)		Repeatability % RSD (n)
			Range	Mean	
Dog chow (dose verification)	Cinmethylin	2	1.73 – 1.85	1.79	- (2)
		100	96.7 – 97.8	97.3	- (2)
		200	188 - 190	189	- (2)
		3000	2920 - 2940	2930	- (2)
		6000	5500 - 5600	5575	- (2)
Dog chow (homogeneity testing)	Cinmethylin	2	2.05 – 2.09	2.06	1.0 (6)
		100	100 - 110	104	3.2 (6)
		200	196 - 202	198	1.3 (6)
		3000	2830 - 3060	2947	3.9 (6)
		6000	6060 - 6620	6280	4.1 (6)

Report:	KCA 4.1.2/7; [redacted] (1985a) Also submitted under KCA 5.3.2/6
Title	A one year dietary feeding study in dogs - sd95481 technical CI-427-002
Guidelines:	None
Deviations	N/A
Previous evaluation:	None

The method in this study is used for the detection of cinmethylin in canine diet. No further information on the exact methodology is available as the time for retention of archived data has expired. However some recovery validation data were available, prepared by fortifying blank diet. these data are presented in Table B.5.1.2.3-3. The results for the determination of cinmethylin the diet as tested for homogeneity of the diet and dose verification are presented in Table B.5.1.2.3-4. The results indicate that the content of cinmethylin in the prepared feed was within ±17% of the target concentrations. The method for analysis is regarded as fit for the purpose of dose verification.

Table B.5.1.2.3-3: Summary of validation data for determination of cinmethylin in 'canine diet'

Matrix	Analyte	Fortification level (mg/kg)	Recoveries		Repeatability % RSD (n)
			Range	Mean	
Canine diet	Cinmethylin	300	82 – 97	91	8.6 (3)
		10000	103-118	109	7.1 (3)

Table B.5.1.2.3-4: Results of the dose verification and homogeneity analysis in 'canine diet'

Matrix	Analyte	Target Dose level (mg/kg)	Cinmethylin content (mg/kg)		Repeatability % RSD (n)
			Range	Mean	
Canine diet (dose verification)	Cinmethylin	300	268 – 338	301	6.4 (19)
		3000	2840 – 3450	3036	7.5 (19)
		10000	9120 - 11100	9723	5.4 (19)
Canine diet (homogeneity testing)	Cinmethylin	300	250 - 327	295	6.1 (20)
		3000	2670 - 3400	2939	5.3 (20)
		10000	8890 - 10300	9506	5.2 (16)

Report:	KCA 4.1.2/8; [redacted] (1988a) Also submitted as KCA 5.3.2/7
Title	One year dietary feeding study in beagle dogs of cinch herbicide CI-427-003
Guidelines:	None
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	KCA 4.1.2/9; [redacted], (1988b)
Title	Cinch herbicide: reversibility of toxicity in beagle dogs (a 12 month feeding study with 6 months reversibility) CI-427-004
Guidelines:	None
GLP	Yes
Deviations	N/A
Previous evaluation:	None

This analytical method supports the following study:

- KCA 5.3.2/7 – CI-427-003

The method in these studies is used for the detection of cinmethylin in canine diet. No further information on the exact methodology is available as the time for retention of archived data has expired. The results for the determination of cinmethylin the diet as tested for homogeneity of the diet and dose verification are presented in Table B.5.1.2.3-5. The results indicate that the content of cinmethylin in the prepared feed was within ±25% of the target concentrations. The method for analysis is regarded as fit for the purpose of dose verification.

Table B.5.1.2.3-5: Results of the dose verification and homogeneity analysis in 'canine diet'

Matrix	Analyte	Target Dose level (mg/kg)	Cinmethylin content (mg/kg)		Repeatability % RSD (n)
			Range	Mean	
Canine diet (dose verification)	Cinmethylin	2	1.51 – 1.92	1.81	6.3 (10)
		30	26.4 – 33.0	30.1	5.6 (10)
		100	91.4 – 104	98.6	3.8 (10)
		2000	193 – 208	200	2.5 (10)
		3000	2735 - 3180	2975	4.5 (10)
Canine diet (homogeneity testing)	Cinmethylin	2	1.61 – 2.08	1.84	7.0 (15)
		30	26.4 – 30.9	29.3	4.5 (15)
		100	92.0 -108	99.2	4.9 (15)
		2000	187 – 222	202.1	5.4 (15)
		3000	2855 - 3355	3027	4.1 (15)

Report:	KCA 4.1.2/10; [redacted] (1985a)
Title	A 2 year feeding study of sd95481 in rats (volume 1 of 8) CI-427-001
Guidelines:	None
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

This analytical method supports the following studies:

- KCA 5.5/2 – CI-427-001
- KCA 5.5/3 – CI-427-007 (corrigendum to study KCA 5.5/2)
- KCA 5.5/4 – CI-427-008 (report amendment to study KCA 5.5/2)
- KCA 5.5/5 – CI-427-006 (report amendment to study KCA 5.5/2)

The method in this study is used for the analysis of cinmethylin in foodstuffs by Soxhlet extraction and GC-FID.

Sample Preparation:

Weigh 20.0 g of diet into a paper extraction thimble. Add to the flask dichloromethane (120 ml) and anti-bumping granules, then assemble the extractor. Extract the sample for 2.5 hours. Allow to cool, then transfer the extract to a 100 ml measuring cylinder, rinsing the flask with fresh dichloromethane to ensure that all of the cinmethylin is transferred. Dilute with dichloromethane to 100 ml.

A diet containing 30 µg g^{-1} produces an extract containing 6.0 µg ml^{-1} of cinmethylin by the procedure described above. More concentrated samples are diluted with dichloromethane to produce samples nominally containing 6.0 µg ml^{-1} of cinmethylin.

Three sets of typical chromatographic conditions were described as follows:

Column length	1m	1m	1m
Column diameter	3mm	3mm	3mm
Packing	2% m/m OV-101 on 100-120 mesh SUPELCOPORT	Ultrabond II on 100-120 mesh SUPELCOPORT	3% OV-17 on 100-120 mesh SUPELCOPORT
Oven temperature	180°C	170°C	180°C
Detector temperature	250°C	250°C	250°C
Carrier gas	N_2	N_2	N_2
Carrier gas flow rate	40 ml min^{-1}	50 ml min^{-1}	40 ml min^{-1}

The results for the routine determination of cinmethylin the diet as tested for dose verification are presented in Table B.5.1.2.3-6.

Table B.5.1.2.3-6: Results of the dose verification analysis in rat diet

Matrix	Analyte	Target dose (mg/kg)	Cinmethylin content (as % of nominal)		Repeatability % RSD (n)
			Range	Mean	
Rat diet	Cinmethylin	100	93 - 107	100	3.6 (43)
		300	92 – 107	99	3.5 (43)
		3000	94 - 106	100	3.0 (43)

Specificity:

The applicant indicates that matrix effects had not been investigated.

Linearity:

The analytical methodology makes use of single point calibration by diluting the sample to a nominal concentration and calculates the actual concentration against a reference standard in DCM, based on area. Therefore, linearity was not investigated as only one concentration is present in the samples.

Accuracy (recovery):

The average recovery is within the limits set in SANCO/3029/99 rev.4, and is therefore acceptable.

Precision (repeatability):

The precision of the method for the detection of cinmethylin was investigated over 53 samples. For the concentration range 6.0 µg ml^{-1}, the RSD produced was 3.9. Therefore the precision of this method is acceptable.

Based on the information above, this method cannot be considered fully validated according to SANCO/3029/99 rev. 4, due to the lack of linearity and specificity data. Linearity data is not considered necessary as all the samples were diluted for measurement to give the same nominal concentration and then quantified against a standard of the same nominal concentration. The applicant proposed that as a high number of determinations (n=43) showed acceptable repeatability the method should be considered fit for purpose.

The results indicate that the content of cinmethylin in the prepared feed was within ±8% of the target concentrations. The method for analysis is regarded as fit for the purpose of dose verification.

Report:	KCA 4.1.2/11; [redacted] (1986a)
Title	Oncogenicity study of sd95481 in the mouse CI-428-001
Guidelines:	None
GLP:	No
Deviations	N/A
Previous evaluation:	None

This analytical method supports the following studies:

- KCA 5.5/7 – CI-428-001
- KCA 5.5/8 – CI-427-002 (report amendment to study KCA 5.5/7)

The method in this study is used for the determination of cinmethylin in mouse diet. Samples were extracted with hexane/acetone (2/1), and analysed by GC-FID. Quantification was accomplished using diphenylpropane as an internal standard. No further information on the exact methodology is available as the time for retention of archived data has expired. The results for the determination of cinmethylin the diet as tested for homogeneity of the diet and dose verification are presented in Table B.5.1.2.3-7. The results indicate that the content of cinmethylin in the prepared feed was within ±12% of the target concentrations. The method for analysis is regarded as fit for the purpose of dose verification.

Table B.5.1.2.3-7: Summary of the dose verification analysis in mouse diet

Matrix	Analyte	Target dose (mg/kg)	Cinmethylin content (mg/kg)		Repeatability % RSD (n)
			Range	Mean	
Mouse diet (dose verification)	Cinmethylin	30	27.0 – 33.7	29.5	7.5 (12)
		100	94.1 – 105	99.4	3.6 (12)
		1000	940 - 1064	997	3.8 (12)
Mouse diet (homogeneity testing)	Cinmethylin	30	27.2 - 31.3	28.7	4.6 (24)
		100	93.0 - 112	101	5.2 (28)
		1000	977 - 1054	1011	2.3 (24)

Report:	KCA 4.1.2/12; [redacted] (1984a) Also submitted under KCA 5.6/1
Title	Two generation reproduction study of cinch herbicide sd95481 in rats CI-432-001
Guidelines:	None
GLP	Yes
Deviations	N/A
Previous evaluation:	None

This toxicity study is considered as invalid based on deficiencies identified during the study conduct. No critical toxicological endpoint is derived from this study. A new toxicity study is available. The new study is presented under KCA 5.6.1/1 and 2.

Report:	KCA 4.1.2/13; Catchpole G., Hidding B., (2017a)
Title	BAS 684 H (Cinmethylin) - Validation of an analytical method for the analysis of BAS 684 H in Isopropanol using GC-FID (control procedure 14/0066_07) 2017/1032967
Guidelines:	SANCO/3029/99 rev. 4
GLP	Yes
Deviations	N/A
Previous evaluation:	None

This analytical method supports the following study:

- KCA 5.2.3/1 - 2017/1068662

The method is used for the determination of cinmethylin in isopropanol solutions that were used as sorbent material for the dose verification of cinmethylin in air.

Sample preparation:

An aliquot of the sample is diluted in isopropanol to obtain a sample concentration within the linear range, prior to analysis by GC-FID using the following conditions:

GC conditions:

Chromatographic system: Agilent 6890 with auto sampler
Analytical column: Optima-delta 3, Machery-Nagel: 30 m, 0.32 mm i.d., film thickness 0.25 µm
Injector temperature: 300 °C
Spilt ratio: 1:20
Injection volume: 1.0 µL
Carrier gas Helium
Flow rate 1.4 mL/min
Temperature program

Initial temperature	70 °C
Hold time	3 min
Ramp rate	20 °C/min to 320 °C
Final hold time	9 min

Detection system: FID
Detector temperature: 340 °C

Validation data are presented in Table B.5.1.2.3-8.

Table B.5.1.2.3-8: Summary of validation data for determination of cinmethylin in isopropanol

Matrix	Analyte	LOQ (mg/100 mL)	Recovery fortification level (mg/100mL)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Isopropanol	Cinmethylin	0.2	0.203	98 – 100 (100)	0.8 (5)	0.16 – 4.78 mg/100mL r = 0.999883
			1.066	98 – 99 (98)	0.7 (5)	
			27.750	100 – 102 (101)	0.6 (5)	

Specificity:

In the chromatograms for the tested samples a peak at around 11 minutes can be seen for the active substance, and while there is a peak present at the same retention time in the matrix sample, it is less than 15% of the LOQ, and therefore acceptable. Therefore the specificity of this method can be considered acceptable.

Linearity:

The linearity of the response was determined from 8 concentrations ranging from 0.16 – 4.78 mg/100mL. The correlation coefficient for cinmethylin was 0.999883. The linearity of this method for the determination of cinmethylin was therefore confirmed to be acceptable.

Accuracy (recovery):

All 5 recoveries reported for this method were within the 70-110% limits, along with the average reported for the concentration group also within this range. The accuracy of this method can therefore be considered acceptable.

Precision (repeatability):

The precision of the method for the detection of cinmethylin was investigated over 3 concentrations, 0.203, 1.066 and 27.750 mg/100mL with 5 determinations at each. The mean RSDs produced at each concentration were 0.8, 0.7 and 0.6 respectively.

The LOQ has been set using the lowest concentration at which an acceptable recovery was made, while also within the linear range.

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4.

Report:	**KCA 4.1.2/14; Daum A. (2017a)**
Title	Analytical report BAS 684 H (Cinmethylin) - Concentration control analyses in paraffin 2017/1145822
Guidelines:	SANCO/3029/99 rev. 4
GLP	Yes
Deviations	N/A
Previous evaluation:	None

The method in this study is used for the determination of cinmethylin in paraffin.

Sample preparation:

Samples are diluted completely with cyclohexane using appropriate volumetric flasks to obtain sample solutions with test substance concentrations that fall within the calibration range. If required, all dilutions are sonicated for 5 minutes to ensure a complete dissolution of the test substance. Samples are analysed by GC-FID using the following conditions:

GC conditions:

Chromatographic system: Shimadzu 2010 with auto sampler
Analytical column: Optima-delta 3, Machery-Nagel: 30 m, 0.32 mm i.d., film thickness 0.25 µm
Injector temperature: 300 °C
Spilt ratio: 1:20
Injection volume: 1.0 µL
Carrier gas Helium
Flow rate 1.4 mL/min
Temperature program

Initial temperature	200 °C
Hold time	3 min
Ramp rate	10 °C/min to 320 °C
Final hold time	5 min

Detection system: FID
Detector temperature: 340 °C

Specificity:

There was no chromatographic peak present in the blank vehicle sample at the retention time of interest and no interference of the analytical peak was observed. Therefore the specificity of this method can be considered acceptable.

Linearity:

The linearity of the response was determined from 6 concentrations ranging from 10.04 – 50.2 mg/100mL. The correlation coefficient r for cinmethylin was 0.999452. The linearity of this method for the determination of cinmethylin was therefore confirmed to be acceptable.

Accuracy (recovery):

A single recovery value was determined at the highest tested concentration in the study (at 75 g/100 g). the recovery of cinmethylin was 96.7%.

Precision (repeatability):

Not determined.

Conclusion:

The analytical method is not fully validated in terms of accuracy and repeatability, as only one recovery sample was determined at the highest tested concentration in the study and no repeatability data was presented. The analytical methodology is considered to be fit for the intended purpose of dose verification in paraffin.

Report:	**KCA 4.1.2/15; [redacted]. (2015a)** **Also submitted under KCA 5.3.1/1**
Title	BAS 684 H (Cinmethylin) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet 2015/1076329
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA 4.1.2/16; [redacted]. (2016 a)** **Also submitted under KCA 5.3.1/2**
Title	BAS 684 H (Cinmethylin) - Repeated-dose 28-day toxicity study in C57BL/6JRj mice - Administration via the diet 2014/1162710
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA 4.1.2/17; Catchpole G., Hidding B. (2017 b)**
Title	BAS 684 H (Cinmethylin) - Validation of an analytical method for the analysis of BAS 684 H in Ground Kliba maintenance diet mouse/rat GLP meal using GC (control procedure 14/066_2) 2017/1123754
Guidelines:	SANCO/3029/99 rev. 4
GLP	Yes
Deviations	N/A
Previous evaluation:	None

This analytical method supports the following studies:

- KCA 5.3.1/1 - 2015/1076329
- KCA 5.3.2/1 - 2014/11228370
- KCA 5.3.2/3 – 2015/1005983
- KCA 5.5/1 - 2017/1093414
- KCA 5.6.1/1 - 2017/1094504 (and amendment KCA 5.6.1/2)

The method in this study is used for the detection of cinmethylin in rat and mouse diet.

Sample preparation:

Samples of feed (5g or 10g depending on concentration) were extracted three times with acetonitrile by shaking for 30 minutes and centrifuged. the supernatants were combined and made up to 100 mL with acetonitrile and filtered prior to analysis by GC-FID

GC-FID conditions:

Chromatographic system:	Shimadzu 2010 with auto sampler
Analytical column:	Optima-delta 3, Machery-Nagel: 30 m, 0.32 mm i.d., film thickness 0.25 µm
Injector temperature:	300 °C
Spilt ratio:	1:20
Injection volume:	1.0 µL
Carrier gas	Helium
Flow rate	1.4 mL/min
Temperature program	

Initial temperature	210 °C
Hold time	10 min
Ramp rate	20 °C/min to 340°C
Hold time	13 min

Detection system:	FID
Detector temperature:	340 °C

Validation data are presented in Table B.5.1.2.3-9 and procedural recovery and validation data in Table B.5.1.2.3-10. In addition, data reported in studies KCA 5.3.2/01, KCA 5.5/1 and KCA 5.6.1/1 is presented in Tables B.5.1.2.3-11 and B.5.1.2.3-12

Table B.5.1.2.3-9: Summary of validation data for determination of cinmethylin in rat and mouse diet [Catchpole G., Hidding B. (2017 b)]

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
GK mouse/rat GLP meal	Cinmethylin	50	50	98 – 104 (100)	2.1 (5)	0.1638-4.3000 mg/100mL r =0.999
			1000	97 – 101 (99)	1.6 (5)	
			10000	102 – 104 (102)	0.7 (5)	

Table B.5.1.2.3-10: Summary of procedural recovery data for determination of cinmethylin in rat and mouse diet [redacted (2015a) and (2016a)]

Matrix	Analyte	Recovery fortification level (mg/100 mL)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
GK mouse/rat GLP meal [redacted] (2015a)	Cinmethylin	30 - 49	91 – 101 (93)	5.4 (5)	0.22 – 3.86 mg/100mL r>0.995
		1250 - 6249	95 – 100 (97)	2.1 (5)	
		6250 - 31249	95 – 98 (97)	1.2 (5)	
GK mouse/rat GLP meal [redacted] (2016a)	Cinmethylin	0.5 – 2.49	99 – 106 (102)	2.6 (5)	0.2 – 3.56 mg/100mL r>0.995

Table B.5.1.2.3-11: Summary of validation data for determination of cinmethylin in rat and mouse diet [redacted (2018), KCA 5.5/1]

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
GK mouse/rat GLP meal	Cinmethylin	50	50-249	99 – 106 (102)	2.6 (5)	0.21-3.56- mg/100mL r =0.999

Table B.5.1.2.3-12: Summary of validation data for determination of cinmethylin in rat and mouse diet [redacted (2018), KCA 5.3.2/1) and [redacted]. (2018), KCA 5.6.1/1]

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
GK mouse/rat GLP meal	Cinmethylin	50	50-249	99 – 106 (102)	2.6 (5)	0.2 – 3.5 mg/100mL r>0.995
			250-1249	102 – 106 (103)	1.4 (5)	
			1250-6249	96 – 100 (98)	1.3 (5)	
			6250-31249	92 – 103 (98)	3.9 (5)	

Specificity:

No significant interferences (> 30% LOQ) were observed at the appropriate retention times. Therefore the specificity of this method can be considered acceptable.

Linearity:

The linearity of the response was determined from a range of concentrations in the studies. In all cases the correlation coefficient for cinmethylin was > 0.99. The linearity of this method for the determination of cinmethylin was therefore confirmed to be acceptable.

Accuracy (recovery):

All recoveries reported for this method were within the 70-110% limits, along with the average reported for the concentration group also within this range. The accuracy of this method can therefore be considered acceptable.

Precision (repeatability):

The precision of the method for the detection of cinmethylin was investigated over a range of concentrations, with 5 determinations at each. The RSDs produced were <20% in all cases.

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4.

Report:	KCA 4.1.2/18; Grauert E., Hidding B (2017a)
Title	Validation of an Analytical method for the analysis of BAS 684H (Cinemthylin) in corn oil using GC (Control Procedure 14/0066_05-02) 2017/1067141
Guidelines:	SANCO/3029/99 rev. 4
GLP	Yes
Deviations	N/A
Previous evaluation:	None

This analytical method supports the following studies:

- KCA 5.4.2/1 - 2018/1048783 (the mouse plasma analysis is covered under 4.1.2/21)
- KCA 5.5/6 - 2017/1094161 (the mouse plasma analysis is covered under 4.1.2/21)

The method in this study is used for the determination of cinmethylin in corn oil.

Sample preparation:

Samples are dissolved in isopropanol to obtain a cinmethylin concentration that falls within the calibration range determined. Analysis was by GC-FID using the following conditions:

GC-FID conditions:

Chromatographic system: Shimadzu 2010 with auto sampler
Analytical column: Optima-delta 3, Machery-Nagel: 30 m, 0.32 mm i.d., film thickness 0.25 µm
Injector temperature: 300 °C
Spilt ratio: 1:20
Injection volume: 1.0 µL
Carrier gas Helium
Flow rate 1.4 mL/min
Temperature program

Initial temperature	200 °C
Hold time	3 min
Ramp rate	10 °C/min to 260 °C
Hold time	0 min
Ramp rate	20 °C/min to 320 °C
Final hold time	4 min

Detection system: FID
Detector temperature: 340 °C

Validation data are presented in Table B.5.1.2.3-13.

Table B.5.1.2.3-13: Summary of validation data for determination of cinmethylin in corn oil

Matrix	Analyte	LOQ (mg/100 mL)	Recovery fortification level (mg/100 mL)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Corn oil	Cinmethylin	24	24	107 – 110 (108)	1.7 (5)	2.01 – 20.12 mg/100 mL r = 0.99
			5000	100 – 102 (101)	1.0 (5)	
			25000	100 – 101 (101)	0.4 (5)	

Specificity:

In the chromatograms for the tested samples a peak at around 6.4 minutes can be seen for the active substance, with no peak present at the same retention time in the blank sample, and is therefore acceptable. Therefore the specificity of this method can be considered acceptable.

Linearity:

The linearity of the response was determined from a range of concentrations from 2.012 – 20.12 mg/100mL. The correlation coefficient r for cinmethylin was 0.999914. The linearity of this method for the determination of cinmethylin was therefore confirmed to be acceptable.

Accuracy (recovery):

All but 1 (110.3%) of the recoveries reported for this method were within the 70-110% limits, along with the average reported for the concentration group also within this range. The accuracy of this method can therefore be considered acceptable.

Precision (repeatability):

The precision of the method for the detection of cinmethylin was investigated over 3 concentrations, 24, 5000 and 25000 mg/kg with 5 determinations at each. The RSDs produced at each concentration were 1.7, 1.0 and 0.4 respectively.

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4.

Report:	**KCA 4.1.2/19; Catchpole G, Hidding B (2018 a)**
Title	BAS 684 H (Cinmethylin) - Stability analysis in acetone 2018/1013043
Guidelines:	None
GLP	Yes
Deviations	N/A
Previous evaluation:	None

The method in this study is used for the detection of cinmethylin in foodstuffs using GC for detection.

Sample preparation:

Sample solution: Samples are diluted completely with 2 – propanol using appropriate volumetric flasks to obtain sample solutions with test substance concentrations that match the calibration range.

If required, all dilutions are sonicated for 5 minutes to ensure a complete dissolution of the test substance.

GC conditions:

Chromatographic system:	Agilent 6890 with autosampler		
Analytical column:	Optima-delta 3, Machery-Nagel: 30 m, 0.32 mm i.d., film thickness 0.25 µm		
Injector temperature:	300 °C		
Spilt ratio:	1:20		
Injection volume:	1.0 µL		
Carrier gas	Helium		
Flow rate	1.4 mL/min		
Temperature program		Initial temperature	70 °C
		Hold time	3 min
		Ramp rate	20 °C/min to 260 °C
		Hold time	9 min
Detection system:	FID		
Detector temperature:	340 °C		

Specificity:

In the chromatograms for the tested samples a peak at around 12.10 minutes can be seen for the active substance, with no peak present at the same retention time in the matrix sample, and is therefore acceptable. Therefore the specificity of this method can be considered acceptable.

Linearity:

The linearity of the response was determined from 6 concentrations ranging from 1.07 – 10.7 mg/100mL. The correlation coefficient r for cinmethylin was 0.9999. The linearity of this method for the determination of cinmethylin was therefore confirmed to be acceptable.

Accuracy (recovery):

Not determined.

Precision (repeatability):

Not determined.

Conclusion:

The analytical method is not fully validated in terms of accuracy and repeatability, however is considered to be fit for the intended purpose of determining cinmethylin in acetone solutions at time points of 0 hours and 4 hours to show stability in the test vehicle.

Report:	**KCA 4.1.2/20; Grauert E., Hidding B. (2017a)**
Title	Validation of an Analytical method for the Analysis of BAS 684 H (Cinmethylin) in 1% carboxymethyl cellulose (as sodium salt) in drinking water with Tween 80 (3 drops/1000 mL) using GC (Control procedure 14/0066_06-02) 2017/1166508
Guidelines:	SANCO/3029/99 rev. 4
GLP	Yes
Deviations	N/A
Previous evaluation:	None

This analytical method supports the following studies:

- KCA 5.3.3/1 - 2017/1094162
- KCA 5.3.3/2 – 2017/1094162 amendment 1
- KCA 5.6.2/2 – 2015/11158053

The method in this study is used for the determination of cinmethylin in aqueous cellulose solutions.

Sample preparation:

Samples are dissolved in 10ml acetonitrile/water (1/1 v/v) and diluted further as necessary with acetonitrile to give a concentration with the calibration range determined. Analysis was by GC-FID using the following conditions:

GC conditions:

Chromatographic system:	Shimadzu 2010 with autosampler
Analytical column:	Optima-delta 3, Machery-Nagel: 30 m, 0.32 mm i.d., film thickness 0.25 µm
Injector temperature:	300 °C
Spilt ratio:	1:20
Injection volume:	1.0 µL
Carrier gas	Helium
Flow rate	1.4 mL/min

Temperature program		Initial temperature	200 °C
		Hold time	3 min
		Ramp rate	10 °C/min to 260 °C
		Hold time	2 min
Detection system:	FID		
Detector temperature:	340 °C		

Validation data are presented in Table B.5.1.2.3-14.

Table B.5.1.2.3-14: Summary of validation data for determination of cinmethylin in aqueous cellulose solutions

Matrix	Analyte	LOQ (mg/100 mL)	Recovery fortification level (mg/100 mL)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Aqueous cellulose solution	Cinmethylin	48	48	107 – 109 (108)	0.6 (5)	3.972 – 19.86 mg/100 mL r = 0.999
			10000	92 – 99 (96)	3.3 (5)	
			25000	96 – 100 (98)	2.0 (5)	

Specificity:

In the chromatograms for the tested samples a peak at around 6.2 minutes can be seen for the active substance, with no peak present at the same retention time in the matrix sample. Therefore the specificity of this method can be considered acceptable.

Linearity:

The linearity of the response was determined from 6 concentrations ranging from 3.972 – 19.86 mg/100 mL. The correlation coefficient r for cinmethylin was 0.999. The linearity of this method for the determination of cinmethylin was therefore confirmed to be acceptable.

Accuracy (recovery):

All of the recoveries reported for this method were within the 70-110% limits, along with the average reported for the concentration group also within this range. The accuracy of this method can therefore be considered acceptable.

Precision (repeatability):

The precision of the method for the detection of cinmethylin was investigated over 3 concentrations, 48, 10000 and 25000 mg/kg with 5 determinations at each. The mean RSDs produced at each concentration were 0.6, 3.3 and 2.0 respectively.

The LOQ has been set using the lowest concentration at which an acceptable recovery was made, while also within the linear range.

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4.

Report:	KCA 4.1.2/21; Catchpole G., Hidding B. (2018)
Title	Validation of an Analytical method for the analysis of BAS 684 H and metabolites in rat plasma using HPLC-MS (Control procedure: 14/0066-11) 2018/1037312
Guidelines:	SANCO/3029/99 rev. 4
GLP	Yes
Deviations	N/A
Previous evaluation:	None

This analytical method supports the following studies:

- KCA 5.4.2/1 - 2018/1048783 (mouse plasma analysis)
- KCA 5.5/6 - 2017/1094161 (mouse plasma analysis)

The method in this study is used for the detection of cinmethylin and 4 metabolites (Reg. No. 6055478, Reg. No. 6055521, Reg. No. 111609 and Reg. No. 6059081) in blood plasma using HPLC-MS for detection.

Sample preparation:

Samples (100 µL) are diluted with 400 µL and mixed by vortex to precipitate any protein. The extracts are centrifuged for 5 minutes. An aliquot of the supernatant (250 µL) is mixed with 250 µL water before analysis by HPLC-MS using the conditions outlined below.

LC-MS/MS conditions:

Chromatographic system: Ultimate 3000 LC-System with autosampler, or similar
Analytical column: YMC Triart C18 ExRS, 50 x 3 mm, particle size 3 µm
Column temperature: 30°C
Injection volume: 5 µL
Mobile phase A: 950 mL acetonitrile + 50 mL water + 0.1 mL formic acid
Mobile phase B: 950 mL water + 50 mL acetonitrile + 0.1 mL formic acid
Flow rate: 0.5 mL/min
Gradient:

Time (min)	Phase A (%)	Phase B (%)
Initial	0	100
6	100	0
8	100	0
8.1	0	100
10	0	100

Detection system: Mass Spectrometer
Ionisation: (ESI)
Analytes:

	m/z	Polarity
cinmethylin	257	Positive
Reg. No. 6055478	319	Negative
Reg. No. 6055521	303	Negative
Reg. No. 111609	151	Negative
Reg. No. 6059081	169	Positive

Validation data are presented in Table B.5.1.2.3-15.

Table B.5.1.2.3-15: Summary of validation data for determination of cinmethylin in plasma

Matrix	Analyte	LOQ (ng/mL)	Recovery fortification level (ng/mL)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Blood plasma	Cinmethylin	100	100.2	84 – 99 (94)	5.9 (5)	Low: 7.904 – 98.8 ng/mL r= 0.998 High: 49.4 – 592.8 ng/mL r= 0.999
			3006	94 – 101 (99)	2.7 (5)	
			100200	96 – 109 (101)	4.9 (5)	
Blood plasma	Reg. No. 6055478	100	98.6	99 – 137 (107)	15.6 (5)	Low: 7.808 – 97.6 ng/mL r= 0.999 High: 48.8 – 585.6 ng/mL r= 0.999
			2958	95 – 99 (97)	1.4 (5)	
			98600	98 – 104 (101)	2.1 (5)	
Blood plasma	Reg. No. 6055521	100	99.4	102 – 107 (105)	2.1 (5)	Low: 8.256 – 103.2 ng/mL r= 0.999 High: 51.6 – 619.2 ng/mL r= 0.999 *49.7 – 497.0 ng/mL *r= 0.999
			2982	98 – 100 (99)	0.9 (5)	
			99400	101 – 106 (104)	1.7 (5)	
Blood plasma	Reg. No. 111609	100	99.4	104 – 106 (105)	0.9 (5)	Low: 8.368 – 104.6 ng/mL r= 0.999 High: 52.3 – 627.6 ng/mL r= 0.999
			2982	98 – 100 (99)	1.2 (5)	
			99400	99 – 104 (101)	2.4 (5)	
Blood plasma	Reg. No. 6059081	100	102	104 – 112 (108)	3.1 (5)	Low: 8.496 – 53.1 ng/mL r= 0.993076 High: 53.1 – 637.2 ng/mL r= 0.999
			3060	96 – 98 (97)	1.0 (5)	
			102000	923 – 100 (96)	2.9 (5)	

*values used for the analysis of data for 100000 ng/mL

Specificity:

In the chromatograms for the tested samples a peak at around 6.2 minutes can be seen for the active substance, with no peak present at the same retention time in the matrix sample, and is therefore acceptable. Therefore the specificity of this method can be considered acceptable.

Linearity:

The linearity of the response for both the low and high ranges for cinmethylin and each of the 4 metabolites was determined from 12 concentrations which can be seen in the table above. The correlation coefficient r was above 0.99 in all cases. The linearity of this method for the determination of cinmethylin and its 4 metabolites is therefore confirmed to be acceptable.

Accuracy (recovery):

All of the recoveries reported for the test substance and the 4 metabolites, except 1, were within the 70-110% limits. The one outlier (136.6% for metabolite 1) was included in the calculation of the mean accuracy, as it did not adversely affect the result. The accuracy of this method can therefore be considered acceptable.

Precision (repeatability):

The precision of the method for the detection of cinmethylin and its 4 metabolites was investigated over 3 concentrations, 100, 3000 and 100000 mg/kg with 5 determinations at each. The mean RSDs produced at each concentration were 0.6, 3.3 and 2.0 respectively.

The LOQ has been set using the lowest target concentration at which an acceptable recovery was made, while also within the linear range.

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4.

Additional toxicology studies that may have methods associated with them

The toxicological evaluation highlighted additional studies where method and validation data could be expected. These have been considered below.

The following study references are for published papers therefore do not contain detailed information on methods of analysis:

- KCA 5.1.2/2 - Miyazawa et al. (2001 a)
- KCA 5.8.1/1 - Maguin K. et al. (2006 a)
- KCA 5.8.1/2 - Masereeuw R. et al. (1995 a)

The following studies did not include any analytical determinations:

- KCA 5.4.2/2 - Smith W.M. and Sawin V.L. (1983 b)
- KCA 5.6.2/3 - Feussner E.L., (1985 b)
- KCA 5.8.2/1 - [redacted] (1983 c)
- KCA 5.8.2/2 - [redacted] (1983 d)

The following studies are either considered supplementary to the core toxicology evaluation or were considered invalid based on the study design therefore have not been relied upon:

- KCA 5.2.3/2 - [redacted]1986 b)
- KCA 5.6.2/4 - [redacted] (1987 b)
- KCA 5.8.3/4 - [redacted] (2011 a)

The following studies contained limited information on methods of analysis as the archiving of raw data has expired and no further information is available:

- KCA 5.6.2/1 - [redacted] (1984 b)

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 studies 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

Method L03378/01 was developed and validated for the determination of cinmethylin in plants. The method is proposed as the method for post-approval control and monitoring and is reported in full under Section B.5.2.1

Report:	**KCA 6.1/1, Spangler, C. (2018a)**
Title	Investigation of the storage stability of BAS 684 H in plant matrices Report number: 2016/1029128 (Study ID: 741160)
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

In this storage stability study an earlier version of BASF method L0337/01 was used to analyse cinmethylin residues in oilseed rape (winter) for storage intervals of 280 ± 7 days, 365 ± 7 days, 545 ± 7 days and 730 ± 7 days. This was because at this point the method was still under development.

Sample preparation:

Oilseed rape (storage intervals of 280 ± 7 days, 365 ± 7 days, 545 ± 7 days and 730 ± 7 days) specimens were extracted with a mixture of acetonitrile/water. After addition of $MgSO_4$, NaCl and buffering citrate salts, the mixture was shaken intensively and centrifuged for phase separation. The organic extract was cleaned up by dispersive SPE (d-SPE), partitioned against cyclohexane after addition of sodium hydroxide solution then centrifuged for phase separation. The cyclohexane phase was evaporated in the presence of 1-octanol.

Analytical method:

Analysis was accomplished by LC-MS/MS with the following conditions noted:

Chromatographic system: Waters Acquity UPLC system
Analytical column: Thermo Fisher Scientific Betasil C18: 100 mm x 2.1 mm, Particle size 5 µm
Target column temperature: 25 °C
Injection volume: 10 µL
Mobile phase A: Water/formic acid (1000/1, v/v)
Mobile phase B: Acetonitrile/formic acid (1000/1, v/v)
Flow rate: 600 µL/min
Gradient (including wash and equilibration):

Time (min)	Phase A (%)	Phase B (%)
0	70	30
0.1	40	50
2.5	40	60
5.5	20	80
5.6	0.1	99.9
7.0	0.1	99.9
7.1	70	30
10.0	70	30

Detection system: AB Sciex API 5000 Mass Spectrometer
Ionisation: Turbo Spray (ESI)
Retention time: BAS 684 H: approximately 4.7 min
Analytes: *m/z* 275 → 153 Quantification
m/z 275 → 105 Confirmatory

A summary of the method validation data is given in Table B.5.1.2.5-1.

Table B.5.1.2.5-1: Summary of validation data for determination of cinmethylin residues in oilseed rape

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity*
Oilseed rape (winter)	BAS 684 H m/z 275 → 153 Quantification	0.01	0.01	71.1 – 77.4 (72.9)	3.6 (5)	0.5 – 25 ng/mL (equivalent to 0.00125 – 0.0625 mg/kg) n = 6 x 2 repeats r = 0.9998
			0.1	75.2 – 83.9 (79.2)	5.0 (5)	
	BAS 684 H m/z 275 → 105 Confirmatory	0.01	0.01	69.2 – 77.4 (73.1)	3.9 (5)	0.5 – 25 ng/mL (equivalent to 0.00125 – 0.0625 mg/kg) n = 6 x 2 repeats r = 0.9999
			0.1	73.9 – 83.3 (79.1)	5.1 (5)	

Specificity and Confirmation of Analyte Identity:

The analysis was performed using LC-MS/MS using two mass transition ions and therefore no other confirmatory method was required.

Linearity:

Linearity of detector response was tested using six calibration standard concentrations in the range of 0.5 ng/mL to 25 ng/mL with correlation coefficients of >0.9990. The calibration standards were prepared in acetonitrile/water (80/20, v/v). Matrix-matched calibration was used for all storage intervals except for 365 ± 7 days and 545 ± 7 days.

Accuracy and Precision:

Samples were spiked with the analyte at LOQ and 10x LOQ. One recovery at 0.1 mg/kg was just outside of the acceptable range (70 – 110 %) at 69.2 %; however, the mean value at this fortification was in the acceptable range. Recoveries at the LOQ are acceptable. The %RSDs at each fortification level are all below 5.1 % and below the acceptable level (< 20 %).

Conclusion:

The method is fully validated in accordance with SANCO/3029/99 rev. 4 for the analysis of cinmethylin residues in oilseed rape (winter) with an LOQ of 0.01 mg/kg.

Report:	KCA 4.1.2/24, Castro M (2018a)
Title	Validation of BASF Method Number L0337/02 for the determination of M684H005 (Reg. No. 6067256) and M684H006 (Reg. No. 6067258) in citrus fruit, dry beans seed, sunflower seeds, lettuce heads, wheat grain, wheat (whole plant) and wheat straw by LC-MS/MS Report number: 2018/3000081 (Study ID: 783329)
Guidelines:	SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The purpose of this validation study was to demonstrate the applicability and repeatability of the method L0337/02 for the determination of M684H005 and M684H006 residues in plant matrices *via* LC-MS/MS.

Reference items:

BAS684H005 ([2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl) phenyl]methyl beta-D-glucopyranoside), BASF Reg. No. 6067256, batch L2017-020, purity 96.5 %, expiry date 01/04/19, CoA provided.

BAS684H006, BASF Reg. No. 6067258, batch L2017-019, purity 88.5 %, expiry date 01/04/19, CoA provided. Impurity specified at 6.4 % is M684H005.

Sample preparation:

Specimens are extracted with acetonitrile and water and a subsequent cleavage of M684H006 to M684H005 is performed by heating under alkaline conditions in aqueous sodium hydroxide. After addition of QuEChERS extraction salt kit ($MgSO_4$, NaCl and buffering citrate salts), the mixture is shaken intensively and centrifuged for phase separation. The organic extract is cleaned up by dispersive SPE (d-SPE) using QuEChERS clean-up kit containing $MgSO_4$, PSA, C18E. An aliquot of the extracts is subsequently diluted with water and acetonitrile/water.

For wheat (straw), the organic extract (after extraction and cleavage) is cleaned up in an additional first step by storage in a freezer for at least 6 hours.

For sunflower (seeds), clean-up is performed using QuEChERS d-SPE EMR-Lipid kit, QuEChERS Final Polish EMR-Lipid kit and a QuEChERS clean-up kit containing $MgSO_4$, PSA, C18E.

Analytical method (L0337/02):

Determination of M684H006 and M684H005, both detected as M684H005 using M684H005 reference standard and giving the total residue as M684H005, was achieved *via* HPLC-MS/MS with the following conditions noted:

Primary chromatographic conditions for all matrices mass transition 453 → 153 for wheat straw, wheat whole plant and sunflower seeds:

Chromatographic system: Waters Acquity UPLC system
Analytical column: Waters XSelect HSS T3 (100 mm x 2.1 mm, Particle size 2.5 µm)
Target column temperature: 45 °C
Injection volume: 25 µL
Mobile phase A: Water/formic acid (1000/1, v/v)
Mobile phase B: Acetonitrile/formic acid (1000/1, v/v)
Flow rate: 600 µL/min
Gradient (including wash and equilibration):

Time (min)	Phase A (%)	Phase B (%)
0	80	20
0.2	80	20
2.0	65	35
4.0	45	55
4.10	0.1	99.9
7.0	0.1	99.9
7.1	70	30
9.0	70	30

Detection system: AB Sciex API 5000 Triple quad Mass Spectrometer
Ionisation: Turbo Spray (ESI positive)
Retention time: M684H005: approximately 4.7 min
Analytes: *m/z* 453 → 291 Quantification
m/z 453 → 153 Confirmatory

Secondary chromatographic conditions were used (confirmatory) mass transition 453 → 291 for wheat straw, wheat whole plant and sunflower seeds:

Chromatographic system: Waters Acquity UPLC system
Analytical column: RESTEK, Pinnacle DB Biphenyl (100 mm x 2.1 mm, Particle size 1.9 µm)

Target column temperature: 45 °C
Injection volume: 25 µL
Mobile phase A: Water/formic acid (1000/1, v/v)
Mobile phase B: Acetonitrile/methanol/ formic acid (400/600/1, v/v/v)
Flow rate: 600 µL/min
Gradient (including wash and equilibration):

Time (min)	Phase A (%)	Phase B (%)
0	90	10
2.5	70	30
10.0	65	45
10.1	10	90
13.0	10	90
13.1	90	10
16.0	90	10

Detection system: Sciex Triple Quad 6500Triple quad Mass Spectrometer
Ionisation: Turbo Spray (ESI positive)
Retention time: M684H005: approximately 7.5 min
Analytes: *m/z* 453 → 291 Confirmatory

A summary of the method validation data is given in Table B.5.1.2.5-2 and B.5.1.2.5-3.

Table B.5.1.2.5-2: Summary of validation data for determination of residues of cinmethylin metabolite M684H005 in plant matrices

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Citrus (whole fruit)	**M684H005** *m/z* 453 → 291	0.01	0.01	92.4 – 109 (98.4)	7.4 (6)	0.04 – 2.5 ng/mL (0.0016 – 0.1 mg/kg) n = 6 x 2 repeats r = 0.9993
			1.0	92.8 - 110 (101)	6.2 (6)	
	M684H005 *m/z* 453 → 153	0.01	0.01	91.9 – 96.9 (94.6)	2.5 (6)	As above r = 0.9988
			1.0	95.3 - 108(103)	6.4 (6)	
Dry beans (seeds)	**M684H005** *m/z* 453 → 291	0.01	0.01	70.1 – 88.2 (80.5)	8.7 (5)	0.04 – 2.5 ng/mL (0.0016 – 0.1 mg/kg) n = 6 x 2 repeats r = 0.9985
			1.0	79.7 – 91.1 (84.6)	5.1 (5)	
	M684H005 *m/z* 453 → 153	0.01	0.01	70.8 – 83.2 (78.3)	6.7 (5)	As above r = 0.9965
			1.0	72.8 – 100 (89.9)	13 (5)	
Sunflower (seeds)	**M684H005** *m/z* 453 → 291 Primary conditions	0.01	0.01	86.8 – 96.9 (91.2)	3.6 (6)	0.04 – 2.5 ng/mL (0.0016 – 0.1 mg/kg) n = 6 x 2 repeats r = 0.9998
			1.0	82.6 – 91.6 (86.3)	3.8 (6)	
	M684H005 *m/z 453 → 291* Secondary conditions	0.01	0.01	72.6 – 90.3 (8.25)	9.2 (6)	As above r = 0.9978
			1.0	95.9 – 107 (101)	5.1 (6)	
Lettuce (heads)	**M684H005** *m/z* 453 → 291	0.01	0.01	77.5 – 86.2 (81.6)	3.8 (6)	0.04 – 2.5 ng/mL (0.0016 – 0.1 mg/kg) n = 6 x 2 repeats r = 0.9996
			1.0	71.2 – 81.9 (77.8)	4.8 (6)	
	M684H005 *m/z* 453 → 153	0.01	0.01	70.7 – 83.5 (79.3)	8.4 (6)	As above r = 0.9997
			1.0	72.1 – 79.1 (75.7)	3.4 (6)	
Wheat grain (seeds)	**M684H005** *m/z* 453 → 291	0.01	0.01	84.8 – 111 (94.6)	10 (6)	0.04 – 2.5 ng/mL (0.0016 – 0.1 mg/kg) n = 6 x 2 repeats r = 0.9993
			1.0	77.6 – 93.6 (85.9)	7.9 (6)	
	M684H005 *m/z* 453 → 153	0.01	0.01	83.1 – 109 (94.6)	11 (6)	As above r = 0.9981
			1.0	74.1 – 84.1 (78.7)	4.2 (6)	

Wheat whole plant	**M684H005** *m/z* 453 → 291	0.01	0.01	79.9 – 91.1 (86.8)	5.9 (6)	0.04 – 2.5 ng/mL (0.0016 – 0.1 mg/kg) n = 6 x 2 repeats r = 0.9999
			1.0	90.8 – 103 (97.2)	4.7 (6)	
	M684H005 *m/z 453 → 291*	0.01	0.01	81.5 – 109 (94.0)	11 (6)	As above r = 0.9996
			1.0	75.3 – 98.5 (87.1)	11 (6)	
Wheat straw	**M684H005** *m/z* 453 → 291	0.01	0.01	71.9 – 82.1 (77.2)	4.6 (6)	0.04 – 2.5 ng/mL (0.0016 – 0.1 mg/kg) n = 6 x 2 repeats r = 0.9999
			1.0	78.4 – 82.9 (80.2)	2.0 (6)	
	M684H005 *m/z 453 → 291* Secondary conditions	0.01	0.01	79.0 – 92.3 (83.4)	6.7 (6)	As above r = 0.9989
			1.0	80.1 – 89.5 (83.1)	4.5 (6)	

Table B.5.1.2.5-3: Summary of validation data for determination of residues of cinmethylin metabolite M684H006 (determined as M684H005) in plant matrices

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Citrus (whole fruit)	**M684H006** (determined as M684H005) m/z 453 → 291	0.01	0.01	81.6 – 113 (96.6)	11 (6)	0.04 – 2.5 ng/mL (0.0016 – 0.1 mg/kg) n = 6 x 2 repeats r = 0.9993
			1.0	104 - 110 (105)	4.4 (6)	
	M684H006 (determined as M684H005) m/z 453 → 153	0.01	0.01	86.4 – 114 (96.7)	12 (6)	As above r = 0.9988
			1.0	79.1 - 114 (99.9)	14 (6)	
Dry beans (seeds)	**M684H006** (determined as M684H005) m/z 453 → 291	0.01	0.01	76.4 – 87.1 (77.8)	8.7 (6)	0.04 – 2.5 ng/mL (0.0016 – 0.1 mg/kg) n = 6 x 2 repeats r = 0.9985
			1.0	74.2 – 87.1 (83.5)	6.0 (6)	
	M684H006 (determined as M684H005) *m/z* 453 → 153	0.01	0.01	64.9 – 87.7 (77.8)	10 (6)	As above r = 0.9965
			1.0	76.8 – 97.8 (90.6)	10 (6)	
Sunflower (seeds)	**M684H006** (determined as M684H005) *m/z* 453 → 291	0.01	0.01	91.5 – 106 (98.5)	6.0 (6)	0.04 – 2.5 ng/mL (0.0016 – 0.1 mg/kg) n = 6 x 2 repeats r = 0.9998
			1.0	84.4 – 94.0 (89.5)	4.2 (6)	
	M684H006 (determined as M684H005) *m/z* 453 → 291	0.01	0.01	87.4 – 113 (103)	9.8 (6)	As above r = 0.9978
			1.0	101 – 109 (107)	2.8 (6)	

Lettuce (heads)	**M684H006** (determined as M684H005) m/z 453 → 291	0.01	0.01	84.0 – 88.9 (87.0)	2.2 (6)	0.04 – 2.5 ng/mL (0.0016 – 0.1 mg/kg) n = 6 x 2 repeats r = 0.9996
			1.0	75.8 – 79.2 (80.1)	5.5 (6)	
	M684H006 (determined as M684H005) m/z 453 → 153	0.01	0.01	83.0 – 97.2 (91.3)	5.9 (6)	As above r = 0.9997
			1.0	78.9 – 86.1 (82.2)	4.4 (6)	
Wheat grain (seeds)	**M684H006** (determined as M684H005) m/z 453 → 291	0.01	0.01	96.2 – 108 (99.9)	4.2 (6)	0.04 – 2.5 ng/mL (0.0016 – 0.1 mg/kg) n = 6 x 2 repeats r = 0.9993
			1.0	70.8 – 81.9 (75.2)	5.9 (6)	
	M684H006 (determined as M684H005) m/z 453 → 153	0.01	0.01	96.2 – 108 (99.9)	4.2 (6)	As above r = 0.9981
			1.0	70.8 – 81.9 (75.2)	5.9 (6)	
Wheat whole plant	**M684H006** (determined as M684H005) *m/z* 453 → 291	0.01	0.01	88.4 – 109 (98.7)	7.8 (6)	0.04 – 2.5 ng/mL (0.0016 – 0.1 mg/kg) n = 6 x 2 repeats r = 0.9999
			1.0	71.1 – 95.8 (98.8)	11 (6)	
	M684H006 (determined as M684H005) *m/z* 453 → 291	0.01	0.01	92.9 – 114 (104)	7.6 (6)	As above r = 0.9996
			1.0	91.8 – 106 (100)	5.4 (6)	
Wheat straw	**M684H006** (determined as M684H005) *m/z* 453 → 291	0.01	0.01	89.1 – 104 (87.9)	3.8 (6)	0.04 – 2.5 ng/mL (0.0016 – 0.1 mg/kg) n = 6 x 2 repeats r = 0.9999
			1.0	91.3 – 110 (83.8)	6.2 (6)	
	M684H006 (determined as M684H005) *m/z* 453 → 291	0.01	0.01	88.7 – 101 (97.8)	4.7 (6)	As above r = 0.9989
			1.0	82.3 – 97.7 (89.4)	7.8 (6)	

Specificity and Confirmation of Analyte Identity:

The primary method is considered specific to the analytes therefore additional confirmation of identity is not required. The ion transitions monitored are appropriate. In the cases of wheat (straw and whole plant) and sunflower (seeds) a second set of chromatographic conditions were used as a confirmatory technique instead of the secondary mass transitions due to significant interferences (> 30% of LOQ) observed at the retention time. Due to the cleavage of M684H006 to M684H005, the method is considered suitable for the determination of residues as the sum of M684H005 and M684H006 only; however, this is in accordance with the proposed residue definition in plants, therefore no further consideration is required.

Linearity:

Linearity of detector response was tested using six calibration standard concentrations in the range of 0.0400 ng/mL to 2.5 ng/mL with correlation coefficients of >0.9900. The calibration standards were prepared in acetonitrile/water (50/50, v/v).

Matrix Effects:

It was shown that the matrix has no significant effects (>20%) on analysis. Therefore calibration standards for all commodities were analysed using solvent-based calibration standards.

Accuracy and Precision:

Samples were spiked with the analyte at LOQ and 100x LOQ. At least two blank control samples were also analysed within the sample sets. Some individual recoveries are outside of the acceptable range (70 – 110 %). However, as all mean recoveries were within the acceptable range, this is considered acceptable. The %RSDs at each fortification level are within the acceptable level (<20% RSD).

Storage stability:

The analytes M684H005 and M684H006 in standard solutions were shown to be stable in acetonitrile/water (50/50 v/v) for up to a period of 60 days when stored in the dark at 5 ± 3 °C.

The differences in recoveries between days 0 and 7 (0 and 8 for sunflower seeds) were all below 20 % for all matrices stored in the dark at 5 ± 3 °C indicating these analytes are stable in final volumes for at least 7 days.

Conclusion:

The method is fully validated in accordance with SANCO/3029/99 rev. 4 for the analysis of M684H005 and M684H006 (as the sum of M684H005 and M684H006) in plant matrices, citrus fruit, dry beans seeds, sunflower seeds, lettuce heads, wheat grain, wheat (whole plant) and wheat straw, with an LOQ of 0.01 mg/kg. The method is also fully validated in accordance with SANCO/825/00 rev.8.1.

Report:	**KCA 4.1.2/25, Rabe U., Forieri I (2017 a)**
Title	Investigation of the extractability of BAS 684 H in samples from 14C plant metabolism studies Report number: 2017/1166468 (Study ID: 777104)
Guidelines:	SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Samples from metabolism studies conducted with wheat and carrots were used to investigate the extraction efficiency of analytical methods L0337/01 and L0337/2 and the multiresidue methods DFG S19 and SweET for residues of cinmethylin and its metabolites M684H005 and M684H006. The extraction procedures used in these methods were compared to the extraction procedures used in the metabolism studies.

Sample preparation:

Method 1 (Method L0377/01):

Samples (5 g) of homogenized wheat forage and carrot leaves were extracted with 10 mL acetonitrile using a homogenizer. 10 mL water and QuEChERS salts were added and the extracts was incubated for 10 min at room temperature. After centrifugation, the volume of the acetonitrile phase was measured, and an aliquot taken for analysis.

Method 2 (Method L0377/02):

Samples (5 g) of homogenized wheat forage were extracted with a mixture of 6 mL water and 10 mL acetonitrile using a homogenizer, while approximately 2 g of homogenized wheat straw was extracted 10 mL water and 10 mL acetonitrile. The extracts were incubated with 1 mL sodium hydroxide (5 M) at 40 °C for 2 h and afterwards the pH was adjusted to 7.0 with sulfuric acid (2 M). QuEChERS salts were added and the extracts incubated at room temperature for 10 min. After centrifugation, the volume of the acetonitrile phase was determined, and an aliquot taken for analysis.

Method 3 (Multiresidue method DFG S19):

For wheat forage and carrot leaves, approximately 25 g of homogenized material were extracted with 20 mL water and 200 mL acetone using a homogenizer. For wheat straw, approximately 20 g of the homogenized sample were incubated with 98 mL water (40 °C). Thereafter, 200 mL acetone were added, and the mixture was extracted using a homogenizer. After centrifugation, the resulting supernatants were filtered, adjusted to defined volumes and analysed.

Method 4 (Multiresidue method SweET):

Samples (10 g) were extracted with a mixture of 3.0 g sodium bicarbonate, 10 g sodium sulphate and ethyl acetate (20 mL or 50 mL for wheat forage, 75 mL for wheat straw and 50 mL for carrot leaves) using a homogenizer assisted by ultrasonication. After centrifugation, the supernatant was filtered, adjusted to a defined volume and analysed.

Metabolism study:

Methanol/water sample extracts of wheat straw and carrot leaves were taken from the respective metabolism studies. For wheat forage a sample from the metabolism study was freshly extracted (as the analytes were found to be unstable in the original sample extracts) as follows: samples were extracted with three aliquots of methanol using a homogenizer. After centrifugation the three extracts were pooled, concentrated and adjusted to a defined volume.

Analysis:

All sample extracts were subjected to LSC (liquid scintillation counting) using the amount of radioactive residue extracted in the respective plant metabolism studies (ERR value) as the reference value for extraction efficiency.

HPLC analyses were performed to quantify the amounts of the metabolites M684H005 and M684H006 in wheat forage and wheat straw extracts and of cinmethylin in the carrot leaves extracts. The method used was method LC01 as described in the plant metabolism studies (see Volume 3 Section B.7.2.1). The relative amounts (sum of metabolite M684H005 and M684H006) were compared to the values found in the metabolism studies.

The results are summarised in Table B.5.1.2.5-4.

Table B.5.1.2.5-4: Summary of extractability of radioactive residues, cinmethylin and the metabolites M850H005 and M850H006 in cereal forage, cereal straw and carrot leaves.

Extraction method	TRR (mg/kg)	Radioactive residues in extract		Cinmethylin		Sum of M684H005 and M684H006	
		mg/kg (% TRR)	Extraction efficiency	mg/kg (% TRR)	Extraction efficiency	mg/kg (% TRR)	Extraction efficiency
Wheat forage (cyclohexane label)							
Methanol	2.678	2.357 (88)	100	Not detected		1.192 (45)	100
Method 1 (L0337/01)		1.703 (64)	72	Not detected		0.786 (29)	66
Method 2 (L0337/02)		2.755 (103)	117	Not detected		1.462 (55)	123
Method 3 (DFG S 19)		2.100 (78)	89	Not detected		1.082 (40)	91
Method 4 (SweEt, 20 mL)		0.108 (4)	5	Not analysed		Not analysed	
Method 4 (SweEt, 40 mL)		0.185 (7)	8	Not analysed		Not analysed	
Wheat forage (cyclohexane label)							
Metabolism study	9.732	8.353 (86)	100	Not detected		2.918 (30)	100
Method 1 (L0337/01)		Not tested					
Method 2 (L0337/02)		6.647 (68)	80	Not detected		2.722 (28.0)	93
Method 3 (DFG S 19)		5.375 (55)	64	Not detected		1.679 (17.3)	58
Method 4 (SweEt)		0.455 (5)	5	Not analysed		Not analysed	
Carrot leaves (cyclohexane label)							
Metabolism study	0.571	0.442 (78)	100	0.225 (39)	100	Not detected	

Method 1 (L0337/01)		0.324 (57)	73	0.182 (32)	81	Not detected
Method 2 (L0337/02)		Not tested				
Method 3 (DFG S 19)		0.329 (58)	74	0.175 (31)	78	Not detected
Method 4 (SweEt)		0.132 (23)	30	Not analysed		Not analysed

Example extraction efficiency calculation: (mg/kg extracted by method 1/ mg/kg extracted by metabolism study)*100

Discussion:

The extractability of radioactive residues from wheat forage and wheat straw was highest for Method 2 (117% and 80%, respectively). For wheat forage, the extractability was also high for Method 3, (89%) and Method 1 (72%). For wheat straw, Method 3 yielded a lower extractability (64%). Extractability of carrot leaves by Methods 1 and 3 (73% and 74%, respectively) was similar. For all three matrices, Method 4 showed the lowest extractability (5 - 30%).

Extraction Method 2 yielded the highest amounts of the metabolites M684H005 and M684H006 for both wheat forage and wheat straw (123% and 93, respectively). Methods 1 and 3 extracted lower amounts of M684H005 and M684H006 in wheat forage (66% and 91%, respectively). For wheat straw, the extraction Method 3 yielded 58% of M684H005 and M684H006. In carrot leaves, Method 1 and Method 3 yielded similar amounts of cinmethylin accounting for 81% and 78%, respectively.

Conclusion:

The extraction efficiency of Method 1, Method 2 and Method 3 was comparable across the three matrices tested. The lowest extraction efficiency was obtained applying Method 4 for all matrices.

Extraction efficiency of method L0377/01 and /02 is addressed for high water (carrot leaves and wheat forage) and difficult (wheat straw) matrices; these were the only matrices investigated. The representative uses include cereals (high protein/starch/dry matrices) and oilseeds (high oil), for which extraction efficiency was not investigated. However, based on the supervised residue trials submitted in support of the representative uses (see Volume 3 Section B.7.3.1 and B.7.3.2) no residues above the LOQ were found in cereal grain or oilseed samples other than whole plant and straw. Extraction efficiency is therefore considered sufficiently addressed.

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

Report:	**KCA 4.1.2/26 [redacted]., 1983 a**
Title	Acute toxicity of technical sd95481 to bluegill sunfish lepomis macrochirus CI-511-002
Guidelines:	None
GLP:	No
Deviations	None
Previous evaluation:	None

Cross reference KCA 8.2.1/7.

Report:	**KCA 4.1.2/27 [redacted]., 1983 b**
Title	Acute toxicity of technical sd95481 to rainbow trout salmo gairdneri CI-511-003
Guidelines:	None
GLP:	No
Deviations	None
Previous evaluation:	None

Cross reference KCA 8.2.1/1.

Report:	KCA 4.1.2/28 Forbis A. et al., 1983 c
Title	Acute toxicity of sd95481 to daphnia magna CI-521-001
Guidelines:	None
GLP:	No
Deviations	None
Previous evaluation:	None

Cross reference KCA 8.2.4.1/1.

Report:	KCA 8.2.1/9 [redacted] 1983 a
Title	Dynamic acute toxicity of sd95481 to bluegill sunfish Lepomis macrochirus CI-512-001
Guidelines:	None
GLP:	No
Deviations	None
Previous evaluation:	None

The four studies listed above all rely on the same HPLC-UV analytical method, the limited validation data from across all three studies are presented below.

An analytical method (HPLC) was required for the determination of the concentration of SD 95481 (BAS 684 H) in application solutions, the conditions are provided below:

HPLC-UV conditions:

Instrument:	Waters Model 6000A solvent delivery system
HPLC:	Column: µBondapak C18 reverse phase column (Waters Associates #27836)
Injector:	Rheodyne Model 7125 injector
Injection volume:	80 µL
Mobile phase:	70 % CH_3CN and 30% deionised water
Flow rate:	1.5 mL/min
PSI:	2000
Detection range:	0.2 AUFS
UV Detection:	Schoeffel Model SF770 Spectroflow UV detector 205 nm (used for quantification)
Retention time:	Approx. 4.50 min

Linearity:

Calculations of the SD 95481 concentrations were made using a normalised standard curve, which determined using the linear regression functions of a Texas Instruments TI-55 calculator. Linearity of detector response was tested using six calibration standard concentrations in the range of 0.52 µg/mL to 20.8 µg/mL with correlation coefficients of > 0.999. Example graphs showing the linear ranges have been provided, however, the acceptability of the graphs is questionable since visually it is difficult to determine where the concentration points would fall.

It is not possible to accept the linearity of this method as the example chromatograms for the highest concentration of the linear range show overloading of the sample, with the detector being saturated giving a broad flat top peak. Therefore, this leads to inaccuracy in the results from the highest concentration and so the results are not acceptable.

Accuracy and precision:

The recoveries of BAS 684 H in test water are summarised in the table below. The method is assessed in a range of 1.04 mg/L to 104 mg/L. The mean recovery values were between 100% and 109%. The relative standard

deviations (RSD, %) for the fortification level 10.4 mg/L was < 20 %, although it is noted that only 3 replicates were completed.

Table B.5.1.2.6-1: Summary of validation data for determination of cinmethylin in application solutions

Analyte	Matrix	Fortification Level [mg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%][1]	RSD [%]
BAS 684 H	Test Water	1.04	1	109	--	106	3.3
		10.4	3	107	0.9		
		104	1	100	--		

Specificity and Confirmation of Analyte Identity:

Specificity was shown in provided chromatograms with no significant interferences from the sample matrix were detected at the retention time corresponding to BAS 684 H in any of the control samples.

LOQ:

The limit of quantitation (LOQ) of the method, defined by the lowest fortification level is 1.04 mg/L, although this is not fully validated.

Conclusion:

The method is not considered validated in accordance with SANCO/3029/99 rev. 4 due to insufficient information being provided. The following deficiencies have been noted:

i) It is not possible to accept the linearity of the method as the example chromatograms for the highest concentration of the linear range show overloading of the sample, with the detector being saturated giving a broad flat top peak.

ii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.

iii) The LOQ is not supported by 5 recovery determinations.

iv) Procedural recoveries have not been completed.

Report:	**KCA 4.1.2/30 [redacted] 1990 a**
Title	WL95481 (Argold): An early life stage test with the fathead minnow (Pimephales promelas) RAFINESQUE
Guidelines:	EPA 540/9-82-024, EPA 72-4
GLP:	Yes
Deviations	None
Previous evaluation:	None

Cross reference KCA 8.2.2.1/1

Sample preparation:

The test samples were sampled in order of increasing nominal concentration. The solutions were sampled centrally within each test vessel, using a pipette (10 mL). Each aliquot was transferred to a screw capped glass scintillation vial then 10 mL hexane added. Portioning was carried out by thorough agitation on a vortex mixer for 1 minute. The phases were allowed to separate before carefully transferring the upper hexane phase to a second glass scintillation vial containing anhydrous sodium sulphate (approximately 2 g). The vial was capped and shaken for about 30 seconds to dry the hexane extract. The samples were the analysed using GC/MSD and the following processing was applied:

i) Untreated control extracts – a portion (5 mL) of extract was concentrated to a 1 mL volume using a gentle stream of nitrogen.

ii) The 0.1 and 0.2 mg/L nominal concentration extracts received no further processing.

iii) The remaining test solution extracts were diluted with hexane as follows:

Nominal concentration (mg/L)	Aliquot after drying (mL)	Final volume for analysis (mL)	Dilution factor
0.5	5	10	2
1.0	5	20	4
2.0	5	50	10
5.0	2	50	25

GC/MSD conditions:

Column:	Fused silica capillary, 25 m x 0.2 mm ID
Stationary phase:	Phenyl (5 %) methyl silicone
Mobile phase:	Helium at approximately 1 mL/min
Injection mode:	Splitless, automatic
Injection temperature:	260 °C
Column temperature programme:	Initial value: 50 °C held for 1 minute Programme rate: 15 °C per minute Final value: 260 °C held for 8 minutes
Detection method:	Selected ion-monitoring, electron impact ionisation
Ions monitored (m/z)	105, 123
Retention time:	15.1 min

Table B.5.1.2.6-2: Summary of validation data for cinmethylin in water

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Water	BAS 684 H	0.1	0.1	92	-	-
			1.0	105, 107, 108 (106.7)	1.4 (3)	
			5.0	106	-	
			35.0	97, 99 (98)	-	

Linearity, specificity, matrix effects, and interference were not further detailed in the study report available.

Accuracy and precision:

The recoveries provided were in the acceptable range of 70 – 120 %, with %RSD of ≤ 20 %. However, five determinations should have been made at two different fortification levels therefore the data do not meet the criteria specified in SANCO/3029/99 rev.4.

Stability:

BAS 684 H was stable in stock and diluted stock solutions for six days.

Conclusion:

The method is not validated in accordance with SANCO/3029/99 rev. 4. The following deficiencies have been noted:

i) No specificity data have been provided and no chromatograms have been submitted to check interferences.

ii) No linearity data have been provided.

iii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.

iv) The LOQ is not supported by 5 recovery determinations.

v) Matrix effects have not been investigated.

vi) Procedural recoveries have not been completed.

Report:	KCA 4.1.2/32 Pearson N., Stephenson R.R., 1987 b
Title	WL95481: Acute toxicity to Gammarus pulex, Lymnaea stagnalis, Tubifex tubifex and Chironomus lugubris CI-521-006
Guidelines:	EPA 540/9-82-024
GLP:	Yes
Deviations	None
Previous evaluation:	None

Cross reference KCA 8.2.4.2/1.

Water samples were extracted by passage through a pre-washed Bond Elut C18 cartridge. Cinmethylin was eluted from the cartridge using an ethyl acetate/hexane mixture. The eluate was concentrated under a stream of nitrogen before analysis by GC-MS, method SAMS 398-2. No specific details for the GC-MS were provided in the study report.

In standard recovery experiments in which test media was fortified with cinmethylin at 0.5, 1.0, 5.0 and 10 mg/L the recovery efficiency of the analysis method was found to be 93 – 126 %. Cinmethylin was not detected (< 0.01 mg/L) in the control, un-dosed media.

Conclusion:

The method is not considered fully validated in accordance with SANCO/3029/99 rev. 4 due to an insufficient information being provided. The following deficiencies have been noted:

i) No specificity data have been provided and no chromatograms have been submitted to check interferences.

ii) No linearity data have been provided.

iii) To assess accuracy and precision, at least 5 recoveries should be provided for at least 2 fortification levels.

iv) The LOQ is not supported by 5 recovery determinations.

v) Matrix effects have not been investigated.

vi) Procedural recoveries have not been completed.

Report:	KCA 4.1.2/33 Pearson N., Girling A., 1989 a
Title	WL95481: Chronic toxicity to Daphnia magna CI-523-001
Guidelines:	None
GLP:	Yes
Deviations	None
Previous evaluation:	None

Cross reference KCA 8.2.5.1/1

Sample preparation:

For each sample of test medium, a 10 mL aliquot was obtained using a pipette and immediately shaken with 10 mL of hexane for one minute then the phases allowed to separate. A portion of the hexane phase was withdrawn, diluted if appropriate, and analysed for BAS 684 H by gas chromatography with mass selective detection.

GC-MS conditions:

Column: 25 m x 0.20 mm ID fused silica coated with phenyl (5 %) methyl silicone (0.5 µm

	thickness)
Carrier gas:	Helium
Flow rate:	1 mL/min
Temperature programme:	Initial: 50 °C held for 1 minute Ramp rate: 15 °C/min Final: 260 °C held for 1 minute
Injector:	Splitless
Injector temperature:	260 °C
Injection volume:	2 µL by auto-injector
Ions monitored:	*m/z* 105 and 123

Table B.5.1.2.6-3: Summary of validation data for BAS 648 H in water

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % (mean)*	Repeatability % RSD (n)	Linearity
Water	BAS 684 H	0.5	0.5	105, 95, 105, 100, 100, 95, 100, 100 (100)	3.8 (8)	-
			5	105, 100, 110, 95, 95, 90, 100, 95 (98.8)	6.4 (8)	

* It is noted that each recovery was completed on a different day and are therefore considered similar to procedural recoveries, rather than method validation data.

Conclusion:

The method is not considered fully validated in accordance with SANCO/3029/99 rev. 4 due to an insufficient information being provided. The following deficiencies have been noted:

i) No specificity data have been provided and no chromatograms have been submitted to check interferences.

ii) No linearity data have been provided.

iii) Matrix effects have not been investigated.

iv) Standard recoveries have not been completed concurrently and could therefore be considered procedural recoveries, rather than validation data.

Report:	**KCA 4.1.2/34 [redacted] 1988 a**
Title	Cineole alcohol: Acute toxicity to rainbow trout Salmo gairdneri and Daphnia magna CI-570-001
Guidelines:	EEC 79/831 A V C, EEC 79/831 A V C 2
GLP:	Yes
Deviations	None
Previous evaluation:	None

Cross reference KCA 8.2.1/10

Sample preparation:

A Bond Elut C18 cartridge was prepared by washing with methanol (5 mL) followed by distilled water (2 x 8 mL). A water sample (50 mL) was passed through the cartridge at a flow rate of 3 – 5 mL/min. The flow rate was adjusted by the use of applied vacuum. The cartridge was washed with distilled water (5 mL) before drying by applying vacuum for at least 5 minutes. The cineole alcohol (M684H003) was eluted from the cartridge with ethyl acetate (2 % volume) in hexane (5 mL). The final extracts were diluted with hexane as appropriate for the final GC-MSD determination. Aqueous solutions of nominal concentration greater than 200 mg/L were diluted tenfold with distilled water prior to analysis.

GC-MSD conditions:

Column:	23 m x 0.2 mm ID fused silica
Stationary phase:	Phenyl (5 %) methyl silicone
Mobile phase:	Helium
Flow rate:	1 mL/min
Injector temperature:	270 °C
Column temperature:	Initial: 50 °C, hold for 1 min Programme rate: 10 °C/min until 140 °C Programme rate: 5 °C/min Final: 160 °C, hold for 1 min
Detector temperature:	200 °C
Detection method:	Positive ion electron impact selective ion monitoring (SIM)
Ions monitored:	*m/z* 43, 112, 170
Retention time:	10 min

Table B.5.1.2.6-4: Summary of validation data for cineole alcohol (M684H003) in aqueous media

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
Aqueous media	M684H003 (cineole alcohol)	10	10 100	89, 90, 96, 96, 93 (93) 96, 105, 99, 98, 105 (101)	3.5 (5) 4.1 (5)	-

Linearity:

The GC-MSD system was calibrated using know concentrations of cineole alcohol (M684H003) in hexane. In order to check the stability of the GC-MSD system during the analysis, injections of these standard solutions were interspersed with sample injections. However, the exact sample concentrations and linear plot have not been provided.

Accuracy and precision:

The mean recoveries were in the acceptable range of 70 – 120 %, with %RSD of ≤ 20 % at each level.

Specificity and Confirmation of Analyte Identity:

The m/z ratios chosen are specific for the analyte. The identification and quantification were based on the selected m/z ratios and the retention time. Under the described conditions the method is specific for the determination of M684H003 in aqueous media. However, no spectra were provided in the study report.

Breakthrough of the cartridge:

A maximum concentration of 300 mg/L M684H003 can be retained on the cartridge before recovery values are significantly lower (< 70 %). Therefore dilution of test solution > 300 mg/L is required before extraction.

Procedural recoveries:

Procedural recoveries were carried out during the studies, results shown below. The recoveries are within the acceptable range of 70 – 120 %, with acceptable RSD, indicating that the method is working correctly in this study giving precise and accurate results.

Table B.5.1.2.6-5: Procedural recovery data for cineole alcohol (M684H003) in aqueous media

Matrix	Analyte	LOQ (mg/L)	Analysed sample	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)
Aqueous media	M684H003 (cineole alcohol)	10	Daphnia study	100	105, 97, 99 (100)	4.1 (3)
			Salmo gairdneri study	100	93, 98, 93, 84, 94, 82, 94, 96 (92)	6.2 (8)

Conclusion:

The method is not considered validated in accordance with SANCO/3029/99 rev. 4 due to insufficient information being provided. The following deficiencies have been noted:

i) Missing data on the specificity (no chromatograms were provided)

ii) Linearity not fully addresses as no calibration curve, equation or standards were provided

Report:	KCA 4.1.2/35 Lockard L.A. et al., 2016 a
Title	Analytical method verification for the determination of BAS 684 H in avian diet 2016/7001370
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	KCA 4.1.2/36 Lockard L.A., Martin K.H., 2017 a
Title	Amended final report - Analytical method verification for the determination of BAS 684 H in avian diet 2017/7017248
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

This analytical method supports the following studies:

- KCA 8.1.1.3/1 - 2016/7009945
- KCA 8.1.1.2/1 - 2017/7008676
- KCA 8.1.1.2/3 - 2017/7008678
- KCA 8.1.1.3/2 - 2017/7016288 (NB: Column used is Restek RTX-5MS (30 m X 0.25 mm I.D., 0.25 µm film thickness); the stationary phase is exactly the same, except that MS-columns show less bleeding and better chromatographic separation.)

Sample preparation:

Samples of avian diet (2.5 g) were weighed into separated 50 mL polypropylene centrifuge tubes. To each centrifuge tube, 25 mL of acetone was added and the samples placed on a Geno/Grinder at ~1200 rpm for ~30 minutes. All samples were then centrifuged at ~4415 RCF for 10 minutes. Samples were diluted as necessary with acetone and submitted for analysis by gas chromatography with flame ionisation detection (GC-FID).

GC-FID conditions (quantification, RTX-5 column):

Instrument: Agilent Technologies Model 5890 Gas Chromatograph (GC)
Detector: Flam Ionization Detector (FID)
Analytical column: Restek RTX-5 column (30 m x 0.25 mm, 0.25 µm film thickness)

Injector temperature:	250 °C	
Run time:	18.6 minutes	
Oven:	Initial temperature:	100 °C
	Initial hold time:	1.50 minutes
	Ramp 1:	10 °C/minute
	Final temperature 1:	200 °C
	Final hold time 1:	0.00 minutes
	Ramp 2:	60 °C/minute
	Final temperature 2:	325 °C
	Final hold time 2:	5.00 minutes
FID detector:	Temperature: 300 °C	
Injection volume:	1.00 µL	
Injection technique:	Splitless	
Carrier gas:	Helium	
Head pressure:	9 psi	
Retention time:	12.0 minutes	

GC-FID conditions (confirmation, DB-624UI column):

Instrument:	Agilent Technologies Model 5890A Series II Gas Chromatograph (GC)	
Detector:	Flam Ionization Detector (FID)	
Analytical column:	DB-624 UI column (30 m x 0.25 mm, 1.40 µm film thickness)	
Injector temperature:	250 °C	
Run time:	23.0 minutes	
Oven:	Initial temperature:	70 °C
	Initial hold time:	1.00 minutes
	Ramp 1:	30 °C/minute
	Final temperature 1:	250 °C
	Final hold time 1:	16.00 minutes
FID detector:	Temperature: 230 °C	
Injection volume:	2.00 µL	
Injection technique:	Splitless	
Carrier gas:	Helium	
Head pressure:	12 psi	
Retention time:	19.97 minutes	

Table B.5.1.2.6-6: Summary of validation data for BAS 684 H in avian diet (quantification Restek RTX-5 column)

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Avian diet	BAS 684 H	45	45	100.5 – 104.0 (102.1)	1.24 (5)	1 – 10 µg/mL (equivalent to 10 – 100 mg/kg) n = 5 R^2 = 1.000
			120	103 – 104 (103)	0.72 (5)	
			1200	98 – 101 (99)	1.07 (5)	
			6000	95.7 – 97.9 (99.4)	0.959 (5)	

Table B.5.1.2.6-7: Summary of validation data for BAS 684 H in avian diet (confirmation DB-624UI column)

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Avian diet	BAS 684 H	45	45 6000	101.8 – 105.1 (103.3) 97.5 – 99.6 (98.6)	1.22 (5) 0.847 (5)	1 – 10 µg/mL (equivalent to 10 – 100 mg/kg) n = 5 R^2 = 0.999

Linearity:

Linearity of detector response was tested using 5 calibration standard concentrations in the range of 1 mg/mL to 10 mg/mL with a correlation coefficient of 1.0000. The calibration standards were prepared in acetone. The range encompasses the LOQ by at least ± 20 %, more concentrated samples were diluted to be within the linear range.

Accuracy and precision:

The mean recoveries were in the acceptable range of 70 – 120 %, with %RSD of ≤ 20 % at each level.

Specificity and Confirmation of Analyte Identity:

Under the described conditions the method is specific for analysis of BAS 684 H in avian diet. Quantification was done by GC-FID. The retention times of the test items in samples matched the retention times in calibration solutions. No peak interferences occurred at the retention times of BAS 684 H.

For confirmation of the method, analysis was performed using a DB-624 UI column (30 m x 0.250 mm, 1.4 µm) for GC-FID.

Matrix effects:

No significant matrix enhancement or suppression was observed hence quantification using solvent standards is acceptable.

Procedural recoveries:

Procedural recoveries were carried out during the studies, results shown below. The recoveries are within the acceptable range of 70 – 120 %, indicating that the method is working correctly and giving accurate results.

Table B.5.1.2.6-8: Summary of procedural recovery data for BAS 684 H in avian diet

Study	Sample	Sample interval	Cinmethylin concentration (mg/kg)		% Recovery
			Nominal	Measured	
KCA 8.1.1.3/1: 2016/7009945	Blank	Day 0, Week 1	0	< LOQ†	-
	Fortification	Day 0, Week 1	160	159	99
	Fortification	Day 0, Week 1	1200	1167	97
	Blank	Day 7, Week 1	0	< LOQ†	-
	Fortification	Day 7, Week 1	160	165	103
	Fortification	Day 7, Week 1	1200	1197	100
	Blank	Day 0, Week 4	0	< LOQ†	-
	Fortification	Day 0, Week 4	160	164	103
	Fortification	Day 0, Week 4	1200	1208	101

Study	Sample	Sample interval	Cinmethylin concentration (mg/kg)		% Recovery
			Nominal	Measured	
	Blank	Day 0 & 7, Week 12	0	< LOQ†	-
	Fortification	Day 0 & 7, Week 12	160	162	102
	Fortification	Day 0 & 7, Week 12	1200	1208	101
	Blank	Day 0, Week 16	0	< LOQ†	-
	Fortification	Day 0, Week 16	160	161	101
	Fortification	Day 0, Week 16	1200	1218	101
	Blank	Day 0 & 7, Week 20	0	< LOQ†	-
	Fortification	Day 0 & 7, Week 20	160	171	107
	Fortification	Day 0 & 7, Week 20	1200	1204	100
KCA 8.1.1.2/1: 2017/7008676	Blank	Day 0 & 5	0	< LOQ	-
	Fortification	Day 0 & 5	120	120	100
	Fortification	Day 0 & 5	6000	5810	97
KCA 8.1.1.2/1: 2017/7008678	Blank	Day 0 & 5	0	< LOQ	-
	Fortification	Day 0 & 5	120	120	100
	Fortification	Day 0 & 5	6000	5810	97
KCA 8.1.1.3/2: 2017/7016288	Blank	Day 0, Week 1	0	< LOQ	-
	Fortification	Day 0, Week 1	120	118	99
	Fortification	Day 0, Week 1	1200	1200	100
	Blank	Day 7, Week 1,Day 0, Week 4	0	< LOQ	-
	Fortification	Day 7, Week 1,Day 0, Week 4	120	133	111
	Fortification	Day 7, Week 1,Day 0, Week 4	1200	1296	108
	Blank	Day 0 & 7, Week 12	0	< LOQ	-
	Fortification	Day 0 & 7, Week 12	120	120	100
	Fortification	Day 0 & 7, Week 12	1200	1145	95
	Blank	Day 0, Week 16	0	< LOQ	-
	Fortification	Day 0, Week 16	120	119	99
	Fortification	Day 0, Week 16	1200	1179	98
	Blank	Day 0 & 7, Week 20	0	< LOQ	-
	Fortification	Day 0 & 7, Week 20	120	124	103
	Fortification	Day 0 & 7, Week 20	1200	1457	121

LOQ was 45 ppm based on method validation except: † where LOQ set at 50 ppm based on the product of the lowest standard (1 ppm) and the dilution factor of the matrix blank extract (50)

Conclusion

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 45 mg/kg.

Report:	KCA 4.1.2/37 Grande A., 2017 a
Title	Validation of BASF method L0378/01 for the determination of BAS 684 H and its metabolites M684H001 and M684H004 by LC/UV BASF study ID 2017/1156774
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), OECD-ENV/JM/MONO/(2007)17
GLP:	Yes
Deviations	
Previous evaluation:	None

This analytical method supports the following studies, additional validation data and procedural recoveries from each study are presented below the overall method validation:

- KCA 8.2.5.1/2 - 2017/1000684
- KCA 8.2.7/3 - 2017/1000221 (water and sediment)
- KCA 8.2.1/3 - 2016/1063240
- KCA 8.2.7/4 - 2017/1000222 (water and sediment)
- KCA 8.2.7/5 - 2017/1000224 (water and sediment)
- KCP 10.2.1/1 - 2017/1106099
- KCP 10.2.1/3 - 2017/1106098
- KCP 10.2.1/4 - 2017/1106097
- KCP 10.2.1/5 - 2017/1013180
- KCA 8.2.4.1/4 - 2017/1069818
- KCA 8.2.7/6 - 2016/1224989
- KCA 8.2.7/8 - 2016/1224988

Sample preparation:

Each samples of 100 mL volume was acidified after fortification by orthophosphoric acid to a pH value of approximately 2 and then applied to a ENVI-18 SPE cartridge (3 mL volume, 500 mg packing material) previously conditioned by sequential washing with twice 5 mL of ethyl acetate and twice 5 mL of deionised water pH 2. Following sample applications the column was dried under vacuum for 5 minutes. The analytes were eluted with 15 mL ethyl acetate. The eluate was evaporated to dryness using vacuum rotary evaporator (at 30 °C). The dry residue was dissolved in acetonitrile:water (1:1 v/v) to be within the linear range. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-UV.

HPLC-UV conditions:

Chromatograph:	Shimadzu, Prominence-*i* LC-2030C 3D
Column:	Agilent Eclipse XDB-C8 5 µm, I = 150 mm, ø = 4.6 mm
Injection volume:	20 µL
Mobile phase:	Acetonitrile : 0.05 % orthophosphoric (V) acid (69 : 31 v/v)
Flow rate:	0.8 mL/min
Wavelength:	215 nm (BAS 684 H, M684H004) 230 nm (M684H001)
Detection system:	Diode Array Detector (DAD)
Retention time:	BAS 684 H: 9.0 min M684H001: 3.4 min M684H004: 3.8 min

Table B.5.1.2.6-9: Summary of validation data for BAS 684 H, M684H001 and M684H004 in tap water and 20xAAP

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Tap water	BAS 684 H	0.002	0.002 (diluted to 0.2 µg/mL)	93, 88, 88, 84, 85 (88)	3.9 (5)	0.05 – 10 µg/mL n = 5 r = 0.999
			0.02	93, 95, 93, 96, 95 (94)	1.3 (5)	
	M684H001	0.002	0.002 (diluted to 0.2 µg/mL)	107, 107, 104, 105, 104 (105)	1.4 (5)	0.05 – 10 µg/mL n = 5 r = 0.999
			0.02	99, 103, 99, 106, 99 (101)	3.1 (5)	
	M684H004	0.002	0.002 (diluted to 0.2 µg/mL)	102, 95, 102, 97, 103 (100)	3.5 (5)	0.05 – 10 µg/mL n = 5 r = 0.999
			0.02	100, 109, 102, 107, 99 (103)	4.5 (5)	
20xAAP*	BAS 684 H	0.002	0.002 (diluted to 0.2 µg/mL)	86, 90, 92, 93, 89 (90)	2.9 (5)	0.05 – 10 µg/mL n = 5 r = 0.999
			0.02	89, 95, 93, 95, 96 (94)	3.1 (5)	
	M684H001	0.002	0.002 (diluted to 0.2 µg/mL)	100, 107, 101, 105, 100 (102)	3.2 (5)	0.05 – 10 µg/mL n = 5 r = 0.999
			0.02	97, 104, 98, 104, 105 (102)	3.8 (5)	
	M684H004	0.002	0.002 (diluted to 0.2 µg/mL)	103, 104, 99, 1110, 99 (103)	4.3 (5)	0.05 – 10 µg/mL n = 5 r = 0.999
			0.02	95, 104, 98, 104, 102 (101)	3.09 (5)	

* Representing the aqueous medium with the highest salt content, hence the most difficult matrix.

Linearity:

Linearity of detector response was tested using six calibration standard concentrations in the range of 0.05 µg/mL to 10 µg/mL with correlation coefficients of > 9998. The calibration standards were prepared in acetonitrile/water (50/50, v/v). The range is appropriate as all samples were diluted to be within the linear range.

Accuracy and precision:

The mean recoveries were in the acceptable range of 70 – 120 %, with %RSD of ≤ 20 % at each level.

Specificity and Confirmation of Analyte Identity:

The identification and quantification of the analytes were based on the retention times and detection wavelengths of the analytes. Confirmation is given by comparison of full UV spectra of calibration standards and fortifications samples.

Matrix effects:

The results from analysis of quality control samples demonstrate, that the matrix-load in the tested quality control samples had negligible influence on the analysis.

Stability in carrier:

All analytes were stable in sample solutions in both matrices for 72 h at 21 – 25 °C and for 96 h at 13 – 17 °C.

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg/L.

Report:	KCA 8.2.5.1/2 Rzodeczko H., 2017 b
Title	BAS 684 H - Daphnia magna reproduction test 2017/1000684
Guidelines:	OECD 211 (2012), EPA 850.1300, EPA 712-C-16-005
GLP:	Yes
Deviations	None
Previous evaluation:	None

The analytical method used within this ecotoxicology study is the same as that evaluated in CA 4.1.2/37 above, however the following differences have been noted:

i) The sample preparation no longer includes the acidification prior to the SPE.

ii) The column used is a Kinetex 5 µ C18 100A, I = 150 mm, ø = 4.6 mm

iii) The mobile phase is acetonitrile : deionised water (80 : 20, v/v)

iv) Flow rate is 0.7 mL/min

v) Retention time is 5.2 min

Table B.5.1.2.6-10: Summary of validation data for cinmethylin in water

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
Water	BAS 684 H	0.002	0.002 (diluted to 0.2 µg/mL)	92, 89, 85, 93, 92 (90.0)	3.9 (5)	0.05 – 10 µg/mL n = 6 R^2 = 0.999
			0.02	95, 90, 95, 90, 95 (93.4)	2.5 (5)	

Procedural recoveries:

Procedural recoveries were carried out during the studies, results shown below. The recoveries are within the acceptable range of 70 – 120 %, with acceptable RSD, indicating that the method is working correctly in this study giving precise and accurate results.

Table B.5.1.2.6-11: Summary of procedural recovery data for cinmethylin in water

Matrix	Analyte	Sample prepared and analysed	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)
Elendt M7 medium	BAS 684 H	Day 0	0.002	95, 90, 95 (93.8)	1.5 (3)
			6.0	89.0, 88.8, 91.8 (88.6)	0.5 (3)
		Day 2	0.002	90, 95, 90 (92.2)	0.7 (3)
			6.0	93.2, 92.7, 93.2 (93.0)	0.3 (3)
		Day 4	0.002	95, 95, 90 (92.6)	1.8 (3)
			6.0	95.2, 94.8, 95.0 (95.0)	0.2 (3)
		Day 7	0.002	90, 90, 90 (89.9)	1.4 (3)

Matrix	Analyte	Sample prepared and analysed	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)
			6.0	96.7, 96.2, 97.2 (96.6)	0.5 (3)
		Day 9	0.002	90, 85, 90 (88.1)	1.8 (3)
			6.0	99.0, 99.5, 99.5 (99.3)	0.3 (3)
		Day 11	0.002	85, 90, 90 (88.7)	1.5 (3)
			6.0	93.0, 92.7, 93.2 (92.9)	0.3 (3)
		Day 14	0.002	95, 95, 95 (96.2)	1.5 (3)
			6.0	100.7, 101.5, 101.3 (101.1)	0.4 (3)
		Day 16	0.002	85, 105, 95 (94.3)	8.2 (3)
			6.0	86.7, 86.8, 86.7 (86.7)	0.0 (3)
		Day 18	0.002	100, 95, 105 (98.6)	4.7 (3)
			6.0	92.2, 92.3, 92.0 (92.2)	0.2 (3)
		Day 21	0.002	100, 105, 100 (102.3)	1.4 (3)
			6.0	95.2, 95.2, 95.2 (95.2)	0.0 (3)

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg/L.

Report:	**KCA 8.2.7/3 Rzodeczko H., 2017 c**
Title	BAS 684 H - Water-sediment Myriophyllum spicatum toxicity test 2017/1000221
Guidelines:	OECD 239 (2014)
GLP:	Yes
Deviations	None relating to the analytical method
Previous evaluation:	None

Report:	**KCA 8.2.7/4 Rzodeczko H., 2018 a**
Title	BAS 684 H, water-sediment Elodea canadensis toxicity test 2017/1000222
Guidelines:	OECD 239 (2014)
GLP:	Yes
Deviations	1 reported although not relating to the analytical method
Previous evaluation:	None

Report:	KCA 8.2.7/5 Rzodeczko H., 2017 d
Title	BAS 684 H - Water-sediment Egeria densa toxicity test 2017/1000224
Guidelines:	OECD 239 (2014)
GLP:	Yes
Deviations	1 reported although not relating to the analytical method
Previous evaluation:	None

The analytical method used to determine the content of BAS 684 H in water within these ecotoxicology studies is the same as that evaluated in CA 4.1.2/37 above, however the following differences have been noted:

i) The sample preparation no longer includes the acidification prior to the SPE.

ii) The column used is a Kinetex 5 µ C18 100A, I = 150 mm, ø = 4.6 mm

iii) The mobile phase is acetonitrile : deionised water (80 : 20, v/v)

iv) Flow rate is 0.7 mL/min

v) Retention time is 5.2 min

Within this study the determination of BAS 684 H is also made in sediment:

Sample preparation:

First, 10 mL of ethyl acetate were added to 10 g of a sediment sample, shaken for 2 minutes, and sonicated for 10 minutes. The sample was centrifuged for 5 minutes 3500 rounds per minute and filtered through anhydrous sodium sulphate (VI). Next, 10 mL of ethyl acetate were added to the sediment again. Shaking, sonication, centrifugation, and filtration were repeated. The combined extracts were evaporated to dryness using vacuum rotary evaporator (at 30 °C). The dry residue was dissolved in a mixture of acetonitrile and deionized water (1:1, v/v) and 20 µL was applied to the chromatographic column. Given the description above, every sample was concentrated before chromatographic analysis. This was done to ensure the result fits within the range of the respective standard curve.

HPLC-UV conditions:

Chromatograph:	Shimadzu, Prominence-*i* LC-2030C 3D
Column:	Kinetex 5 µ C18 100A, I = 150 mm, ø = 4.6 mm (quantification) Gemini 3 µ C6-Phenyl 110A, I = 250 mm, ø = 4.6 mm (confirmation)
Injection volume:	20 µL
Mobile phase:	Acetonitrile : deionised water (63 : 37 v/v)
Flow rate:	0.75 mL/min
Wavelength:	215 nm
Detection system:	Diode Array Detector (DAD)
Retention time:	11.3 min (quantification) 17.1 min (confirmation)

Table B.5.1.2.6-12: Summary of validation data for BAS 684 H in water and sediment

Matrix	Analyte	LOQ	Recovery fortification level	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Water	BAS 684 H	0.002 mg/L	0.002 mg/L (diluted to 0.2 µg/mL) 0.02 mg/L	92, 89, 85, 93, 92 (90.0) 95, 90, 95, 90, 95 (93.4)	3.9 (5) 2.5 (5)	0.05 – 10 µg/mL n = 6 R^2 = 0.999
Sediment	BAS 684 H (quantification column Kinetex 5 µ C18 100A)	0.05 mg/kg	0.05 mg/kg 0.5 mg/kg	110, 104, 100, 92, 102 (101.4) 84, 88, 86, 86, 88(86.2)	6.1 (5) 1.5 (5)	0.05 – 10 µg/mL (0.01 – 2 mg/kg based on a final volume of 2 mL) n = 6 R^2 = 0.999
	BAS 684 H (confirmation column Gemini 3 µ C6-phenyl 110A)	0.05 mg/kg	0.05 mg/kg 0.5 mg/kg	100, 92, 94, 100, 92 (95.6) 86, 82, 88, 88, 84 (86.0)	4.5 (5) 2.9 (5)	0.05 – 10 µg/mL (0.01 – 2 mg/kg based on a final volume of 2 mL) n = 6 R^2 = 0.999

Specificity (sediment):

The analytical method specificity was shown on the basic of the analysis of the chromatograms obtained for the control sediment samples and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experimental conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference is directly apparent and would be observed in the chromatogram of the matrix control sample.

Procedural recoveries:

Procedural recoveries were carried out during the studies, results shown below. The recoveries are within the acceptable range of 70 – 120 %, with acceptable RSD, indicating that the method is working correctly in this study giving precise and accurate results.

Table B.5.1.2.6-13: Summary of procedural recovery data for cinmethylin in media and sediment

KCA 8.2.7/3 Rzodeczko H., 2017c: BAS 684 H - Water-sediment Myriophyllum spicatum toxicity test 2017/1000221					
Matrix	**Analyte**	**Sample prepared and analysed**	**Recovery fortification level**	**Recoveries % (mean)**	**Repeatability % RSD (n)**
Smart and Barko medium	BAS 684 H	Day 0	0.002 mg/L 6.0 mg/L	106.0, 101.5, 101.5 (103.0) 86.0, 86.0, 85.8 (86.0)	2.7 (3) 0.1 (3)
		Day 7	0.002 mg/L 6.0 mg/L	107.5, 105.0, 108.5 (107.0) 88.0, 88.2, 87.8 (86.0)	1.5 (3) 0.2 (3)
		Day 14	0.002 mg/L 6.0 mg/L	99.0, 104.0, 106.5 (103.1) 88.8, 89.3, 89.2 (89.1)	3.5 (3) 0.3 (3)
Sediment	BAS 684 H	Day 7	0.05 mg/kg	108.0, 106.0, 102.0 (105.0)	2.3 (3)

KCA 8.2.7/3 Rzodeczko H., 2017c: BAS 684 H - Water-sediment Myriophyllum spicatum toxicity test 2017/1000221					
Matrix	**Analyte**	**Sample prepared and analysed**	**Recovery fortification level**	**Recoveries % (mean)**	**Repeatability % RSD (n)**
	(quantification column Kinetex 5 µ C18 100A)		6.0 mg/kg	89.8, 90.2 (90.0)	-
		Day 14	0.05 mg/kg	98.0, 100.0, 102.0 (100.0)	2.0 (3)
			6.0 mg/kg	91.3, 90.7, 91.0 (91.1)	0.5 (3)
Sediment	BAS 684 H (confirmation column Gemini 3 µ C6-phenyl 110A)	Day 7	0.05 mg/kg	102.0, 98.0, 102.0 (100.6)	2.7 (3)
			6.0 mg/kg	89.3, 90.2, 90.2 (89.7)	0.7 (3)
		Day 14	0.05 mg/kg	104.0, 98.0, 104.0 (102.0)	3.7 (3)
			6.0 mg/kg	89.7, 90.0, 89.7 (89.9)	0.2 (3)

KCA 8.2.7/4 Rzodeczko H., 2018a: BAS 684 H, water-sediment Elodea canadensis toxicity test 2017/1000222					
Matrix	**Analyte**	**Sample prepared and analysed**	**Recovery fortification level**	**Recoveries % (mean)**	**Repeatability % RSD (n)**
Smart and Barko medium	BAS 684 H	Day 0	0.002 mg/L	94.5, 96.0, 94.0 (95.0)	1.2 (3)
			6.0 mg/L	97.2, 98.0, 97.5 (97.5)	0.4 (3)
		Day 7	0.002 mg/L	96.5, 95.0, 95.5 (95.6)	0.9 (3)
			6.0 mg/L	101.0, 101.2, 100.7 (101.0)	0.3 (3)
		Day 14	0.002 mg/L	98.0, 99.5, 100.5 (99.4)	1.5 (3)
			6.0 mg/L	96.5, 96.3, 96.0 (96.3)	0.3 (3)
Sediment	BAS 684 H (quantification column Kinetex 5 µ C18 100A)	Day 7	0.05 mg/kg	108.0, 106.0, 104.0 (106.0)	1.5 (3)
			6.0 mg/kg	93.0, 93.2, 92.5 (92.9)	0.5 (3)
		Day 14	0.05 mg/kg	108.0, 104.0, 104.0 (105.2)	1.7 (3)
			6.0 mg/kg	98.7, 99.8, 98.8 (99.1)	0.6 (3)
Sediment	BAS 684 H (confirmation column Gemini 3 µ C6-phenyl 110A)	Day 7	0.05 mg/kg	100.0, 100.0, 102.0 (100.7)	1.9 (3)
			6.0 mg/kg	91.0, 92.2, 91.2 (91.4)	0.7 (3)
		Day 14	0.05 mg/kg	102.0, 100.0, 102.0 (100.8)	1.0 (3)
			6.0 mg/kg	99.7, 99.3, 100.2 (99.7)	0.5 (3)

KCA 8.2.7/5 Rzodeczko H., 2017d: BAS 684 H - Water-sediment Egeria densa toxicity test 2017/1000224					
Matrix	**Analyte**	**Sample prepared and analysed**	**Recovery fortification level**	**Recoveries % (mean)**	**Repeatability % RSD (n)**
Smart and Barko medium	BAS 684 H	Day 0	0.002 mg/L	105.0, 105.0, 105.0 (104.3)	1.0 (3)
			6.0 mg/L	90.8, 91.2, 91.8 (91.3)	0.5 (3)
		Day 7	0.002 mg/L	90.0, 95.0, 95.0 (93.0)	1.2 (3)

KCA 8.2.7/5 Rzodeczko H., 2017d: BAS 684 H - Water-sediment Egeria densa toxicity test 2017/1000224					
Matrix	**Analyte**	**Sample prepared and analysed**	**Recovery fortification level**	**Recoveries % (mean)**	**Repeatability % RSD (n)**
			6.0 mg/L	90.8, 90.3, 91.0 (90.7)	0.3 (3)
		Day 14	0.002 mg/L	105.0, 100.0, 105.0 (103.4)	1.3 (3)
			6.0 mg/L	85.3, 86.7, 86.5 (86.2)	0.8 (3)
Sediment	BAS 684 H (quantification column Kinetex 5 µ C18 100A)	Day 7	0.05 mg/kg	88.0, 90.0 (88.9)	-
			6.0 mg/kg	90.8, 91.0, 91.2 (91.0)	0.1 (3)
		Day 14	0.05 mg/kg	104.0, 102.0, 104.0 (102.7)	0.6 (3)
			6.0 mg/kg	97.0, 96.3, 96.8 (96.6)	0.5 (3)
Sediment	BAS 684 H (confirmation column Gemini 3 µ C6-phenyl 110A)	Day 7	0.05 mg/kg	88.0, 84.0, 80.0 (85.6)	2.4 (3)
			6.0 mg/kg	90.3, 90.0 (90.2)	-
		Day 14	0.05 mg/kg	92.0, 102.0, 78.0 (90.7)	8.0 (3)
			6.0 mg/kg	88.0, 88.0, 88.0 (88.0)	0.1 (3)

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with LOQs of 0.002 mg/L in water and 0.05 mg/kg in sediment.

Report:	**KCA 8.2.1/3 [redacted] (2017)**
Title	BAS 684 H (Cinmethylin) – Carp, Acute Toxicity Test BASF Study Identification Number: 2016/1063240
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	Corrections were made to the report and therefore the study is only valid in combination with BASF DocID 2018/20168368 – these corrections do not affect the analytical method validation
Previous evaluation:	None

The analytical method used to determine the content of BAS 684 H in water within this ecotoxicology study is the same as that evaluated in CA 4.1.2/37 above, specific method validation details from the study are presented below:

Sample preparation:

From each sample a volume 10 – 100 mL was applied to ENVI-18 (3 mL, 500 mg) column conditioned previously by sequential washing twice with ethyl acetate (5 mL) and twice with deionised water (5 mL). Following the sample introduction, the column was dried for 5 mins. The part of the sample with affinity to the column was eluted with ethyl acetate (10 mL). Eluate was evaporated to dryness using vacuum rotary evaporator (at 30 °C). The dry residue was dissolved in acetonitrile and 20 µL was applied to the chromatographic column.

Every sample was concentrated, to ensure the results fits within the range of the linear range the samples were diluted before chromatographic analysis.

HPLC-UV conditions:

Chromatographic System: High Performance Liquid Chromatography (HPLC)
Chromatograph: Shimadzu, Prominence-*I*
Analytical Column: Kinetex 5µm C18 100A, l = 150 mm, diameter = 4.6 mm

Injection Volume:	20 µL
Mobile Phase:	acetonitrile : deionized water (90: 10, v/v)
Flow Rate:	0.7 mL/min
Wavelength:	215 nm
Detection System:	Diode Array Detector (DAD, Shimadzu Corporation, Prominence-*I*)
Analyte:	BAS 684 H
Retention Time:	approx. 3.7 min

Table B.5.1.2.6-14: Summary of validation data for cinmethylin in water

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Water	BAS 684 H	0.002	0.002 0.2 2.0	98.5 – 105.5 (102.7) 95.8 – 99.8 (97.5) 92.7 – 93.7 (93.2)	2.4 (5) 1.5 (5) 0.4 (5)	0.05 – 10.0 µg/mL n = 6 R^2 = 0.999

Procedural Recoveries:

Procedural recoveries were carried out during the study, results shown below. The recoveries are within the acceptable range of 70 – 120 %, with acceptable RSD, indicating that the method is working correctly in this study giving precise and accurate results.

Table B.5.1.2.6-15: Procedural recovery data for cinmethylin in water

Matrix	Analyte	LOQ (mg/L)	Analysed sample	Recovery fortification level (mg/L)	Recoveries % range (mean)	Repeatability % RSD (n)
Water	BAS 684 H	0.002	At exposure initiation	0.002	89 – 94 (92)	2.88 (3)
				20.000	96 – 97 (96)	0.77 (3)
			At exposure termination	0.002	92 – 96 (94)	2.41 (3)
				20.000	96 – 97 (97)	0.68 (3)

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg/L.

Report:	**KCP 10.2.1/1 [redacted] (2017a)**
Title	BAS 684 03 H - Common carp, acute toxicity test BASF Study Identification Number: 2017/1106099
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The analytical method used within this ecotoxicology study is the same as that evaluated in CA 4.1.2/37 above, however the following differences have been noted:

i) Samples were 10 – 100 mL

ii) UV detection only at 215 nm

Table B.5.1.2.6-16: Summary of validation data for cinmethylin in tap water

Matrix	Analyte	LOQ (mg/mL)	Recovery fortification level (mg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Tap Water	BAS 684 H	0.002	0.002	84 – 93 (88)	3.9 (5)	0.05 – 10 µg/mL n = 5 R^2 = 0.999
			0.02	93 – 96 (94)	1.3 (5)	

Procedural recoveries:

Procedural recoveries were carried out during the studies, results shown below. The recoveries are within the acceptable range of 70 – 120 %, with acceptable RSD, indicating that the method is working correctly in this study giving precise and accurate results.

Table B.5.1.2.6-17: Procedural recovery data for cinmethylin in tap water

Matrix	Analyte	Sample prepared and analysed	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)
Tap water	BAS 684 H	At exposure initiation	0.0021	95.2, 104.8, 95.2 (99.8)	7.2 (3)
			14.73	95.2, 94.8, 94.6 (94.8)	0.3 (3)
		At exposure termination	0.0021	90.5, 100.0, 90.5 (95.7)	5.0 (3)
			14.73	95.5, 95.7, 96.3 (95.8)	0.1 (3)

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg/L.

Report:	**KCP 10.2.1/3 Turek, T (2017a)**
Title	BAS 684 03 H - *Daphnia* magna, acute immobilisation test BASF Study Identification Number: 2017/1106098
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The analytical method used within this ecotoxicology study is the same as that evaluated in CA 4.1.2/37 above, however the following differences have been noted:

i) The sample preparation no longer includes the acidification prior to the SPE.

ii) The column used is a Kinetex 5 µ C18 100A, I = 150 mm, ø = 4.6 mm

iii) The mobile phase is acetonitrile : deionised water (80 : 20, v/v)

iv) Flow rate is 0.7 mL/min

v) Retention time is 5.2 min

Table B.5.1.2.6-18: Summary of validation data for cinmethylin in Elendt M7 medium

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Elendt M7 medium	BAS 684 H	0.002	0.002 0.02	85 – 96 (88) 81 – 82 (82)	5.2 (5) 0.4 (5)	0.05 – 10 µg/mL n = 5 R^2 = 0.999

Specificity and Confirmation of Analyte Identity:

The primary method is considered specific to the analytes therefore additional confirmation of identity is not required. Acceptable specificity was shown in provided chromatograms with no significant interferences from the sample matrix were detected at the retention time corresponding to cinmethylin in any of the control samples. Chromatographs of solvent blank, formulation blank, reference standard and formulation solution were provided with RT match between reference standard and fortified solutions.

Matrix Effects:

Quality control samples at LOQ were prepared and analysed for Elendt M7 medium in order to assess the impact of the matrix load on the overall instrument performance. The mean recovery results of the quality control samples of BAS 684 H (Ref No 900292) 99% for the Elendt M7 medium. The obtained results demonstrate that the matrix-load in the tested quality control samples had negligible influence on the analysis – there were no significant matrix effects (<30 %) observed for any matrices.

Procedural recoveries:

Procedural recoveries were carried out during the studies, results shown below. The recoveries are within the acceptable range of 70 – 110 %, with acceptable RSD, indicating that the method is working correctly in this study giving precise and accurate results.

Table B.5.1.2.6-19: Procedural recovery data for cinmethylin in Elendt M7 medium

Matrix	Analyte	Sample prepared and analysed	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)
Elendt M7 medium	BAS 684 H	At exposure initiation	0.0021 36.83	104.8, 104.8, 90.5 (102.2) 98.4, 98.5, 98.4 (98.4)	2.2 (3) 0.0 (3)
		At exposure termination	0.0021 36.83	100.0, 100.0, 100.0 (100.0) 99.3, 99.2, 99.1 (99.2)	0.0 (3) 0.1 (3)

Conclusion:

The method is considered fully validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg/L.

Report:	KCP 10.2.1/4 Nierzedska, E (2017a)
Title	BAS 684 03 H - *Pseudokirchneriella subcapitata* SAG 61.81, growth inhibition test BASF Study Identification Number: 2017/1106097
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The analytical method used within this ecotoxicology study is the same as that evaluated in CA 4.1.2/37 above, however the following differences have been noted:

i) The sample preparation no longer includes the acidification prior to the SPE.

ii) The column used is a Kinetex 5 µ C18 100A, I = 150 mm, ø = 4.6 mm

iii) The mobile phase is acetonitrile : deionised water (80 : 20, v/v)

iv) Flow rate is 0.7 mL/min

v) Retention time is 5.2 min

Table B.5.1.2.6-20: Summary of validation data for cinmethylin in water

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Water	BAS 684 H	0.002	0.002 (diluted to 0.2 µg/mL)	92, 89, 85, 93, 92 (90.0)	3.9 (5)	0.05 – 10 µg/mL n = 6 R^2 = 0.999
			0.02	95, 90, 95, 90, 95 (93.4)	2.5 (5)	

Procedural recoveries:

Procedural recoveries were carried out during the studies, results shown below. The recoveries are within the acceptable range of 70 – 120 %, with acceptable RSD, indicating that the method is working correctly in this study giving precise and accurate results.

Table B.5.1.2.6-21: Procedural recovery data for cinmethylin in water

Matrix	Analyte	Sample prepared and analysed	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)
AAP medium	BAS 684 H	At exposure initiation	0.0021	104.8, 100.0, 109.5 (106.2)	1.5 (3)
			73.66	98.0, 98.2, 98.4 (98.2)	0.2 (3)
		At exposure termination	0.0021	109.5, 104.8, 104.8 (108.5)	2.1 (3)
			73.66	101.5, 101.4, 101.5 (101.5)	0.0 (3)

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg/L.

Report:	KCP 10.2.1/5 Rzodecko, H (2017a)
Title	BAS 684 03 H - *Lemna gibba* CPCC 310 growth inhibition test BASF Study Identification Number: 2017/1013180
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The analytical method used within this ecotoxicology study is the same as that evaluated in CA 4.1.2/37 above, however the following differences have been noted:

i) Samples were 10 – 100 mL

ii) UV detection only at 215 nm

Table B.5.1.2.6-22: Summary of validation data for cinmethylin in 20xAAP

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
20xAAP	BAS 684 H	0.002	0.002 (diluted to 0.2 µg/mL)	86, 90, 92, 93, 89 (90)	2.9 (5)	0.05 – 10 µg/mL n = 5 R2 = 0.999
			0.02	89, 95, 93, 95, 96 (94)	3.1 (5)	

Procedural Recoveries:

Procedural recoveries were carried out during the studies, results shown below. The recoveries are within the acceptable range of 70 – 120 %, with acceptable RSD, indicating that the method is working correctly in this study giving precise and accurate results.

Table B.5.1.2.6-23: Procedural recovery data for cinmethylin in 20xAAP

Matrix	Analyte	Sample prepared and analysed	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)
20xAAP	BAS 684 H	Day 0	0.0021	92.4, 103.8, 103.3 (101.6)	7.8 (3)
			14.73	98.7, 98.8, 99.0 (98.8)	0.1 (3)
		Day 7	0.0021	100.5, 105.2, 106.7 (104.1)	3.4 (3)
			14.73	101.7, 101.7, 100.8 (101.4)	0.0 (3)

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg/L.

Report:	KCA 8.2.4.1/4 Turek T., 2018 a
Title	Reg. No. 6055521 (Metabolite of BAS 684 H, M684H001) Daphnia magna, acute immobilisation test 2017/1069818
Guidelines:	OECD 202 (2004)
GLP:	Yes
Deviations	None
Previous evaluation:	None

The analytical method used within this ecotoxicology study is the same as that evaluated in CA 4.1.2/37 above, however the following differences have been noted:

i) The sample preparation no longer includes the acidification prior to the SPE, and elution is with methanol

ii) The column used is a Kinetex 5 µ C18 100A, I = 150 mm, ø = 4.6 mm

iii) The mobile phase is acetonitrile : 0.05 % orthophosphoric acid (60 : 40 v/v)

iv) Flow rate is 0.7 mL/min

v) Detection at 229 nm

vi) Retention time is 4.48 min

Table B.5.1.2.6-24: Summary of validation data for M684H001 in Elendt M7 medium

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
Elendt M7 medium	M684H001 (Reg. 6055521)	0.002	0.002 (diluted to 0.2 µg/mL)	99.5, 103.0, 99.0, 101.0, 101.5 (100.8)	1.7 (5)	0.05 – 10 µg/mL n = 6 R^2 = 0.999
			0.02	96.4, 96.5, 96.5, 96.3, 96.5 (96.4)	0.1 (5)	

Procedural recoveries:

Procedural recoveries were carried out during the studies, results shown below. The recoveries are within the acceptable range of 70 – 120 %, with acceptable RSD, indicating that the method is working correctly in this study giving precise and accurate results.

Table B.5.1.2.6-25: Procedural recovery data for M684H001 in Elendt M7 medium

Matrix	Analyte	Sample prepared and analysed	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)
Elendt M7 medium	M684H001 (Reg. 6055521)	At exposure initiation	0.002	100.0, 105.0, 100.0 (100.4)	3.67 (3)
			200.0	104.1, 104.1, 103.4 (103.9)	0.39 (3)
		At exposure termination	0.002	105.0, 105.0, 110.0 (105.8)	1.38 (3)
			200.0	105.1, 105.2, 105.3 (105.2)	0.07 (3)

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg/L.

Report:	**KCA 8.2.7/6 Rzodeczko H., 2017 e**
Title	Reg.No. 6055521 (metabolite of BAS 684 H, M684H001) - Lemna gibba CPCC 310 growth inhibition test 2016/1224989
Guidelines:	OECD 221 (2006)
GLP:	Yes
Deviations	
Previous evaluation:	None

The analytical method used within this ecotoxicology study is the same as that evaluated in CA 4.1.2/37 above, however the following differences have been noted:

i) The sample preparation no longer includes the acidification prior to the SPE, and elution is with methanol

ii) The column used is a Kinetex 5 µ C18 100A, I = 150 mm, ø = 4.6 mm

iii) The mobile phase is acetonitrile : 0.05 % orthophosphoric acid (60 : 40 v/v)

iv) Flow rate is 0.7 mL/min

v) Detection at 229 nm

vi) Retention time is 4.4 min

Table B.5.1.2.6-26: Summary of validation data for M684H001 in 20xAAP

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
20xAAP	M684H001 (Reg. 6055521)	0.002	0.002 (diluted to 0.2 µg/mL)	88.6, 93.0, 88.1, 91.5, 92.6 (90.7)	2.5 (5)	0.05 – 10 µg/mL n = 6 R^2 = 0.999
			0.02	92.6, 90.5, 93.6, 91.7, 91.3 (91.9)	1.3 (5)	

Procedural recoveries:

Procedural recoveries were carried out during the studies, results shown below. The recoveries are within the acceptable range of 70 – 120 %, with acceptable RSD, indicating that the method is working correctly in this study giving precise and accurate results.

Table B.5.1.2.6-27: Procedural recovery data for M684H001 in 20xAAP

Matrix	Analyte	Sample prepared and analysed	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)
20xAAP	M684H001 (Reg. 6055521)	Day 0 10/08/2017	0.002	102.5, 92.5, 96.0 (96.9)	5.2 (3)
			150.0	98.2, 98.3, 98.2 (98.2)	0.1 (3)
		Day 7 17/08/2017	0.002	99.0, 95.0, 99.5 (97.9)	2.5 (3)
			150	98.3, 98.3, 98.3 (98.3)	0.0 (3)

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg/L.

Report:	KCA 8.2.7/8 Rzodeczko H., 2017 f
Title	Reg.No. 6055480 (metabolite of BAS 684 H, M684H004) - Lemna gibba CPCC 310 growth inhibition test 2016/1224988
Guidelines:	OECD 221 (2006)
GLP:	Yes
Deviations	
Previous evaluation:	None

The analytical method used within this ecotoxicology study is the same as that evaluated in CA 4.1.2/37 above, however the following differences have been noted:

i) The sample preparation no longer includes the acidification prior to the SPE, and elution is with methanol

ii) The column used is a Kinetex 5 µ C18 100A, I = 150 mm, ø = 4.6 mm

iii) The mobile phase is acetonitrile : 0.05 % orthophosphoric acid (80 : 20 v/v)

iv) Flow rate is 0.7 mL/min

v) Detection at 215 nm

vi) Retention time is 3.0 min

Table B.5.1.2.6-28: Summary of validation data for M684H004in 20xAAP

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
20xAAP	M684H004 (Reg. 6055480)	0.005	0.005 (diluted to 0.5 µg/mL)	89.4, 86.4, 87.8, 92.8, 88.8 (89.0)	2.4 (5)	0.1 – 20 µg/mL n = 6 R^2 = 0.999
			5.0	92.4, 95.0, 94.8, 93.4, 93.9 (94.0)	1.2 (5)	
			50.0	96.1, 98.6, 102.8, 99.0, 98.4 (99.0)	2.2 (5)	

Procedural recoveries:

Procedural recoveries were carried out during the studies, results shown below. The recoveries are within the acceptable range of 70 – 120 %, with acceptable RSD, indicating that the method is working correctly in this study giving precise and accurate results.

Table B.5.1.2.6-29: Procedural recovery data for M684H004in 20xAAP

Matrix	Analyte	Sample prepared and analysed	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)
20xAAP	M684H004 (Reg. 6055480)	Day 0 21/08/2017	0.005	99.0, 91.8, 95.4 (95.4)	3.8 (3)
			50.0	98.0, 97.7, 95.9 (97.2)	1.2 (3)
		Day 7 28/08/2017	0.005	92.8, 99.8, 98.4 (97.0)	3.8 (3)
			50.0	99.8, 99.1, 101.7 (100.2)	1.4 (3)

Conclusion:

The guidance recommends fortification levels of LOQ and 10xLOQ, within this ecotoxicology study fortification levels are LOQ, 1000xLOQ and 10000xLOQ. However, this is not of significant concern as these data support the validation data presented in CA 4.1.2/37 Grande A., 2017a where acceptable recoveries were presented for M684H004 in 20xAAP medium at 0.002 and 0.02 mg/L. The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.005 mg/L.

Report:	**KCA 4.1.2/38 Friedemann A.,Stroemel C., 2017 a**
Title	Effect of BAS 684 03 H on vegetative vigour of ten species of terrestrial plants under greenhouse conditions 2017/1134475
Guidelines:	OECD 227 July 2006, EPA 850.4150 - Vegetative Vigour (2012)
GLP:	Yes
Deviations	None
Previous evaluation:	None

Cross reference KCP 10.6.2/1

Report:	KCA 4.1.2/39 Friedemannn A.,Stroemel C., 2018 a
Title	Effect of BAS 684 03 H on seedling emergence and seedling growth of ten species of terrestrial plants under greenhouse conditions 2017/1134474
Guidelines:	OECD 208 (2006), EPA 850.4100 - Seedling Emergence and Seedling Growth (2012)
GLP:	Yes
Deviations	None relevant to the analytical method
Previous evaluation:	None

Cross reference KCP 10.6.2/2

Remark: As a much higher concentration range was used within this study compared to the method validation data for L0378/01 as presented in KCA 4.1.2/37, analytical details are summarised separately.

Sample preparation:

The defrosted spray solution was homogenised by treatment in an ultrasonic bath for 20 minutes using a vortex mixer. The homogenised application solution (0.5 mL) was transferred into a 100 mL volumetric flask, and diluted using acetonitrile/ water (1/1, v/v – dilution factor: 200). The solution was manually shaken for final homogenization, before an aliquot was taken for HPLC-UV determination.

HPLC-UV conditions:

Instruments:	Varian ProStar 230 solvent delivery modular, Varian ProStar 410 HPLC autosampler, Dionex STH 585 column oven, and Varian ProStar 335 diode array detector.
HPLC Column:	Agilent Zorbax Eclipse C_8 (150 x 4.6 mm, 5µm particle size)
Injection Volume:	20 µL
Column Oven:	30 °C
Mobile Phase:	acetonitrile + 0.1 % orthophosphoric acid (69 : 31, v/v)
Flow Rate:	0.8 mL/min
UV Detection:	215 nm (used for quantification)
Retention time:	Approx. 10.0 min

Table B.5.1.2.6-30: Summary of validation data for BAS 684 H in water

Matrix	Analyte	LOQ (g/L)	Recovery fortification level (g/L)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
Water	BAS 684 H (2017/1134475)	3	3 9	101, 102, 101, 100, 102 (101) 98, 98, 99, 100, 99 (99)	0.76 (5) 0.72 (5)	5.0 – 100 mg/L n = 8 R^2 = 0.9991
Water	BAS 684 H (2017/1134474)	3	3 9	102, 101, 102, 102, 101 (102) 98, 100, 100, 100, 100 (99)	0.63 (5) 0.69 (5)	5.0 – 100 mg/L n = 8 R^2 = 0.9999

NB – the recoveries are labelled as procedural (concurrent) recoveries in the study report – these data support the previously validated method L0378/01 (KCA 4.1.2/37).

Linearity:

Linearity of detector response was tested using eight calibration standard concentrations in the range of 5.0 mg/L to 100 mg/L (corresponding to 1 – 20 g/L) with correlation coefficients of > 999. The calibration standards were prepared in acetonitrile/water (1/1, v/v).

Accuracy and precision:

The mean recoveries were in the acceptable range of 70 – 120 %, with %RSD of ≤ 20 % at each level.

Specificity and Confirmation of Analyte Identity:

The identification and quantification of the analyte was based on the retention time and detection wavelength. Therefore, the HPLC/UV method is sufficiently specific for the determination of BAS 684 H in aqueous application solutions as no other significant signals were shown to interfere with the peak of the analyte under the described conditions.

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 3 g/L. Stability of the extracts was demonstrated in the overall method validation (KCA 4.2.1/37) however storage stability of the samples has not been provided. It has been noted that the samples are stored frozen at ≤ -18 °C from 31 July 2017 to the analysis date of 24/25 October 2017. As the analytical method was only used for verification of the content of BAS 684 H in aqueous application solutions which showed acceptable results (99 – 101 % nominal content), and acceptable frozen storage stability has also been proven for BAS 684 H in OECD test medium (KCA CA 8.2.6.1/2, 2016/1001944), it can be assumed the samples remained stable. No additional data have been requested to address this.

Report:	**KCA 4.1.2/40 Andre M., 2017 a**
Title	Validation of BASF Method L0361/01 for the determination of pesticides in water by LC-MS/MS 2017/1065621
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), OECD-ENV/JM/MONO/(2007)17, EFSA Panel on Plant Protection Products and their Residues (PPR)
GLP:	Yes
Deviations	None
Previous evaluation:	None

This analytical method supports the following studies:

KCP 10.2.1/6 – 2017/1000861

Sample preparation:

Samples were prepared for analysis by weighing 5 g (equal to 5 mL) of the specimen into a 20 mL amber vial. To this, 5 mL of D1 (acetonitrile/water/formic acid, 40/60/0.2, v/v/v) was added and mixed thoroughly. If residues are > 1 µg/L then the sample was further diluted. An aliquot of the sample was used for LC-MS/MS analysis.

LC-MS/MS conditions:

Chromatographic system:	Agilent A1290 with CTC autosampler
Analytical column:	Pinnacle DB AQ C18, 50 x 2.1 mm, 1.9 µm particle size, Restek
Guard column:	Raptor C18, 5 x 2.1 mm, 2.7 µm particle size, Restek
Column temperature:	35 °C
Injection volume:	10 µL
Mobile phase:	A: water/formic acid, 1000/1, v/v B: acetonitrile/formic acid, 1000/1, v/v
Flow rate:	600 µL/min

Gradient	Time (min)	Phase A	Phase B
	0.0	95	5
	0.5	70	30
	3.0	10	90
	4.0	10	90
	4.1	95	5
	5.0	95	5
Divert valve:	Yes		
Switching intervals:	To waste 0 – 0.5		
	To LC-MS/MS 0.5 – 4.0		
	To waste 4.0 – 5.0		
Detection system:	Sciex API 5500 Mass Spectrometer		
Ionisation:	Electrospray (ESI)		
Retention time:	Approx. 2.6 min		
Transitions:	*m/z* 275 → 153 (quantification)		
	m/z 275 → 105 (qualification)		

Table B.5.1.2.6-31: Summary of validation data for BAS 684 H in tap water and M4-medium

Matrix	Analyte	LOQ (µg/L)	Recovery fortification level (µg/L)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
Tap water	BAS 684 H 275 → 153 (quantification)	1.0	1.0	97, 94, 94, 93, 96 (95)	1.6 (5)	0.1 – 3 ng/mL n = 7 R^2 = > 0.998
			10	97, 99, 99, 95, 95 (97)	2.0 (5)	
	BAS 684 H 275 → 105 (qualification)	1.0	1.0	95, 94, 96, 97, 100 (96)	2.3 (5)	0.1 – 3 ng/mL n = 7 R^2 = > 0.998
			10	94, 99, 97, 93, 96 (96)	2.3 (5)	
M4-medium	BAS 684 H 275 → 153 (quantification)	1.0	1.0	95, 100, 101, 94, 99 (98)	3.2 (5)	0.1 – 3 ng/mL n = 7 R^2 = > 0.998
			10	96, 97, 92, 94, 95 (95)	2.2 (5)	
	BAS 684 H 275 → 105 (qualification)	1.0	1.0	102, 98, 99, 97, 100 (99)	2.0 (5)	0.1 – 3 ng/mL n = 7 R^2 = > 0.998
			10	102, 99, 96, 99, 96 (99)	2.5 (5)	

Linearity:

Linearity of detector response was tested using seven calibration standard concentrations in the range of 0.1 ng/mL to 3 ng/mL (corresponding to 0.2 – 6 µg/L in the sample) with correlation coefficients of > 0.999. The calibration standards were prepared in the respective matrix/(acetonitrile/water/HCOOH, 40/60/0.2, v/v/v) (50/50, v/v).

Accuracy and precision:

The mean recoveries were in the acceptable range of 70 – 120 %, with %RSD of ≤ 20 % at each level.

Specificity and Confirmation of Analyte Identity:

LC-MS/MS is a highly specific detection technique, analysis is possible at two different mass transitions, therefore no confirmatory technique is required.

Matrix effects:

No significant matrix effects were observed (deviation of matrix matched standards from standards prepared in acetonitrile/water/HCOOH (20/80/0.01, v/v/v) was < 20 %).

Stability:

BAS 684 H was stable in calibration solutions in tap water matrix for 28 days and in M4-medium matrix for 29 days, when stored refrigerated at 2 – 8 °C in the dark. It was stable in fortification solutions for 28 days, when stored at 2 – 8 °C in the dark. The stability of specimen final volume extracts was not investigated during this study, as storage stability of matrix matched standards was proven and composition of matrix matched standards and specimen final volume extract was equal.

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 1.0 µg/L.

Report:	**KCP 10.2.1/6 Janson G.-M., 2017 a**
Title	Effect of BAS 684 03 H on the growth of the aquatic plant Glyceria maxima 2017/1000861
Guidelines:	OECD 239 (2014)
GLP:	Yes
Deviations	None
Previous evaluation:	None

The analysis was conducted based on BASF analytical method L0361/01 (developed in study IF-17/04022633 BASF DocID 2017/1065621, CA 4.1.2/40) fully validated above. The analytical method used for determination of residues of BAS 684 03 H in aqueous specimens was validated with a reduced validation set for Smart&Barko medium in this study according to the required guidelines.

Sample preparation:

A 10 mL specimen aliquot was mixed with 10 mL of acetonitrile/water/formic acid (40/60/0.2, v/v/v). An aliquot of the diluted specimen was transferred into a HPLC vial, then an aliquot injected into the LC-MS/MS instrument for quantification.

Table B.5.1.2.6-32: Summary of validation data for cinmethylin in Smart&Barko medium

Matrix	**Analyte**	**LOQ (µg/L)**	**Recovery fortification level (µg/L)**	**Recoveries % (mean)**	**Repeatability % RSD (n)**	**Linearity**
Smart& Barko medium	BAS 684 H 275 → 153 (quantification)	1.55	1.55 2216	102, 101, 95.3 (100) 104, 106, 105 (105)	3.7 (3) 0.6 (3)	0.15 – 9.3 ng/mL n = 8 R^2 = 0.9998
	BAS 684 H 275 → 105 (qualification)	1.55	1.55 2216	106, 104, 97.9 (103) 103, 105, 107 (105)	4.1 (3) 2.1 (5)	0.15 – 9.3 ng/mL n = 8 R^2 = 0.9985

Procedural recoveries:

Procedural recoveries were carried out during the studies, results shown below. The recoveries are within the acceptable range of 70 – 120 %, with acceptable overall RSD, indicating that the method is working correctly in this study giving precise and accurate results.

Table B.5.1.2.6-33: Procedural recovery data for cinmethylin in Smart&Barko medium

Analyte	Matrix	Fortification level (µg/L)	n	Recoveries (%)	Mean recovery (%)	SD (%)	RSD (%)
BAS 684 H	Bulk solution	1.5	1	106	-	-	-
		2216	1	107	-	-	-
	Mixed solution	1.5	2	102, 90.6	96.0	7.7	8.0
		2216	2	106, 103	105	2.0	1.9
		Overall (1.5 µg/L)	3	-	99.5	8.1	8.1
		Overall (2216 µg/L)	3	-	105	2.0	1.9
		Overall	**6**	**-**	**102**	**6.2**	**6.0**

Conclusion:

Overall, the analytical method L0361/01 is validated in accordance with SANCO/3029/99 rev. 4. Full validation data have provided for BAS 684 H in tap water and M4-medium, in addition to a limited data set for BAS 684 H in Smart&Barko medium. The LOQ for BAS 684 H in Smart&Barko medium is 1.55 µg/L.

Report:	**KCA 8.2.3/1 [redacted]., 2020**
Title	BAS 684 H – Amphibian Metamorphosis Assay with African Clawed Frog (*Xenpous laevis*) 2020/2032686
Guidelines:	-
GLP:	Yes
Deviations	None relevant to the analytical method
Previous evaluation:	None

Cross reference KCA 8.2.3/1

Remark: The analytical method used within this ecotoxicology study, L0361/01 is the same as that evaluated in CA 4.1.2/40 above. Additional procedural recovery data to demonstrate the applicability of the method to this study were provided and are discussed below

Procedural recoveries:

Procedural recoveries were carried out during the study, results shown below. The recoveries are within the acceptable range of 70 – 120 %, with the exception of one recovery at the fortification level of 0.2 mg/L (123%). The study report states that as the other recoveries at this level were within the acceptable range and that the results for the samples analysed at the same time as this high recovery were consistent with the samples taken at other intervals that this does not impact on the results of the study. For all three recovery levels an acceptable RSD was obtained. Overall the data indicate that the method is working acceptably in this study.

Table B.5.1.2.6-34: Procedural recovery data for cinmethylin in exposure solutions

Matrix	Analyte	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)
Exposure solutions	BAS 684 H	0.01	91.6, 118, 98.5, 107 (104)	11 (4)
		0.2	101, 123, 103, 103 (108)	9.7 (4)
		2.0	83.0, 105, 101, 105 (98.5)	11 (4)

Conclusion:

The method was considered fully validated in accordance with SANCO/3029/99 rev. 4 in tap water and M4-medium, under study CA 4.1.2/40, and is shown be acceptable in support of the current study with an LOQ of 1.0 µg/L.

Report:	**KCA 8.2.3/2 [redacted] 2020**
Title	BAS 684 H: Zebrafish (*Danio rerio*), Short term reproduction assay, flow through conditions BASF Study ID: 887576 2019/2054638
Guidelines:	-
GLP:	Yes
Deviations	Analytical verification of the test-item pre-exposure was non-GLP
Previous evaluation:	None

Cross reference KCA 8.2.3/2

Remark: The analytical method used within this ecotoxicology study, L0361/01 is the same as that evaluated in CA 4.1.2/40 above, however the following differences have been noted:

i) The column used was a Aquity BEH C18, 50 x 2.1 mm, 1.9 µm

Additional data to demonstrate the applicability of the method to this study were provided and are discussed below

Linearity:

Linearity of detector response was tested using nine calibration standard concentrations in the range of 0.2 ng/mL to 10 ng/mL with correlation coefficients of > 999. The calibration standards were prepared in acetonitrile/water (2:8 v/v)+ 0.1% formic acid.

Procedural recoveries:

Procedural recoveries were carried out during the study, results shown below. The recoveries are within the acceptable range of 70 – 120 %, with acceptable RSD, indicating that the method is working correctly in this study giving precise and accurate results.

Table B.5.1.2.6-35: Procedural recovery data for cinmethylin in coper free water medium

Matrix	**Analyte**	**Recovery fortification level (µg/mL)**	**Recoveries % (mean)**	**Repeatability % RSD (n)**
Copper-free water	BAS 684 H	1.00	94.0, 91.4, 97.4 (94.3)	3.2 (3)
		1240	93.4, 94.5, 96.3 (94.8)	1.5 (3)

Specificity and Confirmation of Analyte Identity:

The identification and quantification of the analyte was based on the retention time. LC-MS/MS is a highly specific detection technique, analysis is possible at two different mass transitions. Therefore, the method is sufficiently specific for the determination of cinmethylin in aqueous application solutions. No significant matrix effects were observed.

Conclusion:

The method was considered fully validated in accordance with SANCO/3029/99 rev. 4 in tap water and M4-medium, under study CA 4.1.2/40, and has is shown be acceptably validated in support of the current study with an LOQ of 1.0 µg/L.

Report:	**KCA 4.1.2/41 Grande A., 2017 b**
Title	Validation of BASF Method L0382/01 for the determination of M684H003 in water and 20xAAP medium by GC-FID 2017/1156775
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), OECD-ENV/JM/MONO/(2007)17
GLP:	Yes
Deviations	None relevant to the analytical method
Previous evaluation:	None

This analytical method supports the following studies:

KCA 8.2.4.1/5 - 2017/1069817

KCA 8.2.7/7 - 2017/1032136

Sample preparation:

Each sample of 100 mL was alkalized after fortification by ammonia solution 20 % to a pH value of 9 and the applied to a ENVI-18 SPE-cartridge (3 mL volume, 500 mg packing material) previously conditioned by sequential washing with twice 5 mL acetone, 5 mL methanol and twice 5 mL deionised water pH 9. Following sample application the column was dried under vacuum for 0.5 minutes. The analytes were eluted with 10 mL methanol and 10 mL acetone. The eluate was evaporated to dryness using vacuum rotary evaporation (at 30 °C). The dry residue was dissolved in acetone for quantification using GC-FID.

Given the description above, every sample was concentrated before chromatographic analysis. This was done to ensure the result fits within the range of the respective standard curve.

GC-FID conditions (quantification):

Analytical column:	Agilent DB-5 (30 m x 0.32 mm); film thickness 0.25 µm
Injection volume:	2.0 µL
Temperature:	Initial column temperature: 80 °C (3.0 min) 1. Gradient 20 °C/min to temperature of 170 µC (0.5 minutes)
Injector temperature:	200 °C
Detection system:	Flame ionization detector (FID)
Detector temperature:	300 °C
Retention time:	Approx. 5.8 min

GC-FID conditions (confirmation):

Analytical column:	Agilent DB-17 (30 m x 0.32 mm); film thickness 0.25 µm
Injection volume:	2.0 µL
Temperature:	Initial column temperature: 80 °C (3.0 min) 1. Gradient 20 °C/min to temperature of 170 µC (0.5 minutes)
Injector temperature:	260 °C
Detection system:	Flame ionization detector (FID)
Detector temperature:	300 °C
Retention time:	Approx. 6.8 min

Table B.5.1.2.6-36: Summary of validation data for M684H003 in tap water and 20xAAP medium

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
Tap water	M684H003 Quantification	0.002	0.002 0.02	109, 96, 89, 93, 88 (95) 91, 86, 93, 95, 90 (91)	8.8 (5) 3.8 (5)	0.1 – 10 µg/mL n = 7 R^2 = 0.9994
	M684H003 Qualification	0.002	0.002 0.02	100, 98, 102, 98, 96 (99) 94, 88, 93, 91, 93 (91)	2.2 (5) 2.8 (5)	0.1 – 10 µg/mL n = 7 R^2 = 0.9998
20xAAP medium	M684H003 Quantification	0.002	0.002 0.02	101, 102, 94, 94, 86 (95) 83, 85, 88, 93, 79 (85)	6.8 (5) 6.4 (5)	0.1 – 10 µg/mL n = 7 R^2 = 0.9994
	M684H003 Qualification	0.002	0.002 0.02	100, 101, 90, 96, 92 (96) 84, 97, 87, 97, 99 (93)	5.3 (5) 7.2 (5)	0.1 – 10 µg/mL n = 7 R^2 = 0.9998

Linearity:

Linearity of detector response was tested using seven calibration standard concentrations in the range of 0.1 µg/mL to 10 µg/mL with correlation coefficients of > 9994. The calibration standards were prepared in acetone.

Accuracy and precision:

The mean recoveries were in the acceptable range of 70 – 120 %, with %RSD of ≤ 20 % at each level.

Specificity and Confirmation of Analyte Identity:

The identification and quantification of the analyte was based on the retention time of the analyte. Confirmation is given by the use of a different column.

Matrix effects:

It was demonstrated that the matrix-load in the tested quality control samples had no significant influence on the detection of M684H003.

Stability in carrier:

M684H003 was stable in fortified samples (10x LOQ) in tap water for 96 h at 13-17 °C and in 20x AAP medium for 96 h at 21-25 °C.

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg/L.

Report:	KCA 8.2.4.1/5 Turek T., 2018 b
Title	Reg.No. 4539586 (Metabolite of BAS 684 H, M684H003) - Daphnia magna, acute Immobilisation test 2017/1069817
Guidelines:	OECD 202 (2004)
GLP:	Yes
Deviations	None relevant to the analytical method
Previous evaluation:	None

The analytical method used within this ecotoxicology study is the same as that evaluated in CA 4.1.2/41 above, the following information is specific to the ecotoxicology study.

Sample preparation:

Samples with volumes between 10 and 100 mL were acidified after fortification by ammonia solution 25 % to a pH value of approximately 9 and then applied to column (ENVI-18 SPE- cartridge, 500 mg packing material, 3 mL volume), which was previously conditioned. The resulting eluate was evaporated to dryness using vacuum rotary evaporator (at 30 °C). The dry residue was dissolved in acetone for further quantification was performed using GC - FID.

Given the description above, every sample was concentrated before chromatographic analysis. This was done to ensure the result fits within the range of the respective standard curve.

Table B.5.1.2.6-37: Summary of validation data for M684H003 in Elendt M7

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
Elendt M7	M684H003	0.002	0.002 0.02	99, 87, 98, 106, 91 (96) 94, 100, 100, 93, 92 (96)	7.4 (5) 4.0 (5)	0.1 – 10 µg/mL n = 7 R^2 = 0.9994

Procedural recoveries:

Procedural recoveries were carried out during the studies, results shown below. The recoveries are within the acceptable range of 70 – 120 %, with acceptable RSD, indicating that the method is working correctly in this study giving precise and accurate results.

Table B.5.1.2.6-38: Procedural recovery data for M684H003 in Elendt M7

Matrix	Analyte	LOQ (mg/L)	Analysed sample	Recovery fortification level (mg/L)	Recoveries % range (mean)	Repeatability % RSD (n)
Elendt M7	M684H003	0.002	At exposure initiation	0.002	91.5 – 97.5 (93.5)	3.5 (3)
				120.0	101.4 – 107.6 (105.5)	3.3 (3)
			At exposure termination	0.002	94.0 – 107.0 (98.5)	7.7 (3)
				120.0	99.7 – 104.6 (102.8)	2.6 (3)

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg/L.

Report:	KCA 8.2.7/7 Turek T., 2018 c
Title	Reg.No. 4539586 (Metabolite of BAS 684 H, M684H003) - Lemna gibba CPCC 310 growth inhibition test 2017/1032136
Guidelines:	OECD 221 (2006)
GLP:	Yes
Deviations	None relevant to the analytical method
Previous evaluation:	None

The analytical method used within this ecotoxicology study is the same as that evaluated in CA 4.1.2/41 above, the following information is specific to the ecotoxicology study.

Sample preparation:

Samples with volumes between 10 and 100 mL were acidified after fortification by ammonia solution 25 % to a pH value of approximately 9 and then applied to column (ENVI-18 SPE- cartridge, 500 mg packing material, 3 mL volume), which was previously conditioned. The resulting eluate was evaporated to dryness using vacuum rotary evaporator (at 30 °C). The dry residue was dissolved in acetone for further quantification was performed using GC - FID.

Given the description above, every sample was concentrated before chromatographic analysis. This was done to ensure the result fits within the range of the respective standard curve.

Procedural recoveries:

Procedural recoveries were carried out during the studies, results shown below. The recoveries are within the acceptable range of 70 – 120 %, with acceptable RSD, indicating that the method is working correctly in this study giving precise and accurate results.

Table B.5.1.2.6-39: Procedural recovery data for M684H003 in Elendt M7

Matrix	Analyte	LOQ (mg/L)	Analysed sample	Recovery fortification level (mg/L)	Recoveries % range (mean)	Repeatability % RSD (n)
Elendt M7	M684H003	0.002	At exposure initiation	0.002	93.0 – 103.0 (98.6)	5.3 (3)
				120.0	95.3 – 101.7 (97.7)	3.6 (3)
			At exposure termination	0.002	94.0 – 101.5 (97.3)	3.9 (3)
				120.0	97.0 – 100.3 (98.3)	1.8 (3)

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.002 mg/L.

Report:	KCA 4.1.2/42 Catchpole G., Hidding B., 2017 c
Title	BAS 684 H (Cinmethylin) - Validation of an analytical method for the analysis of BAS 684 H in test water using HPLC-MS (control procedure 14/0066_08-02) 2017/1047671
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	Yes
Deviations	None
Previous evaluation:	None

This study supports the following ecotoxicology studies:

KCA 8.2.1/2 – 2017/1134335

KCA 8.2.1/5 – 2017/1111618

KCA 8.2.2.1/2 – 2017/1176649

Sample preparation:

Samples are diluted 1:1 (v/v) with acetonitrile. If required, all dilutions are sonicated for five minutes to ensure a complete dissolution of the test substance. If the amount of test substance in the sample solution is outside the calibration range, an additional adequate dilution step with matrix solution is performed to reach the described concentration range. The samples are filtered if required (cellulose filter, 0.2 µm) prior to HPLC-MS analysis.

HPLC-MS conditions:

System:	Ultimate 3000 with autosampler, Excalibure-Software (Thermo Fischer Scientific), TSQ Quantum Access Max, or equivalent system
Column:	Length: 100 mm Inner diameter: 4.6 mm
Stationary phase:	Ascentis Express, 2.7 µm, Phenomenex or equivalent
Mobile phase:	A: 950 mL acetonitrile mixed with 50 mL water and 0.1 mL formic acid B: 950 mL water mixed with 50 mL acetonitrile and 0.1 mL formic acid
Isocratic:	Mobile phase A: 80 % Mobile phase B: 20 %
Injection volume:	80 µL
Flow rate:	0.5 mL/min
Detection:	Ionization: Electrospray ionization (positive mode) MRM (multiple reaction monitoring) Parent ion (*m/z*): 257.1 Molecular mass with a water molecule split off product ions (*m/z*): 105.1
Column temperature:	40 °C
Run time:	Approximately 8 min

Table B.5.1.2.6-40: Summary of validation data for cinmethylin in water

Matrix	Analyte	LOQ (ng/mL)	Recovery fortification level (ng/mL)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
Test water	BAS 684 H	2.5	2.5	82.3, 86.9, 78.8, 88.8, 97.0 (86.8)	7.96 (5)	0.998 – 9.98 ng/mL n = 6 R^2 = 0.9993
			25	73.0, 80.1, 79.2, 84.1, 81.3 (79.5)	5.14 (5)	
			2500	85.4, 90.3, 89.7, 85.3, 93.0 (88.8)	3.77 (5)	

Linearity:

The linearity of detector response was tested using six calibration standard concentrations in the range of 0.998 ng/mL to 9.98 ng/mL with correlation coefficients of > 0.999. The calibration standards were prepared in matrix solution. The following linear ranges were presented in each of the individual ecotoxicology studies:

KCA 8.2.1/2 – 2017/1134335: 0.964 – 9.64 ng/mL, n = 6, R^2 = 0.9978

KCA 8.2.1/5 – 2017/1111618: 1.01 – 10.1 ng/mL, n = 6, R^2 = 0.9987

KCA 8.2.2.1/2 – 2017/1176649: 0.998 – 9.98 ng/mL, n = 6, R^2 = 0.998

Accuracy and precision:

The mean recoveries were in the acceptable range of 70 – 120 %, with %RSD of ≤ 20 % at each level.

Specificity and Confirmation of Analyte Identity:

The method allows the specific quantification of BAS 684 H in test water using HPLC-MS. Detection is accomplished by MS using the mass transition 257 → 105 m/z. As there was no chromatographic peak present in the blank vehicle sample at the retention time of interest, no interference of the analytical peak was observed. As the method is only used for dose verification of known substances and known nominal concentrations, no additional confirmatory technique is necessary. No significant interferences (> 30% LOQ) were observed at the appropriate retention time and using the given mass transition.

Matrix effects:

Matrix effects were not determined; however, matrix-matched standards were used.

Extract stability:

As the duration of a complete analytical run is always below 24 hours, this test was not considered to be mandatory as part of this validation work. Sample solutions are always promptly injected after sample preparation.

Storage stability (KCA 8.2.1/2 – 2017/1134335):

A sample (12c) was analysed to demonstrate the storage stability of samples which had been stored in the freezer. After the storage period, samples 12c was found to contain 85 % of its initial concentration. This result thus proves the adequate stability of the stored samples.

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 2.5 ng/mL. It has been noted that no procedural recoveries have been provided, although as the analytical method was only used for verification of the content of BAS 684 H in test water solutions no additional validation data have been requested.

Report:	**KCA 4.1.2/43 Kleebaum K., 2016 a**
Title	Repeated exposure of BAS 684 H to honey bee (Apis mellifera) larvae under laboratory conditions (in vitro) 2016/1044854 Analytical phase report BASF study ID 777066
Guidelines:	OECD 239 (2016) SANCO/3029/99 rev. 4 ENV/JM/MONO (2007)17
GLP:	Yes
Deviations	None
Previous evaluation:	None

This study is cross referenced with KCA 8.3.1.3/1.

This analytical method is also used in the following studies, although each has been validated in turn below:

KCA 8.3.1.1.1/2 – 2017/1140992

KCP 10.3.1.3/1 – 2017/103667

Sample preparation:

For sample measurement the samples (0.2 g) were allowed to reach room temperature and homogenised by shaking with a Multitube-Vortexer. For the extraction procedure 5 mL of water and 5 mL of acetonitrile as well as QuEChERS citrate extraction mix containing 0.5 g magnesium sulphate, 0.12 g sodium chloride, 0.06 g sodium hydrogencitrate sesquihydrate and 0.12 g of sodium citrate were added to a sample aliquot of 0.2 g. The mixture was shaken vigorously for 3 minutes with a Multitube-Vortexer and centrifuged for 2 minutes at 3000 g. Aliquots of the acetonitrile phase were diluted and injected into the HPLC. All diluted samples contained the same amount of QuEChERS blank extract (15 %). The following dilution steps were applied:

Sample	Ident-ification	Sampling time	Nominal conc. after extraction (mg/L)	Sample extract volume (ml)	Add (mL) of blank extract	Add (ml) of ACN	Final volume (ml) in water	Final Conc. (µg/L)
Test item	AT	D3,D4, D5, D6	26.01	0.015	0.135	0.35	1.00	390.1
	BT	D3,D4, D5, D6	13.00	0.030	0.120	0.35	1.00	390.1
	CT	D3,D4, D5, D6	6.502	0.050	0.100	0.35	1.00	325.1
	DT	D3,D4, D5, D6	3.521	0.100	0.050	0.35	1.00	325.1
	ET	D3,D4, D5, D6	1.625	0.150	0.000	0.35	1.00	243.8
Control	AC /BC	D3,D4, D5, D6	0.000	0.150	0.000	0.35	1.00	0.000

Doses and overall dilution factors (DF):

AT = 650 mg a.s./kg food, DF = 1667; BT = 325 mg a.s./kg food, DF = 833

CT = 163 mg a.s./kg food, DF = 500; DT = 81 mg a.s./kg food, DF = 250

ET = 41 mg a.s./kg food, DF = 167

HPLC-MS conditions:

HPLC-MS system: Agilent 1200 with a 6410 triple quadrupole mass spectrometric detector
Mobile phase: A: Water containing 0.1 % formic acid and 5 mM ammonium formate
B: Methanol containing 0.1 % formic acid and 5 mM ammonium formate
Flow rate: 0.40 mL/min
Gradient:
0.00 min 5 % B
1.50 min 50 % B
8.50 min 100 % B
10.00 min 100 % B
12.5 min Stop
Column: ZORBAX Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm
Detection: ESI positive
m/z 257→239 (quantifier)
m/z 257→105 (qualifier 1)
m/z 257→157 (qualifier 2)
Retention time: 7.8 min

Table B.5.1.2.6-41: Summary of validation data for BAS 684 H in bee larvae diet

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Bee larvae diet	BAS 684 H	18.8 mg/kg	18.8 (113.3 µg/L once diluted)* 945.4 (567.3 µg/L once diluted)†	82 – 93 (87) 85 – 95 (92)	5.3 (5) 4.0 (5)	21.29 – 709.8 µg/L (equivalent to 3.5 – 118.3 mg/kg based on the overall dilution factor of the lowest concentration sample, 167) n = 6 R^2 = 0.9983

* Dilution factor = 167
† Dilution factor = 1667

Linearity:

Linearity of detector response was tested using six calibration standard concentrations in the range of 21.29 µg/L to 709.8 µg/L (corresponding to 3.549 mg/kg to 118.3 mg/kg based on the overall dilution factor of the lowest concentration, 167) with correlation coefficients of > 0.99. The calibration standards were prepared in water/acetonitrile/test medium (50/35/15). The range encompasses the LOQ by at least ± 20 %, all samples were diluted to be within the linear range.

Accuracy and precision:

The mean recoveries were in the acceptable range of 70 – 120 %, with %RSD of ≤ 20 % at each level.

Specificity and Confirmation of Analyte Identity:

LC-MS/MS is a highly specific self-confirmatory technique. Under the described conditions the method is specific for the determination of BAS 684 H in feeding solutions. A peak at 4.3 minutes was observed that belongs to a contaminant in the test medium since the peak appears in all samples except the reagent blank. The retention time of BAS 684 H is 7.8 minutes therefore there is no interference from this contaminant on the evaluation of BAS 684 H. The ion transitions monitored are appropriate. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary.

Matrix effects:

Matrix effects were not determined within this study, however matrix-matched standards were used.

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 18.8 mg/kg.

Report:	KCA 8.3.1.1.1/2 Amsel K., 2017
Title	Acute toxicity of BAS 684 H to the bumblebee Bombus terrestris L. under laboratory conditions 2017/1140992 Analytical phase report BASF study ID 815891
Guidelines:	OECD Guideline 246 and 247
GLP:	Yes
Deviations	N/A Document is only valid in combination with the document 2018/1000903 (CA 8.3.1.1.1/3)
Previous evaluation:	None

Sample preparation:

a) Contact toxicity test (acetone)

The control and treated samples were allowed to reach room temperature and homogenised by shaking. Sample aliquots were diluted into the range of the calibration curve with 50/50 (v/v) MeOH/H_2O.

b) Oral toxicity test (50 % w/v sucrose, 5 % v/v acetone and 1 % v/v Tween)

The samples were extracted according to the QuEChERS method prior to sample analysis. The control and treated samples (0.2 mL) were allowed to reach room temperature and homogenised by shaking with a Multitube-Vortexer. For the extraction of treated, untreated and validation samples, 5 mL of water and 5 mL of acetonitrile as well as QuEChERS citrate extraction mix containing 0.5 g magnesium sulphate, 0.12 g sodium chloride were added to a sample aliquot of 0.2 mL. The mixture was shaken vigorously for 3 minutes with a Multitube-Vortexer and centrifuged for 2 minutes at 3000 g. Aliquots of the acetonitrile phase were diluted into the range of the calibration curve with dilution medium.

HPLC-MS conditions:

HPLC-MS system:	Agilent 1200 with a 6410 triple quadrupole mass spectrometric detector	
Mobile phase:	A: Water containing 0.1 % formic acid and 5 mM ammonium formate	
	B: Methanol containing 0.1 % formic acid and 5 mM ammonium formate	
Flow rate:	0.40 mL/min	
Gradient:	0.00 min	5 % B
	1.50 min	50 % B
	8.50 min	100 % B
	10.00 min	100 % B
	12.5 min	Stop
Column:	ZORBAX Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm	
Detection:	ESI positive	
	m/z 257→239 (quantifier)	
	m/z 257→105 (qualifier 1)	
	m/z 257→157 (qualifier 2)	
Retention time:	8.7 min	

Table B.5.1.2.6-42: Summary of validation data for BAS 684 H in bee contact and oral toxicity tests

Matrix	Analyte	LOQ (g/L)	Recovery fortification level (g/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Contact toxicity test	BAS 684 H m/z 257→239 quantification	3.12	3.12 (299 ng/mL once diluted)	78 – 100 (95)	10 (5)	57.6 – 1047 ng/mL (equivalent to 19.2 – 134 ng/L based on the overall dilution factor of the lowest concentration sample, 10417) n = 6 R^2 = 0.999
			130 (783 ng/mL once diluted)	102 – 117 (108)	7.2 (4)*	

Matrix	Analyte	LOQ (g/L)	Recovery fortification level (g/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Oral toxicity test	BAS 684 H m/z 257→105 qualification	3.12	3.12 (299 ng/mL once diluted) 130 (783 ng/mL once diluted)	78 – 99 (95) 102 – 118 (107)	10 (5) 6.7 (4)*	57.6 – 1047 ng/mL (equivalent to 19.2 – 134 ng/L based on the overall dilution factor of the lowest concentration sample, 10417) n = 6 R^2 = 0.999
	BAS 684 H m/z 257→239 quantification	0.154	0.154 (198 ng/mL once diluted) 6.57 (526 ng/mL once diluted)	76 – 83 (80) 80 – 86 (84)	3.4 (5) 2.7 (5)	40.4 – 734 ng/mL (equivalent to 31.5 – 573 ng/L based on the overall dilution factor of the lowest concentration sample, 781) n = 6 R^2 = 0.999
	BAS 684 H m/z 257→105 qualification	0.154	0.154 (198 ng/mL once diluted) 6.57 (526 ng/mL once diluted)	80 – 85 (82) 81 – 86 (84)	2.7 (5) 2.6 (5)	40.4 – 734 ng/mL (equivalent to 31.5 – 573 ng/L based on the overall dilution factor of the lowest concentration sample, 781) n = 6 R^2 = 0.999

* Due to an outlier confirmed with the Dixon test (recovery = 155 and 153 %, α = 0.01) mean values and RSD were calculated from 4 replicates.

Linearity:

Linearity of detector response was tested using six calibration standard concentrations in the range of 57.6 ng/mL to 1047 ng/mL (corresponding to 19.2 – 134 ng/L based on the overall dilution factor of the lowest concentration, 10417) for contact toxicity tests and in the range of 40.4 – 734 ng/mL (corresponding to 31.5 – 573 ng/L based on the overall dilution factor of the lowest concentration, 781) for oral toxicity with correlation coefficients of > 0.99. The range encompasses the LOQ by at least ± 20 %, all samples were diluted to be within the linear range.

Accuracy and precision:

The mean recoveries were in the acceptable range of 70 – 120 %, with %RSD of ≤ 20 % at each level. An outlier was noted in the contact toxicity samples at the higher fortification level. The outlier was confirmed according to the Dixon test with α = 0.01 therefore this value was discounted from the mean and RSD calculations.

Specificity and Confirmation of Analyte Identity:

LC-MS/MS is a highly specific self-confirmatory technique. Under the described conditions the method is specific for the determination of BAS 684 H in both contact and oral toxicity matrices. The ion transitions monitored are appropriate. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary.

Matrix effects:

For contact toxicity matrix effects needed not to be considered since the sample matrix was acetone only and samples were further diluted with 50/50 (v/v) MeOH/H_2O.

For oral toxicity matrix effects were taken into account by spiking calibration solutions with 16 % of blank extract obtained from extraction of 0.2 mL of untreated sample matrix. Thus, all measuring samples contained the same amount of original sample matrix.

Procedural recoveries:

Procedural recoveries were carried out during the studies, results shown below. The recoveries are within the acceptable range of 70 – 120 %, indicating that the method is working correctly in this study giving accurate results.

Table B.5.1.2.6-43: Procedural recovery data for cinmethylin in bee contact and oral toxicity tests

Test	Sample description	Test solution ID	Nominal conc. (mg/L)	Analysed conc. (mg/L)	% Recovery
Contact test	Control	CC	0.00	<LOD	-
	Lowest dose	ET	6.25	6.03	97
	Highest does	AT	100	80.8	81
Oral test	Control	BC	0.00	<LOD	-
	Lowest dose	ET	312	280	89
	Highest does	AT	5000	5124	102

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 3.12 g/L for contact toxicity tests and 0.154 g/L for oral toxicity tests.

Report:	**KCP 10.3.1.3/1 Kleebaum, K., 2017**
Title	Repeated exposure of honey bee (Apis mellifera) larvae to BAS 684 03 H under laboratory conditions 2017/1036677 Analytical phase report BASF study ID 777067
Guidelines:	OECD Guidance Document for testing chemicals, No. 239
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Sample preparation:

The samples were extracted according to the QuEChERS method prior to sample analysis. The control and treated samples (0.5 g) were allowed to reach room temperature and homogenised by shaking with a Multitube-Vortexer. For the extraction of treated, untreated and validation samples, 5 mL of water and 5 mL of acetonitrile as well as QuEChERS citrate extraction mix containing 0.5 g magnesium sulphate, 0.12 g sodium chloride were added to a sample aliquot of 0.5 g. The mixture was shaken vigorously for 3 minutes with a Multitube-Vortexer and centrifuged for 2 minutes at 3000 g. Aliquots of the acetonitrile phase were diluted into the range of the calibration curve with dilution medium.

HPLC-MS conditions:

HPLC-MS system:	Agilent 1200 with a 6460 triple quadrupole mass spectrometric detector	
Mobile phase:	A: Water containing 0.1 % formic acid and 5 mM ammonium formate	
	B: Methanol containing 0.1 % formic acid	
Flow rate:	0.35 mL/min	
Gradient:	0.00 min	5 % B
	1.50 min	50 % B

	5.00 min	100 % B
	7.00 min	100 % B
	7.00 min	100 % B
	Post time	3 min
Column:	ZORBAX Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm	
Detection:	ESI positive	
	m/z 257→239 (quantifier)	
	m/z 257→157 (qualifier)	
Retention time:	6.1 min	

Table B.5.1.2.6-44: Summary of validation data for BAS 684 H in bee larvae diet

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Bee larvae diet	BAS 684 H m/z 257→239 quantification	19.12	19.12 (80.29 ng/mL once diluted)	92 – 113 (102)	7.4 (5)	22.65 – 338.1 ng/mL (equivalent to 5.394 – 80.50 mg/kg based on the overall dilution factor of the lowest concentration sample, 238) n = 7 R^2 = 0.999 Non-linear quadratic fit
			801.7 (216.5 ng/mL once diluted)	91 – 101 (97)	4.7 (5)	
	BAS 684 H m/z 257→157 qualification	19.12	19.12 (80.29 ng/mL once diluted)	97 – 119 (106)	7.6 (5)	22.65 – 338.1 ng/mL (equivalent to 5.394 – 80.50 mg/kg based on the overall dilution factor of the lowest concentration sample, 238) n = 7 R^2 = 0.999 Non-linear quadratic fit
			801.7 (216.5 ng/mL once diluted)	97 – 107 (103)	4.7 (5)	

Linearity:

Linearity of the detector response was tested using seven calibration standard concentrations in the range of 22.65 – 338.1 ng/mL (corresponding to 5.394 – 80.50 mg/kg based on the overall dilution factor of the lowest concentration, 238) with correlation coefficients of > 0.99. Matrix effects were taken into consideration by spiking the calibration solution with 42 % of QuEChERS blank extract. The range encompasses the LOQ by at least ± 20 %, all samples were diluted to be within the range. It has been noted that the range is slightly non-linear with a quadratic fit. The study report states LC-MS calibrations are often non-linear, or the linear range is quite small. This is due to the ionization process in the electrospray interface and is dependent on the analyte and the matrix. No further data or explanation are required, the calibration plot is considered acceptable for the intended purpose.

Accuracy and precision

The mean recoveries were in the acceptable range of 70 – 120 %, with %RSD of ≤ 20 % at each level.

Specificity and Confirmation of Analyte Identity

LC-MS/MS is a highly specific self-confirmatory technique. Under the described conditions the method is specific for the determination of BAS 684 H in feeding solutions. The ion transitions monitored are appropriate. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary.

Matrix effects

Matrix effects were taken into account by spiking the calibration solutions with 42 % of QuEChERS blank extract obtained from extraction of 0.5 g of untreated sample matrix. Thus all measuring samples contained the same amount of original sample matrix.

Conclusion

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 19.12 mg/kg. It has been noted that no procedural recoveries have been provided, although as the analytical method was only used for verification of the content of BAS 684 H in bee larvae diet no additional validation data have been requested.

Report:	**KCA 4.1.2/44 Haerthe N., 2016 a**
Title	Acute toxicity of BAS 684 H (Cinmethylin) to Daphnia magna STRAUS in a 48 hour static test 2016/1001943
Guidelines:	OECD 202, EPA 850.1010 draft April 1996
GLP:	Yes
Deviations	None
Previous evaluation:	None

Remark: Analytical method APL0500/03 was developed as a multi-compound method based on LC-MSD technique. The analysis of BAS 684 H in this multimethod was validated within the ecotoxicological study DocID 2016/1001943.

This analytical method supports the following studies:

KCA 8.2.4.1/2 - 2016/1001943 (includes method validation presented below, cross referenced as CA 4.1.2/44)

KCA 8.2.6.1/2 - 2016/1001944

KCA 8.2.7/2 - 2015/1029520

Sample preparation:

Test samples were directly dissolved with acetonitrile and 0.5 % formic acid and if necessary further diluted with a mixture of M4-medium/acetonitrile/formic acid 80:20:0.1 (v/v/v) into the range of the calibration solutions. Quantification was completed by reversed phase UHPLC with MS-detection.

HPLC-MS conditions:

Column: Acquity UPLC BEH C18 1.7 µm, 2.1 x 50 mm
Mobile phase: A: Water/formic acid = 1000/1
B: Acetonitrile/formic acid = 1000/1
Gradient:

Time (min)	% B
0.00	30.0
1.50	80.0
2.00	80.0
2.01	100.0
2.50	100.0
2.51	30.0
3.00	30.0

Injection volume: 50 µL
Flow rate: 0.8 mL/min
Column temperature: 40 °C
MS-detection (ESI^+) *m/z* 257 (BAS 684 H $[M\text{-}H_2O]^+$)
Expected retention time: 1.6 min

Table B.5.1.2.6-45: Summary of validation data for BAS 684 H in M4-medium

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
M4-medium	BAS 684 H	0.01	0.01 12.5	104, 102, 103, 102, 106 (103) 116, 103, 106, 103, 105 (107)	1.6 (5) 5.1 (5)	0.002 – 0.04 mg/L n = 5 R^2 = 0.9994

Linearity:

Linearity of detector response was tested using five calibration standard concentrations in the range of 0.002 mg/L to 0.04 mg/L with correlation coefficients of > 0.995. The calibration standards were prepared in M4-medium/acetonitrile/formic acid (80/20/0.1, v/v/v).

Accuracy and precision:

The mean recoveries were in the acceptable range of 70 – 120 %, with %RSD of ≤ 20 % at each level.

Specificity and Confirmation of Analyte Identity:

The method allows the specific determination of BAS 684 H in M4-medium using HPLC-MS at *m/z* 257. Specificity is accomplished by mass detection and comparison of the mean retention time of the reference item with the mean retention time of the corresponding peak of the test item during HPLC-MS analysis. As the method is only used for dose verification of known substances and known nominal concentrations, no additional confirmatory technique has been provided.

Matrix effects:

Matrix effects were not determined; however, matrix-matched standards were used.

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.01 mg/L.

Report:	**KCA 8.2.6.1/2 Kauf A., 2017 a**
Title	Effect of BAS 684 H (Reg.No.: 900202) on the growth of the green alga Pseudokirchneriella subcapitata 2016/1001944
Guidelines:	OECD 201, EPA 850.4500, OECD-ENV/JM/MONO(2002)/9
GLP:	Yes
Deviations	None
Previous evaluation:	None

Sample preparation and HPLC-MS conditions are identical to those presented above. The following validation data are specific to this ecotoxicology study.

Table B.5.1.2.6-46: Summary of validation data for cinmethylin in OECD medium

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
OECD medium	BAS 684 H	0.135	0.135	100, 105, 102, 103, 107 (104)	2.6 (5)	0.01 – 0.1 mg/L n = 5 R^2 = 0.9961
			116.5	99.8, 101, 103, 96.4, 100 (100)	2.2 (5)	0.045 – 0.225 mg/L n = 5 R^2 = 0.9917

Linearity:

Linearity of detector response was tested using five calibration standard concentrations in the ranges of 0.01 – 0.1 mg/L and 0.045 – 0.225 mg/L with correlation coefficients of > 0.99. The calibration standards were prepared in OECD-medium/acetonitrile/formic acid (80/20/0.1, v/v/v).

Accuracy and precision:

The mean recoveries were in the acceptable range of 70 – 120 %, with %RSD of ≤ 20 % at each level.

Specificity and Confirmation of Analyte Identity:

The method allows the specific determination of BAS 684 H in OECD-medium using HPLC-MS at *m/z* = 257. Specificity is accomplished by mass detection and comparison of the mean retention time of the reference item with the mean retention time of the corresponding peak of the test item during HPLC-MS analysis. As the method is only used for dose verification of known substances and known nominal concentrations, no additional confirmatory technique has been provided.

Procedural recoveries:

Procedural recoveries were carried out during the studies, results shown below. The recoveries are within the acceptable range of 70 – 120 %, however, as only 2 determinations were made it is not possible to report a repeatability value. The study report states that the overall standard deviation is 4.3 %. It is noted that the lower recovery fortification level is lower than the validated LOQ, however this is within the LOQ as reported in the overall method validation (0.01 mg/L) therefore this is considered acceptable.

Table B.5.1.2.6-47: Procedural recovery data for cinmethylin in OECD medium

Matrix	Analyte	LOQ (mg/L)	Analysed sample	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)
OECD medium	**BAS 684 H**	**0.135**	**05/04/2017**	0.05	105.1, 106.4 (106)	- (2)
				110	98.2, 98.2 (98.2)	- (2)
			19/04/2017	0.05	109.0, 109.6 (109)	- (2)
				110	104.5, 105.4 (105)	- (2)

Matrix effects:

Matrix effects were not determined; however, matrix-matched standards were used.

Storage stability:

The storage stability of BAS 684 H in fortification samples in OECD medium was investigated within this study. The fortification samples Z1_K (0.135 mg/L) and Z2_G (111.7 mg/L) were stored deep frozen at

approximately -18°C in the dark for a maximum duration of 4 months (121 days). After this period the concentration of BAS 684 H was measured against freshly prepared standards within one analytical queue.

Stability tests confirmed that the analyte BAS 684 H was stable for a maximum duration of 4 months (121 days) in fortification samples, when stored deep frozen at approximately -18 °C in the dark. Mean recoveries were in an acceptable range of 100% to 106% over the tested time period. As the stability was confirmed over the concentration range investigated, it can be concluded that concentration dependency is not given.

Sample name	Nominal conc. [mg/L]	Date of sample preparation	Date of sampling	Date of analytical determ.	Found 1.inj. [mg/L]	Found 2.inj. [mg/L]	Found mean [mg/L]	Found mean [%]
Z1_K	0.135	19.12.2016	19.12.2016	19.12.2016	0.135	--	0.135	100
Z1_K	0.135	19.12.2016	19.04.2017	19.04.2017	0.142	0.146	0.144	106
Z2_G	111.7	19.12.2016	19.12.2016	19.12.2016	113	--	113	101
Z2_G	111.7	19.12.2016	19.04.2017	19.04.2017	117	116	117	105

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.135 mg/L.

Report:	**KCA 8.2.7/2 Vlechev S., 2017 b**
Title	Effects of BAS 684 H on the growth of the aquatic plant Glyceria maxima 2015/1029520
Guidelines:	OECD 221, OECD 219, OECD 239 (2016), ASTM E 1913-0
GLP:	Yes
Deviations	None
Previous evaluation:	None

Sample preparation and HPLC-MS conditions are identical to those presented above. The following validation data are specific to this study.

Table B.5.1.2.6-48: Summary of validation data for BAS 684 H in Smart&Barko-medium

Matrix	**Analyte**	**LOQ (mg/L)**	**Recovery fortification level (mg/L)**	**Recoveries % (mean)**	**Repeatability % RSD (n)**	**Linearity**
Smart & Barko-medium	BAS 684 H	0.005	0.005	103, 109, 101, 114, 108 (107)	5.1 (5)	0.001 – 0.02 mg/L n = 5 R^2 = 0.9996
			4	106, 97, 105, 100, 99 (101)	3.8 (5)	0.002 – 0.04 mg/L n = 5 R^2 = ≥ 0.995

Linearity:

Linearity of detector response was tested using five calibration standard concentrations in the ranges of 0.001 – 0.02 mg/L and 0.002 – 0.04 mg/L with correlation coefficients of ≥ 0.995. The calibration standards were prepared in Smart&Barko-medium/acetonitrile/formic acid (80/20/0.1, v/v/v).

Accuracy and precision:

The mean recoveries were in the acceptable range of 70 – 120 %, with %RSD of ≤ 20 % at each level.

Specificity and Confirmation of Analyte Identity:

The method allows the specific determination of BAS 684 H in Smart&Barko-medium using HPLC-MS at *m/z* = 257. Specificity is accomplished by mass detection and comparison of the mean retention time of the reference

item with the mean retention time of the corresponding peak of the test item during HPLC-MS analysis. As the method is only used for dose verification of known substances and known nominal concentrations, no additional confirmatory technique has been provided.

Procedural recoveries:

A procedural recovery with a concentration of 0.01 mg/L, analysed concurrently with the test samples was 106 %. This recovery confirms the applicability of the applied method in addition to the recoveries for method validation (above).

Sample name	Nominal conc. [mg/L]	Date of sample preparation	Date of sample work-up	Date of analytical determ.	Found 1.inj. [mg/L]	Found 2.inj. [mg/L]	Found mean [mg/L]	Found [%]
ZK8	0.0100	04.07.2016	04.07.2016	04.07.2016	0.0106	0.0106	0.0106	106

Matrix effects:

Matrix effects were not determined; however, matrix-matched standards were used.

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.005 mg/L.

Report:	**KCA 4.1.2/45 Kauf A., 2017 a**
Title	Effect of BAS 684 H (Reg.No.: 900202) on the growth of the blue alga Anabaena flos-aquae 2016/1001945
Guidelines:	EPA 850.4500, EPA 850.4550, OECD 201, OECD-ENV/JM/MONO(2002)/9
GLP:	Yes
Deviations	None
Previous evaluation:	None

Cross reference KCA 8.2.6.2/1.

Sample preparation:

Samples were prepared by dissolving 1600 µL of the medium to a volume of 2 mL with solvent solution (acetonitrile/formic acid (99.5/0.5 v/v)) and mixed thoroughly. Based on the nominal concentration of the samples, a defined amount of sample material was diluted with diluent (AAP medium/acetonitrile/formic acid (80/20/0.1 v/v/v)) to obtain a sample solution with a test substance concentration that matched the calibration range. Analysis was completed using HPLC-MS.

HPLC-MS conditions:

Instrument: Agilent Technologies 1290 Infinity Binary LC system
Agilent Technologies 6490 Triple Quadrupole MS
Column: Agilent Technologies, Eclipse XDB-C18, 50 x 2.1 mm, 1.8 µm
Column temperature: 30 °C
Column flow rate: 0.3 mL/min
Injection volume: 3 µL
Mobile phase composition: A: 0.1 % formic acid in water
B: 0.1 % formic acid in methanol

Gradient

Time (min)	A (%)	B (%)
0.0	80	20
0.2	40	60
3.0	0	100
4.0	0	100
4.1	80	20
6.0	80	20

Retention time: Approx. 3.2 min
Ionization mode: ESI (ESI Jet Stream ion source)

Polarity:	Positive
Drying gas flow:	15 L/min
Gas temperature:	150 °C
Sheath gas temperate:	300 °C
Sheath gas flow:	10 L/min
Capillary voltage:	3000 V

m/z:

	Precursor ion	Product ion
Quantifier:	275.2 [M-H$^+$]	105
Qualifier:	275.2 [M-H$^+$]	153.1

Table B.5.1.2.6-49: Summary of validation data for cinmethylin in AAP medium

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
AAP medium	BAS 684 H	0.2	0.2	93, 101, 89, 88, 89, 88, 88 (91)	5.3 (7)	Approx. 10 – 60 µg/L n = 6 R^2 = > 0.996
			100	88, 63*, 91, 102, 88, 96, 102 (95)	6.9 (6)	

* Value was identified as an outlier and not taken into account for the calculation of mean recovery and relative standard deviation.

Linearity:

Linearity of detector response was tested using six calibration standard concentrations in the range of about 10 µg/L to 60 µg/L with correlation coefficients of > 0.996. The calibration standards were prepared in AAP medium/acetonitrile/formic acid (80/20/0.1, v/v/v).

Accuracy and precision:

The mean recoveries were in the acceptable range of 70 – 120 %, with %RSD of ≤ 20 % at each level. One recovery at the 100 mg/L fortification level was identified as an outlier.

Specificity and Confirmation of Analyte Identity:

LC-MS/MS monitoring two mass transitions, is a highly specific detection technique and therefore no confirmatory technique is required.

Matrix effects:

Influence of matrix effects was not determined within this study. No interference > 30% at the elution time of the analyte was observed. However, matrix-matched standards were used for quantification.

Procedural recoveries:

Calibration control solutions were injected twice within the sequence run each. The resulting data are given in the following tables (first table for samples GLP-013/16-1 to -12, second table for samples GLP-013/16-15 to -38).

Table B.5.1.2.6-50: Procedural recovery data for cinmethylin in AAP medium

Samples	Injection	Response of Reference Item No.1	Concentration of Reference Item No.1 (µg/L)	Nominal Concentration of Reference Item No.1 (µg/L)	Recovery (%)
GLP-013/16-1 to -12	1	7480	30.45	30.24	101
	2	7416	30.18	30.24	100
	1	7188	29.22	29.30	100
	2	7207	29.30	29.30	100
GLP-013/16-15 to -38	1	5446	32.85	31.43	105
	2	5382	32.45	31.43	103
	1	5321	32.06	31.08	103
	2	5292	31.88	31.08	103

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.2 mg/L.

Report:	**KCA 4.1.2/46 Vlechev S., 2017 a**
Title	Effect of BAS 684 H on the growth of Lemna gibba 2015/1029521
Guidelines:	OECD 221, EPA 850.4400, ASTM E 1415-91
GLP:	Yes
Deviations	None
Previous evaluation:	None

Cross reference KCA 8.2.7/1.

For controlled and treated specimens, 0.5 mL of the thawed water specimen was added into a vial containing 0.5 mL of acetonitrile/water/formic acid (40/60/0.2 v/v/v). An aliquot of the diluted specimen was transferred into a HPLC vial then injected into the LC-MS/MS instrument for quantification. Samples are diluted with acetonitrile/water/formic acid (40/60/0.2, v/v/v) to obtain a sample solution with a test substance concentration that falls within the calibration range determined.

LC-MS/MS conditions:

Analytical column: Betasil C18, 100 x 2.1 mm, 5 µm particle size, Thermo
Column temperature: 25 °C
Injection volume: 50 µL*
Mobile phase: A: Ultrapure water/formic acid, 1000/1 (v/v)
Acetonitrile/formic acid, 1000/1 (v/v)
Flow rate: 700 µL/min
Gradient:

Time (min)	A (%)	B (%)
0	70	30
0.1	40	60
2.5	40	60
5.5	20	80
5.6	0.1	99.9
7	0.1	99.9
7.1	70	30
10	70	30

Switching intervals: To waste 0 – 2
To LC-MS/MS 2 – 6
To waste 6 – 10
Detection system: Sciex API 5500 Mass Spectrometer
Ionisation: Electro Spray (ESI)

Transitions: Retention time = approx.. 4.3 min
m/z 275 → 153 (quantification)
m/z 275 → 105 (qualification)
* In deviation to method L0337/01, where 10 µL were used as injection volume

Table B.5.1.2.6-51: Validation data for cinmethylin in 20x AAP medium

Matrix	Analyte	LOQ (µg/L)	Recovery fortification level (µg/L)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
20x AAP medium	BAS 684 H 275 → 153 (quantification)	0.2	0.2	98.9, 96.0, 103, 104, 107 (102)	4.2 (5)	0.02 – 1.4 ng/mL n = 7 R^2 = 0.9991
			300	106, 107, 108, 107, 108 (107)	0.8 (5)	
	BAS 684 H 275 → 105 (qualification)	0.2	0.2	94.2, 97.2, 94.9, 101, 106 (99)	4.9 (5)	0.02 – 1.7 ng/mL n = 8 R^2 = 0.9979
			300	106, 107, 106, 106, 106 (106)	0.4 (5)	

Linearity:

Linearity of detector response was tested using seven or eight calibration standard concentrations in the range of 0.02 ng/mL to 1.7 ng/mL with correlation coefficients of > 0.997. The calibration standards were prepared in matrix solution.

Accuracy and precision:

The mean recoveries were in the acceptable range of 70 – 120 %, with %RSD of ≤ 20 % at each level.

Specificity and Confirmation of Analyte Identity:

LC-MS/MS is a highly specific self-confirmatory technique. Under the described conditions the method is specific for the determination of BAS 684 H in 20x AAP medium. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary.

Matrix effects:

As matrix-matched standards were used in this study, there was no need to determine the influence of matrix effects on the detection of BAS 684 H.

Procedural recoveries:

Procedural recoveries were carried out during the studies, results shown below. The recoveries are within the acceptable range of 70 – 120 %, with acceptable RSD, indicating that the method is working correctly in this study giving precise and accurate results.

Table B.5.1.2.6-52: Procedural recvoery data for cinmethylin in 20x AAP medium

Analyte	Fortification Level [µg/L]	n	Recoveries [%]	Mean Recovery [%]	SD [%]	RSD [%]
BAS 684 H	0.2	4	106, 107, 102, 98	103	4.2	4.0
	304	4	110, 110, 110, 103	108	3.7	3.4
	Overall	**8**	--	**106**	**4.5**	**4.2**

Conclusion:

The method is validated in accordance with SANCO/3029/99 rev. 4 with an LOQ of 0.2 µg/L

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

Report:	**KCA 2.5/1 Daum A; 2017 a**
Title	Water solubility of Cinmethylin (BAS 684 H) pure active ingredient (PAI) 2017/1077867
Guidelines:	EC A6, OECD 105, EPA 830.7840, SANCO/3029/99 rev 4.
GLP:	Yes
Deviations	None
Previous evaluation:	None

The following method was used in the solubility in water study reported at B.2.5/01

Sample preparation:

Sample aliquots (4 mL) were taken from the solubility samples and centrifuged at 20°C and 5000rpm for 10 minutes. 1 mL of the clear supernatant was taken and diluted to 10 mL with acetonitrile: water 7:3 (v/v) prior to analysis by HPLC-UV with the following conditions:

Column	Nucelosil 100-5, C18; 250 x 4.6 mm
Column temperature	40 °C
Injection volume	45 µL
Gradient	Isocratic
Eluent	Acetonitrile: water: formic acid (700/300/0.5 v/v/v)
Flow rate	1.0 mL/min
Analysis run time	15 min
Retention time	Approx. 7.7 min
Detector	UV at 208 nm

Table B.5.1.2.7-1: Validation of analytical method for the determination of solubility of cinmethylin in water

LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity	Specificity
2	2	101.6 – 105.7 (105)	2.9 (n=5)	1.0 – 48.6 mg/L n=5 r=0.9993 y = 1.3009x + 0.8450	Retention time match with analytical standard.
	20	99.9 – 102.1 (101)	0.9 (5)		

Identity:

The identity of the active substance cinmethylin was confirmed by comparison of the retention times with those of a reference standard.

Specificity:

No significant interference was observed

Linearity:

Linearity was measured using a series of 5 calibration standards in a concentration range of 1.0 – 48.6 mg/L The concentrations extend over an appropriate range, and the correlation coefficient of >0.99 demonstrates an acceptable linear correlation.

Accuracy:

The accuracy of the method was assessed by analysing five sample solutions fortified with pure cinmethylin at concentrations of 2 mg/L and 20 mg/L. Recoveries were found to be in the range 99.9 – 105.7 % at both fortification levels.

Precision:

The precision of the method was assessed *via* analysis of the accuracy samples. The reported %RSDs were <20% at both levels tested.

Conclusion:

The analytical method is fully validated according to SANCO/3029/99 rev. 4 for the determination of the active substance cinmethylin in solution from the solubility in water study. The LOQ is 2 mg/L.

B.5.2. METHODS FOR POST-APPROVAL CONTROL AND MONITORING PURPOSES

B.5.2.1. Methods for residues in or on food and feed of plant origin

Report:	KCA 4.1.2/22, Spangler, C. et al (2016a)
Title	Validation of analytical method L0337/01 for the determination of BAS 684 H residues in plant matrices by LC-MS/MS Report number: 2016/1029129 (Study ID: 741162)
Guidelines:	SANCO/3029/99 rev. 4, SANCO/825/00 rev.8.1
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	KCA 4.1.2/23, Spangler, C. et al (2018b)
Title	Amendment 1: Validation of analytical method L0337/01 for the determination of BAS 684 H residues in plant matrices by LC-MS/MS Report number: 2018/1044640 (Study ID: 741162)
Guidelines:	SANCO/3029/99 rev. 4, SANCO/825/00 rev.8.1
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The purpose of this study was the validation of analytical method L0337/01 for the determination of cinmethylin in plant matrices *via* LC-MS/MS.

Reference items:

Cinmethylin (BASF Reg. No. 900202), batch L87-84, purity 99.0 %w/w (pure), CoA provided, expiry 01/08/17

Sample preparation:

Samples (5 g all samples except cereal straw – 2 g) are extracted with acetonitrile or acetonitrile/sodium hydroxide solution (grapes). After addition of the QuEChERS extraction salt kit (containing magnesium sulphate, sodium chloride and buffering citrate salts) the samples are shaken then centrifuged. The organic phase is cleaned-up by dispersive SPE (d-SPE) using the QuEChERS clean-up kit. For rape (seeds), d-SPE is performed using the QuEChERS d-SPE EMR-Lipid kit and the QuEChERS Final Polish EMR-Lipid kit. In case of wheat (straw), the organic extract after d-SPE clean-up is supplemented with sodium hydroxide solution, partitioned against cyclohexane and centrifuged for phase separation. An aliquot of the cyclohexane phase is evaporated to dryness in the presence of 1-octanol and reconstituted in acetonitrile/water 80/20 (v/v) prior to analysis.

Analysis was accomplished by LC-MS/MS with the following conditions noted:

Chromatographic system:	Waters Acquity UPLC system
Analytical column:	Thermo Fisher Scientific Betasil C18: 100 mm x 2.1 mm, Particle size 5 µm
Target column temperature:	25 °C
Injection volume:	10 µL
Mobile phase A:	Water/formic acid (1000/1, v/v)
Mobile phase B:	Acetonitrile/formic acid (1000/1, v/v)
Flow rate:	600 µL/min

Gradient (including wash and equilibration):

Time (min)	Phase A (%)	Phase B (%)
0.0	70	30
0.1	40	50
2.5	40	60
5.5	20	80
5.6	0.1	99.9
7.0	0.1	99.9
7.1	70	30
10.0	70	30

Detection system: AB Sciex API 5000 Triple quad Mass Spectrometer
Ionisation: Turbo Spray (ESI positive)
Retention time: Cinmethylin: approximately 4.7 min
Ions monitored: m/z 275 → 153 Quantification
m/z 275 → 105 Confirmatory

A summary of the method validation data is given in Table B.5.2.1-1.

Table B.5.2.1-1: Summary of method validation data for determination of cinmethylin residues in plant matrices

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
No group: Barley (whole plant without roots)	Cinmethylin *m/z* 275 → 153	0.01	0.01	87.0 – 95..5 (90.9)	3.9 (5)	0.5 – 25 ng/mL (equivalent to 0.00125 – 0.0625 mg/kg) (n = 6) x 3 repeats r = 0.9988
			0.1	87.0 - 89.3 (88.2)	1.2 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	88.8 – 96.0 (91.3)	3.6 (5)	As above r=0.9992
			0.1	88.3 – 93.0 (90.6)	2.3 (5)	
High water group: Beans (pods with seeds)	Cinmethylin *m/z* 275 → 153	0.01	0.01	80.0 – 84.8 (82.5)	2.5 (5)	0.5 – 25 ng/mL (equivalent to 0.00125 – 0.0625 mg/kg) (n = 6) x 3 repeats r = 0.9988
			0.1	83.5 – 86.3 (85.4)	1.4 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	79.5 – 82.0 (80.4)	1.2 (5)	As above r=0.9992
			0.1	81.3 – 85.3 (83.7)	1.9 (5)	
High acid group: Grapes (fruits)	Cinmethylin *m/z* 275 → 153	0.01	0.01	75.3 – 87.8 (82.6)	6.5 (5)	0.5 – 25 ng/mL (equivalent to 0.00125 – 0.0625 mg/kg) (n = 6) x 3 repeats r = 0.9988
			0.1	79.5 – 85.3 (82.2)	2.6 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	79.5 – 87.5 (86.4)	6.5 (5)	As above r=0.9992
			0.1	80.3 – 85.0 (83.4)	2.7(5)	

High oil group: Oilseed rape seed	Cinmethylin *m/z* 275 → 153	0.01	0.01	66.5 – 77.3 (71.0)	6.5 (5)	0.5 – 25 ng/mL (equivalent to 0.00125 – 0.0625 mg/kg) (n = 6) x 3 repeats r = 0.9988
			0.1	66.8 – 76.5 (71.0)	5.5 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	69.0 – 77.3 (72.1)	5.7 (5)	As above r=0.9992
			0.1	70.5 – 76.0 (72.2)	3.0 (5)	
High protein group: Dried beans (seeds)	Cinmethylin *m/z* 275 → 153	0.01	0.01	80.3 – 85.8 (84.0)	3.0 (5)	0.5 – 25 ng/mL (equivalent to 0.00125 – 0.0625 mg/kg) (n = 6) x 3 repeats r = 0.9988
			0.1	79.8 – 87.8 (83.3)	3.5 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	83.0 – 89.0 (86.2)	3.6 (5)	As above r=0.9992
			0.1	79.5 – 84.8 (83.1)	2.5 (5)	
High starch group: Wheat (grain)	Cinmethylin *m/z* 275 → 153	0.01	0.01	87.8 – 94.3 (91.5)	3.6 (5)	0.5 – 25 ng/mL (equivalent to 0.00125 – 0.0625 mg/kg) (n = 6) x 3 repeats r = 0.9988
			0.1	89.3 – 95.3 (93.6)	3.2 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	92.0 – 95.0 (93.5)	1.4 (5)	As above r=0.9992
			0.1	91.8 – 98.3 (95.3)	2.8 (5)	
No group: Wheat (straw)	Cinmethylin *m/z* 275 → 153	0.01	0.01	89.1 – 104 (92.9)	6.6 (6)	0.5 – 25 ng/mL (equivalent to 0.00125 – 0.0625 mg/kg) (n = 6) x 3 repeats r = 0.9988
			0.1	91.3 – 110 (98.9)	7.4 (6)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	86.9 – 98.8 (93.1)	4.7 (6)	As above r=0.9992
			0.1	90.9 - 104 (94.5)	5.4 (6)	

Specificity and Confirmation of Analyte Identity

The primary method is considered specific to the analytes therefore additional confirmation of identity is not required. The ion transitions monitored are appropriate.

Linearity:

Linearity of detector response was tested using at least six calibration standard concentrations over appropriate concentration ranges with correlation coefficients of ≥0.99. The calibration standards were prepared in acetonitrile/water (80/20, v/v) except for calibration and quantification of rape (seeds) which was quantified using matrix-matched standards.

Matrix Effects:

Matrix effects (>20 %) were identified for rape (seeds). Therefore, matrix-matched standards were used for quantification of cinmethylin in rape (seeds). In the other matrices, the matrix effect was negligible and solvent-based standards were used for quantification.

Accuracy and Precision:

Samples were spiked at LOQ and 10x LOQ fortification levels with a minimum of two unfortified control samples also analysed. Some individual recoveries were outside of the acceptable range (70 – 110 %); however, as mean recoveries for all levels are within the acceptable range, this is considered acceptable. The %RSD at each fortification level was within the acceptable level (<20 % RSD).

Mean procedural recoveries were also all in the range acceptable (70 – 110 %) with %RSDs <20 %, giving further evidence for the accuracy and precision of the method.

Storage stability:

Cinmethylin was shown to be stable in standard solutions for 30 days when stored at 4 °C in the dark and in sample solutions for 7 days when stored refrigerated at 4 °C in the dark.

Conclusion:

The method is fully validated in accordance with both SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1. for the determination of cinmethylin residues *via* HPLC-MS/MS in high water (beans with pods, barley whole plant), high acid (grapes), high oil (rape seeds), dry high protein/starch or (wheat grain and dried beans) and difficult (wheat straw) plant matrices, with an LOQ of 0.01 mg/kg.

Extraction efficiency was evaluated in the study by Rabe U., Forieri I. (KCA 4.1.2/25, 2017a) and it was concluded that the extraction methodology within this study is comparable to that of the metabolism study, and therefore that the extraction efficiency is addressed for high water (carrot leaves and wheat forage) and difficult (wheat straw) matrices, as these were the only matrices investigated. The representative uses in this dossier include cereals (high protein/starch/dry matrices) and oilseeds (high oil), for which extraction efficiency was not investigated. However, based on the supervised residue trials submitted in support of the representative uses (see Volume 3, Section B.7.3) no residues above the LOQ were found in cereal grain or oilseed samples other than whole plant and straw. Extraction efficiency is therefore considered sufficiently addressed.

Report:	**KCA 4.2/1, Bodsch, J. (2018a)**
Title	Independent laboratory validation of BASF method L0337/01 for the determination of BAS 684 H residues in plant matrices by LC-MS/MS Report number: 2017/1202457 (Study ID: 765933)
Guidelines:	SANCO/3029/99 rev.4, SANCO/825/00/rev. 8.1
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The objective of this study was to perform an independent laboratory validation (ILV) of the analytical method L0337/01 for the determination of cinmethylin in plant matrices.

Deviations from the primary method validation study:

For the analysis of cinmethylin an injection volume of 1µL instead of 10µL was used.

A summary of the method validation data for method is given in Table B.5.2.1-2:

Table B.5.2.1-2: Summary of Independent Laboratory validation data for determination of cinmethylin residues in plant matrices

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
No group: Barley (whole plant without roots)	Cinmethylin *m/z* 275 → 153	0.01	0.01	89.9 – 98.6 (95.5)	3.7 (5)	0.8 – 60.4 ng/mL (0.002 – 0.15 mg/kg) (n =8) r = 0.9999
			0.1	84.8 – 93.2 (88.4)	1.9 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	93.9 – 98.2 (96.6)	1.7 (5)	As above r=0.9993
			0.1	86.1 – 91.9 (88.4)	3.5 (5)	
High water group: Beans (pods with seeds)	Cinmethylin *m/z* 275 → 153	0.01	0.01	72.3 – 89.0 (79.4)	8.1 (5)	0.8 – 60.4 ng/mL (0.002 – 0.15 mg/kg) (n =8) r = 1.000
			0.1	76.7 – 81.4 (79.0)	2.2 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	72.5 – 90.2 (81.2)	7.8 (5)	As above r=0.9993
			0.1	77.9 – 79.2 (78.7)	0.93 (5)	
High acid group: Grapes (fruits)	Cinmethylin *m/z* 275 → 153	0.01	0.01	70.2 – 77.0 (72.1)	4.1 (5)	0.8 – 60.4 ng/mL (0.002 – 0.15 mg/kg) (n =8) r = 0.9999
			0.1	72.4 – 73.9 (73.0)	0.87 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	70.0 – 74.2 (72.3)	2.6 (5)	As above r=0.9999
			0.1	71.1 – 73.2 (72.1)	1.0 (5)	
High oil group: Oilseed rape seed	Cinmethylin *m/z* 275 → 153	0.01	0.01	79.6 – 89.4 (85.5)	4.7 (5)	0.8 – 60.4 ng/mL (0.002 – 0.15 mg/kg) (n =8) r = 0.9999
			0.1	75.5 – 81.6 (78.5)	2.8 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	77.7 – 90.9 (82.9)	6.6 (5)	As above r=0.9999
			0.1	76.1 – 78.8 (78.3)	1.7 (5)	
High protein group: Dried beans (seeds)	Cinmethylin *m/z* 275 → 153	0.01	0.01	84.4 – 89.3 (85.2)	3.8 (5)	0.8 – 60.4 ng/mL (0.002 – 0.15 mg/kg) (n =8) r = 1.000
			0.1	90.7 – 96.5 (93.6)	2.5 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	82.3 – 86.3 (84.3)	2.5 (5)	As above r=0.9999
			0.1	92.0 – 97.8 (94.7)	2.5 (5)	

High starch group: Wheat (grain)	Cinmethylin *m/z* 275 → 153	0.01	0.01	90.4 – 94.8 (92.7)	3.1 (5)	0.8 – 60.4 ng/mL (0.002 – 0.15 mg/kg) (n =8) r = 0.9997
			0.1	82.8 – 89.8 (86.9)	2.8 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	88.7 – 94.4 (91.7)	2.8 (5)	As above r=0.9999
			0.1	83.0 – 90.5 (87.7)	3.5 (5)	
No group: Wheat (straw)	Cinmethylin *m/z* 275 → 153	0.01	0.01	76.8 – 84.2 (81.5)	4.0 (5)	0.8 – 60.4 ng/mL (0.002 – 0.15 mg/kg) (n =8) r = 0.9999
			0.1	76.5 – 82.2 (80.1)	2.7 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	76.8 – 82.5 (80.5)	2.7 (5)	As above r=0.9999
			0.1	77.9 – 82.1 (80.7)	2.1 (5)	

Linearity:

Linearity of detector response was tested using 8 calibration standard concentrations over appropriate concentration ranges with correlation coefficients of ≥0.9999. The calibration standards were prepared in acetonitrile/water (80/20, v/v).

Accuracy and Precision:

Samples were spiked with the analyte at LOQ and 10x LOQ. At least two unfortified control samples and a reagent blank were also analysed at each level. All individual and mean recoveries were within the acceptable ranges. The %RSD at each fortification level are within the acceptable level (<20% RSD).

Conclusion:

The stated deviation from the primary method description is not considered to significantly impact on the method performance or the study. The method L0337/01 has been acceptably independently validated in accordance with the EU guidance SANCO/825/00 rev. 8.1 in all matrices with an LOQ of 0.01 mg/kg.

B.5.2.2. Methods for residues in or on food and feed of animal origin

Report:	**KCA 4.2/2, Asekunowo J. (2018)**
Title	Validation of BASF analytical method L0385/01 for the determination of BAS 684 H animal matrices. Report number: 2017/1202142
Guidelines:	SANCO 825/00/rev. 8.1, SANCO 3029/99/rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The objective of this study was to validate the analytical method L0385/01 for the determination of cinmethylin in animal matrices by LC-MS/MS.

Sample preparation:

Samples (5 g for all commodities except fat – 2 g) are extracted with pure acetonitrile. Samples with low water content (< 80%, e.g. fat) require the addition of water before the initial extraction to get a total of approximately 10 g of water. After addition of the QuEChERS extraction salts kit, the mixture is shaken intensively and centrifuged for phase separation.

The organic extract is cleaned up by dispersive SPE (d-SPE) using QuEChERS clean up-kit containing $MgSO_4$, PSA, C18EC and GCB. For bovine meat, d-SPE is performed using QuEChERS clean-up kit containing $MgSO_4$, PSA and C18. For eggs, d-SPE is performed using the QuEChERS d-SPE EMR-Lipid kit and the QuEChERS Final Polish EMR-Lipid kit and an additional freeze out was included to separate the analyte from the fat matrix. An aliquot (0.8ml) of the cleaned-up extracts were taken and supplemented with water (0.2ml) before analysis. The final determination is achieved by liquid chromatography coupled to tandem mass spectrometric detection (LC-MS/MS).

LC-MS/MS conditions (Used for all matrices except fat):

Chromatographic system: Agilent 1290 series LC system
Analytical column: Thermo Fisher Scientific Betasil C18: 100 mm x 2.1 mm, Particle size 5 µm
Target column temperature: 30 °C
Injection volume: 10 µL
Mobile phase A: Water/formic acid (1000/1, v/v)
Mobile phase B: Acetonitrile/formic acid (1000/1, v/v)
Flow rate: 600 µL/min
Gradient (including wash and equilibration):

Time (min)	Phase A (%)	Phase B (%)
0	70	30
2.5	40	60
5.5	20	80
5.6	0.1	99.9
7	0.1	99.9
7.1	70	30
10.0	70	30

Detection system: AB Sciex API 5000 Triple quad Mass Spectrometer
Ionisation: Turbo Spray (ESI positive)
Retention time: BAS 684 H: approximately 4.8 min
Ions monitored: m/z 275 → 153 Quantification
m/z 275 → 105 Confirmatory

LC-MS/MS conditions (Used only for fat:)

Chromatographic system: Agilent 1290 series LC system
Analytical column: Machery Nagel Nucleoshell RP-Plus C18: 100 mm x 3.0 mm, Particle size 2.7 µm
Target column temperature: 40 °C
Injection volume: 10 µL
Mobile phase A: Water/formic acid (1000/1, v/v)
Mobile phase B: Acetonitrile/formic acid (1000/1, v/v)
Flow rate: 600 µL/min
Gradient (including wash and equilibration):

Time (min)	Phase A (%)	Phase B (%)
0	70	30
0.1	40	60
3.0	40	60
6.5	20	80
6.6	0.1	99.9
8.0	0.1	99.9
8.1	70	30
10.0	70	30

Detection system: AB Sciex API 5000 Triple quad Mass Spectrometer
Ionisation: Turbo Spray (ESI positive)
Retention time: Cinmethylin: approximately 5.9 min

Ions monitored: m/z 275 → 153 Quantification
m/z 275 → 105 Confirmatory

A summary of the method validation data is given in Tables B.5.2.2-1

Table B.5.2.2-1: Summary of method validation data for determination of cinmethylin residues in animal matrices

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Milk	Cinmethylin *m/z* 275 → 153	0.01	0.01	73.5 – 80.5 (76.9)	3.3 (5)	0.5 – 50 ng/mL (0.001 – 0.1 mg/kg) (n = 7) r = 0.9997
			0.1	76.5 – 79.5 (78.7)	1.6 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	74.0 – 78.3 (76.9)	2.2 (5)	As above r=0.9998
			0.1	75.3 – 79.5 (77.7)	2.0 (5)	
Egg	Cinmethylin *m/z* 275 → 153	0.01	0.01	95.3 – 99.5 (97.1)	2.0 (5)	0.5 – 50 ng/mL (0.001 – 0.1 mg/kg) (n = 7) r = 0.9996
			0.1	82.8 – 95.5 (91.2)	5.5 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	94.3 – 97.1 (95.9)	1.3 (5)	As above r=0.9997
			0.1	81.8 – 95.6 (91.3)	6.1 (5)	
Meat	Cinmethylin *m/z* 275 → 153	0.01	0.01	95.0 – 99.1 (97.0)	1.6 (5)	0.5 – 50 ng/mL (0.001 – 0.1 mg/kg) (n = 7) r = 0.9999
			0.1	97.0 – 102 (98.6)	2.0 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	98.3 – 102 (99.3)	1.8 (5)	As above r=0.9999
			0.1	97.3 – 103 (99.9)	2.2 (5)	
Liver	Cinmethylin *m/z* 275 → 153	0.01	0.01	71.5 – 76.0 (73.7)	2.3 (5)	0.5 – 50 ng/mL (0.001 – 0.1 mg/kg) (n = 7) r = 1.000
			0.1	74.0 – 75.6 (74.5)	0.92 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	71.8 – 75.5 (73.6)	2.3 (5)	As above r=1.000
			0.1	73.8 – 75.5 (74.5)	1.0 (5)	
Kidney	Cinmethylin *m/z* 275 → 153	0.01	0.01	71.5 – 75.0 (73.0)	2.1 (5)	0.5 – 50 ng/mL (0.001 – 0.1 mg/kg) (n = 7) r = 0.9999
			0.1	70.3 – 74.1 (72.3)	1.9 (5)	

	Cinmethylin *m/z* 275 → 105	0.01	0.01	71.3 – 74.3 (72.1)	1.7 (5)	As above r=1.000
			0.1	70.0 – 73.3 (71.6)	1.6 (5)	
Fat	Cinmethylin *m/z* 275 → 153	0.01	0.01	84.4 – 89.4 (86.8)	2.1 (5)	0.3 – 25 ng/mL (0.002 – 0.167 mg/kg) (n = 7) r = 0.9999
			0.1	82.5 – 88.1 (84.8)	2.5 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	88.1 – 98.8 (92.6)	4.2 (5)	As above r=0.9992
			0.1	84.4 – 86.9 (86.1)	1.3 (5)	

Specificity and Confirmation of Analyte Identity:

The primary method is considered specific to the analytes therefore additional confirmation of identity is not required.

The interferences of the analyte measured in the control sample were below 20 % of the limit of quantification (LOQ) for each matrix and each mass transition.

Linearity:

Linearity of detector response was tested using at least 7 calibration standard concentrations over appropriate concentration ranges with correlation coefficients of ≥0.999. The calibration standards were prepared in acetonitrile/water (80/20, v/v).

Matrix Effects:

The matrix effect was tested for each matrix and mass transition. In all cased the matrix effect was negligible and solvent-based standards were used for quantification.

Accuracy and Precision

Samples were spiked with the analyte at LOQ and 10x LOQ. At least two unfortified control samples one reagent blank were also analysed at each level. All recoveries were within the acceptable range (70 – 110%). The %RSD at each fortification level is below the acceptable level (<20% RSD).

Storage stability

Cinmethylin was shown to be stable in standard solutions for 30 days when stored at 4 °C in the dark and in sample solutions for 7 days when stored refrigerated at 4 °C in the dark.

It is noted that the mean procedural recoveries are also all in the range (70 – 110%) with %RSDs < 20% giving further evidence for the accuracy and precision of the method.

Conclusion:

The method is fully validated in accordance with SANCO/825/00 rev. 8.1for the determination of cinmethylin in animal matrices milk, egg, meat, liver, kidney and fat with an LOQ of 0.01 mg/kg.

Report:	**KCA 4.2/3, Ford K., (2018)**
Title	Independent Laboratory Validation of BASF analytical method L0385/01 for the determination of BAS 684 H animal matrices. Report number: 2017/1202456 (Study ID: 765936)
Guidelines:	SANCO 825/00/rev. 8.1, SANCO 3029/99/rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Method L0385/01 for the determination of cinmethylin was independently validated in milk, egg, meat, liver, kidney and fat.

Deviations from the primary method validation study:

In the ILV study all matrices were analysed using the same LC-MS/MS conditions – sperate conditions for the analyses of fat samples were not used.

A summary of the method validation data is given in Table B.5.2.2-2.

Table B.5.2.2-2: Summary of independent laboratory validation data for determination of cinmethylin residues in animal matrices

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Milk	Cinmethylin *m/z* 275 → 153	0.01	0.01	80.4 – 81.7 (81.7)	1.7 (5)	0.5 – 50 ng/mL (0.001 – 0.1 mg/kg) (n = 7) r = 1.0000
			0.1	81.6 – 84.3 (82.7)	1.3 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	80.6 – 82.7 (81.7)	1.2 (5)	As above r=1.0000
			0.1	81.9 – 83.7 (82.9)	1.0 (5)	
Egg	Cinmethylin *m/z* 275 → 153	0.01	0.01	85.5 – 94.8 (91.2)	4.3 (5)	0.5 – 50 ng/mL (0.001 – 0.1 mg/kg) (n = 7) r = 0.9998
			0.1	88.5 – 90.6 (89.3)	0.9 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	86.6 – 95.0 (91.6)	4.1 (5)	As above r=0.9997
			0.1	88.9 – 90.4 (89.6)	0.7 (5)	
Muscle (bovine)t	Cinmethylin *m/z* 275 → 153	0.01	0.01	84.6 – 87.9 (85.8)	1.5 (5)	0.5 – 50 ng/mL (0.001 – 0.1 mg/kg) (n = 7) r = 1.0000
			0.1	82.6 – 86.1 (83.7)	1.5 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	89.1 – 90.8 (89.5)	1.5 (5)	As above r=1.0000
			0.1	83.0 – 85.9 (84.5)	1.3 (5)	
Liver (bovine)	Cinmethylin *m/z* 275 → 153	0.01	0.01	82.1 – 86.0 (84.4)	2.0 (5)	0.5 – 50 ng/mL (0.001 – 0.1 mg/kg) (n = 7) r = 0.9999
			0.1	82.2 – 89.4 (87.0)	3.2 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	76.8 – 81.9 (79.8)	2.4 (5)	As above r=0.9999
			0.1	88.6 – 91.0 (88.3)	3.6 (5)	

Kidney (bovine)	Cinmethylin *m/z* 275 → 153	0.01	0.01	78.8 – 83.0 (80.9)	2.1 (5)	0.5 – 50 ng/mL (0.001 – 0.1 mg/kg) (n = 7) r = 1.0000
			0.1	80.9 – 84.0 (82.1)	0.8 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	94.3 – 97.1 (81.6)	1.7 (5)	As above r=0.9999
			0.1	80.9 – 83.9 (82.5)	1.5 (5)	
Fat (bovine)	Cinmethylin *m/z* 275 → 153	0.01	0.01	87.8 – 90.7 (87.7)	2.3 (5)	0.3 – 25 ng/mL (0.002 – 0.167 mg/kg) (n = 7) r = 0.9998
			0.1	89.1 – 92.7 (91.4)	1.7 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	86.5 – 90.9 (88.7)	2.0 (5)	As above r=0.9998
			0.1	89.7 – 93.7 (91.3)	1.9(5)	

Linearity:

Linearity of detector response was tested using at 7 calibration standard concentrations over appropriate concentration ranges with correlation coefficients of ≥0.995. The calibration standards were prepared in acetonitrile/water (80/20, v/v).

Accuracy and Precision:

Samples were spiked with the analyte at LOQ and 10x LOQ. At least two unfortified control samples one reagent blank were also analysed at each level. All recoveries were within the acceptable range (70 – 110%). The %RSD at each fortification level is below the acceptable level (<20% RSD).

Conclusion:

The stated deviation from the primary method description is not considered to significantly impact on the method performance or the study. The method has been acceptably independently validated in accordance with SANCO/825/00 rev. 8.1 for the determination of cinmethylin in animal matrices with an LOQ of 0.01 mg/kg.

Report:	**KCA 4.2/4, [redacted] (2018 a)**
Title	Investigation of the extractability of BAS 684 H in liver from a 14C goat metabolism study (enforcement methods) Report number: 2017/1192630 (Study ID: 765938)
Guidelines:	SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Samples of liver from the goat metabolism study were used to investigate the extraction efficiency of the multiresidue methods DFG S19 and QuEChERS for residues of cinmethylin. Liver was chosen as the only matrix from the metabolism studies that contained significantly high residues of cinmethylin to allow comparison to be made. The extraction procedures used in these methods were compared to the extraction procedure (methanol extraction) used in the metabolism study.

Sample preparation:

Method 1 (Multiresidues method DFG S19):

Samples (25 g) were extracted with 82.5 mL water and 200 mL acetone using a homogenizer. Thereby the water content of the sample was considered (liver: approximately 70%). 35 g sodium chloride and 100 mL of a mixture of cyclohexane and ethyl acetate were added, and the sample was further extracted using a homogenizer. The mixture was centrifuged, and the supernatant was filtered (cotton) into a separating funnel. The solid extraction residue was discarded. The organic phase and the water phase were separated, the volumes determined, and analysed.

Method 2 (Multiresidue method QuEChERS – module E6):

Samples (5 g) were mixed with 6.5 mL water and 10 mL acetonitrile and agitated on a shaker. Afterwards, QuEChERS salts were added, and the sample was forcefully shaken. The pH of the sample was checked (approximately pH 5). After centrifugation, the acetonitrile phase was removed for analysis.

Metabolism study:

Samples were extracted with three aliquots of methanol using a homogenizer. After centrifugation the three extracts were pooled, concentrated and adjusted to a defined volume.

Analysis:

For both extracts, subsamples were evaporated almost to dryness using a rotary evaporator (operated at 40 °C), taken up in mixtures of Triton X 100, acetonitrile and water, assisted by ultrasonication, and adjusted to a defined volume with water. Aliquots of both samples were subjected to LSC measurement and HPLC analysis. The method used was the same as method LC05 used in the goat metabolism study (See Volume 3 Section B.7.2.3)

The amount of radioactive residue and cinmethylin residue extracted in the respective goat metabolism study was used as the reference value for extraction efficiency.

The results are summarised in Table B.5.2.2-3:.

Table B.5.2.2-3: Summary of extractability of radioactive residues and cinmethylin in goat liver.

Extraction method	TRR (mg/kg)	Radioactive residues in extract		Cinmethylin	
		mg/kg (% TRR)	Extraction efficiency	mg/kg (% TRR)	Extraction efficiency
Goat liver - phenyl label					
Metabolism study	0.681	0.423 (62)	100	0.097 (14)	100
Method 1 (DFG S 19)		0.201 (29)	48	0.090 (13)	93
Method 2 (QuEChERS)		0.301 (44)	71	0.068 (10)	70

Example extraction efficiency calculation: (mg/kg extracted by method 1/ mg/kg extracted by metabolism study)*100

Conclusion:

The extractability of total residues was higher using the QuEChERS method (71%) than in the DFG S19 method (48%), however extractability of cinmethylin residues from goat liver using DFG S19 was similar to the metabolism study and was lower using extraction method according to QuEChERS.

The general criteria acceptable extraction efficiency of a method is that is can extract at least 70% of the TRR and 50% of the residue definition components. On this basis neither method is acceptable. However, it is noted that the extraction procedure used in the metabolism study only released 62% TRR. Furthermore, on the basis of the representative uses, significant residues in animal products are not expected, therefore extraction efficiency does not need to be addressed further. This conclusion may need to be revised if residues in animals products are considered to be significant.

Report:	KCA 4.2/10, Spangler, C., (2020)
Title	Validation of BASF analytical method L0337/03 for the determination of BAS 684 H (Reg. No. 900202) in honey by LC-MS/MS. Report number: 2020/2002775
Guidelines:	OECD-ENV/JM/MONO/(2007)17, SANCO 825/00/rev. 8.1, SANCO 3029/99/rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The objective of this study was to validate the analytical method L0337/03 for the determination of cinmethylin in honey by LC-MS/MS.

Sample preparation:

Samples (1 g) are extracted with 5ml acetonitrile/water (80/20 v/v) and centrifuged. A 0.8ml aliquot of the acetonitrile phase from the supernatant is taken and supplemented with 0.2ml water before analysis.

The final determination is achieved by liquid chromatography coupled to tandem mass spectrometric detection (LC-MS/MS).

LC-MS/MS conditions:

Chromatographic system: Acquity UPLC LC system
Analytical column: Thermo Scientific Betasil C18: 100 mm x 2.1 mm, Particle size 5 µm
Target column temperature: 25 °C
Injection volume: 25 µL
Mobile phase A: Water/formic acid (1000/1, v/v)
Mobile phase B: Acetonitrile/formic acid (1000/1, v/v)
Flow rate: 600 µL/min
Gradient (including wash and equilibration):

Time (min)	Phase A (%)	Phase B (%)
0	70	30
0.1	40	60
2.5	40	60
5.5	20	80
5.6	0.1	99.9
7	0.1	99.9
7.1	70	30
10.0	70	30

Detection system: Triple quad 5500+ Mass Spectrometer
Ionisation: Turbo Spray (ESI positive)
Retention time: BAS 684 H: approximately 4.7 min
Ions monitored: m/z 275 → 153 Quantification
m/z 275 → 105 Confirmatory

A summary of the method validation data is given in Table B.5.2.2-4

Table B.5.2.2-4: Summary of method validation data for determination of cinmethylin residues in honey

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Honey	Cinmethylin *m/z* 275 → 153	0.01	0.01	90.9 – 96.6 (93.7)	2.4 (5)	0.5 – 25 ng/mL (0.0025 – 0.125 mg/kg) (n = 6) r = 0.9997
			0.1	89.7 – 94.5 (92.9)	2.1 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	93.8 – 103 (97.7)	3.6 (5)	As above r=0.9999
			0.1	90.8 – 92.1 (91.6)	0.6 (5)	

Specificity and Confirmation of Analyte Identity:

The primary method is considered specific to the analytes therefore additional confirmation of identity is not required.

The interferences of the analyte measured in the control sample were below 25 % of the limit of quantification (LOQ) for each mass transition.

Linearity:

Linearity of detector response was tested using at least 6 calibration standard concentrations over appropriate concentration ranges with correlation coefficients of ≥0.999. The calibration standards were prepared in acetonitrile/water (80/20, v/v).

Matrix Effects:

The matrix effect was tested for each mass transition. In all cases the matrix effect was negligible and solvent-based standards were used for quantification.

Accuracy and Precision

Samples were spiked with the analyte at LOQ and 10x LOQ. At least two unfortified control samples and one reagent blank were also analysed at each level. All recoveries were within the acceptable range (70 – 110%). The %RSD at each fortification level is below the acceptable level (<20% RSD).

Storage stability

Cinmethylin was shown to be stable in standard solutions for 28 days when stored at 4 °C in the dark and in sample solutions for 7 days when stored refrigerated at 4 °C in the dark.

It is noted that the mean procedural recoveries are also all in the range (70 – 110%) with %RSDs < 20% giving further evidence for the accuracy and precision of the method.

Conclusion:

The method is fully validated in accordance with SANCO/825/00 rev. 8.1for the determination of cinmethylin in honey with an LOQ of 0.01 mg/kg.

Report:	**KCA 4.2/11, Link T., Walsch M., (2020)**
Title	Independent Laboratory Validation of BASF analytical method L0337/03 for the determination of BAS 684 H in honey by LC-MS/MS. Report number: 2020/2004119
Guidelines:	OECD-ENV/JM/MONO/(2007)17, SANCO 825/00/rev. 8.1, SANCO 3029/99/rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Method L0337/03 for the determination of cinmethylin in honey was independently validated.

Deviations from the primary method validation study:

The LC-MS/MS conditions were altered as follows:

The injection volume was reduced to 5 µL in the ILV and 25 µL in the primary study

The mobile phase gradient program did not include the step at 0.1 minutes i.e. mobile phase remained in the ratio 70:30 from 0.00 until 2.5 min. The retention time for cinmethylin was approximately 1 minute later under these conditions, however the chromatograms submitted indicated that chromatography is still acceptable under these slightly different conditions.

A summary of the method validation data is given in Table B.5.2.2-5.

Table B.5.2.2-5: Summary of independent laboratory validation data for determination of cinmethylin residues in honey

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Honey	Cinmethylin *m/z* 275 → 153	0.01	0.01	85.2 – 89.7 (86.9)	2.1 (5)	0.3 – 33 ng/mL (0.0015 – 0.165 mg/kg) (n = 8) r = 0.9999
			0.1	93.1 – 95.4 (94.1)	1.0 (5)	
	Cinmethylin *m/z* 275 → 105	0.01	0.01	83.6 – 87.1 (85.5)	1.7 (5)	As above r=0.9999
			0.1	92.9 – 95.4 (94.4)	1.2 (5)	

Linearity:

Linearity of detector response was tested using at 8 calibration standard concentrations over appropriate concentration ranges with correlation coefficients of ≥0.999. The calibration standards were prepared in acetonitrile/water (80/20, v/v).

Accuracy and Precision:

Samples were spiked with cinmethylin at LOQ and 10x LOQ. At least two unfortified control samples one reagent blank were also analysed at each level. All recoveries were within the acceptable range (70 – 110%). The %RSD at each fortification level is below the acceptable level (<20% RSD).

Conclusion:

The stated deviations from the primary method description are not considered to significantly impact on the method performance or the study. The method has been acceptably independently validated in accordance with SANCO/825/00 rev. 8.1 for the determination of cinmethylin in honey with an LOQ of 0.01 mg/kg.

B.5.2.3. Methods for residues in soil and sediment

Method L0308/01 was developed and validated for the determination of the enantiomers of cinmethylin (Reg. No. 5925632 and Reg. No. 5925581) in soil and sediment. This method was also used to generate data in support of authorisation and is reported in full under B.5.1.2.1.

B.5.2.4. Methods for residues in water

Report:	KCA 4.2./5, Obermann, M., Arndt S., (2018a)
Title	Validation of analytical method L0366/01 for the enantiomers Reg. No. 5925632 and Reg. No. 5925581 of BAS 684 H in water by reversed-phase chiral LC-MS/MS Report number: 2017/1194948 (Study ID: 738433)
Guidelines:	SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4, EPA 850.6100
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Method L0366/01 was developed and validated for the determination of the enantiomers of cinmethylin (Reg. No. 5925632 and Reg. No. 5925581) in ground and surface water. The ground water was a sample of well water supplied by Wasserwerk Schifferstadt, and the surface water was a typical sample from Kelmetschwiher, Germany. Certificates of analysis covering total and dissolved organic carbon content, pH, conductivity and hardness were provided.

Sample preparation:

Samples (100 mL) of ground or surface water are acidified with 100 µL formic acid and extracted using C18 solid phase extraction (SPE) eluting twice with methanol. The extracts are combined, evaporated to dryness and reconstituted in 3 mL acetonitrile/water (80/20, v/v) prior to final determination by LC-MS/MS for which typical conditions are provided below:

Chromatographic system: Waters Acquity UPLC system
Analytical column: ChiralPak A-3 (150 mm x 4.6 mm, Particle size 3 µm)
Target column temperature: 10 °C
Injection volume: 10 µL
Mobile phase A: Water/formic acid (1000/1, v/v)
Mobile phase B: Acetonitrile/formic acid (1000/1, v/v)
Flow rate: 800 µL/min
Gradient (including wash and equilibration):

Time (min)	Phase A (%)	Phase B (%)
0	40	60
8.0	30	70
8.1	20	80
15.0	20	80
15.1	40	60
20.0	40	60

Detection system: AB Sciex API 6500 Mass Spectrometer
Ionisation: Turbo Spray (ESI positive)
Retention time: Reg. No. 5925581: approximately 6.1 min
Reg. No. 5925632: approximately 6.4 min
Mass transitions: *m/z* 275 → 105 Quantification
m/z 275 → 153 Confirmatory

The LOQ was 0.03 µg/L. A summary of the method validation data is given in Table B.5.2.4-1:

Table B.5.2.4-1: Summary of method validation data for determination of cinmethylin enantiomers in ground and surface water

Matrix	Analyte	LOQ (µg/L)	Recovery fortification level (µg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Ground water	**Reg. 5925581 (1S,2R,4R)** *m/z* 275 → 105	0.03	0.03	85 – 90 (89)	2.1 (5)	0.25 – 25 ng/mL (0.0075 – 0.75 µg/L) (n = 7) r = 0.9998
			0.3	88 – 93 (91)	2.0 (5)	
	Reg. 5925581 (1S,2R,4R) *m/z* 275 → 153	0.03	0.03	85 – 90 (88)	2.7 (5)	As above r=0.9999
			0.3	89 – 93 (91)	1.8 (5)	
	Reg. 5925632 (1R,2S,4S) *m/z* 275 → 105	0.03	0.03	82 – 91 (87)	3.5 (5)	0.25 – 25 ng/mL (0.0075 – 0.75 µg/L) (n = 7) r = 0.9994
			0.3	90 – 97 (92)	3.1 (5)	
	Reg. 5925632 (1R,2S,4S) *m/z* 275 → 153	0.03	0.03	85 – 87 (86)	1.5 (5)	As above r=0.9997
			0.3	79 – 89 (86)	4.8 (5)	
Surface water	**Reg. 5925581 (1S,2R,4R)** *m/z* 275 → 105	0.03	0.03	79 – 85 (82)	2.6 (5)	0.25 – 25 ng/mL (0.0075 – 0.75 µg/L) (n = 7) r = 0.9994
			0.3	85 – 90 (88)	2.8 (5)	
	Reg. 5925581 (1S,2R,4R) *m/z* 275 → 153	0.03	0.03	85 – 98 (90)	5.6 (5)	As above r=0.9997
			0.3	84 – 93 (88)	4.1 (5)	
	Reg. 5925632 (1R,2S,4S) *m/z* 275 → 105	0.03	0.03	79 – 84 (81)	2.4 (5)	0.25 – 25 ng/mL (0.0075 – 0.75 µg/L) (n = 7) r = 0.9994
			0.3	88 – 93 (91)	2.5 (5)	
	Reg. 5925632 (1R,2S,4S) *m/z* 275 → 153	0.03	0.03	78 – 81 (80)	1.6 (5)	As above r=0.9997
			0.3	83 – 91 (87)	4.3 (5)	

Specificity and Confirmation of Analyte Identity:

LC-MS/MS is a highly specific self-confirmatory technique therefore additional confirmation of identity is not required. The ion transitions monitored are appropriate. Analysis of unfortified control samples and reagent blanks demonstrated no significant interference (> 30% of the LOQ) at the retention times of interest.

Linearity:

Linearity of detector response was tested using seven calibration standard concentrations covering a concentration range of 0.25 – 25 ng/mL (equivalent to 0.0075 – 0.75µg/L in the samples). The calibration standards were prepared in acetonitrile/water (80/20, v/v). Correlation coefficients of ≥0.99 were obtained for both enantiomers and both ion transitions.

Matrix Effects:

Matrix effects were tested preparing matrix-matched standards for each matrix. The matrix effects were negligible and solvent-based standards were used for quantification.

Accuracy and Precision:

Five individual replicates of samples of ground and surface water were fortified at the LOQ and 10x LOQ levels. Mean recoveries at both levels were within the acceptable range for both analytes and both ion transitions. The %RSD at each fortification level was within the acceptable level (<20 % RSD).

Storage stability:

Stability was confirmed for Reg. No. 5925632 and Reg. No. 5925581 in stock and calibration solutions for 28 days, when stored refrigerated at 4°C in the dark. Both analytes were stable for up to 6 days in ground water and 7 days in surface water when stored refrigerated at 4 °C in the dark.

Conclusion:

The method is fully validated in accordance with SANCO/825/00 rev. 8.1. for the determination of cinmethylin enantiomer residues via HPLC-MS/MS in surface and ground water with an LOQ of 0.03 µg/L.

Report:	**KCA 4.2./6, Obermann, M., Arndt S., (2018c)**
Title	Validation of analytical method L0366/02 for the determination of Metabolites M684H001 (Reg.No. 6055521) and M684H004 (Reg.No. 6055480) in drinking (ground) and surface-water by LC-MS/MS Report number: 2017/1011310 (Study ID: 783156)
Guidelines:	SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4, EPA 850.6100
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Method L0366/02 was developed and validated for the determination of cinmethylin metabolites M684H001 and M684H004 in ground and surface water. The ground water was a sample of well water supplied by Wasserwerk Schifferstadt, and the surface water was a typical sample from Kelmetschwiher, Germany. Certificates of analysis covering total and dissolved organic carbon content, pH, conductivity and hardness were provided.

Sample preparation:

Samples (100 mL) of ground or surface water are acidified with 0.5mL 1M hydrochloric acid and extracted using C18 solid phase extraction (SPE) eluting with methanol. The extract is evaporated to dryness, reconstituted in 1 mL acetonitrile/water (50/50, v/v) and diluted further as necessary (for high concentration samples) prior to final determination by LC-MS/MS for which typical conditions are provided below:

Chromatographic system:	Waters Acquity UPLC system
Analytical column:	Thermo Aquasil C18 (150 mm x 3 mm, Particle size 3 µm)
Target column temperature:	25 °C
Injection volume:	30 µL
Mobile phase A:	Water/formic acid (1000/1, v/v)
Mobile phase B:	Acetonitrile/formic acid (1000/1, v/v)
Flow rate:	800 µL/min

Gradient (including wash and equilibration):

Time (min)	Phase A (%)	Phase B (%)
0	95	5
1.0	95	5
9.0	25	75
9.01	5	95
12.00	5	95
12.01	95	5
15.00	95	5

Detection system: AB Sciex API 6500 Mass Spectrometer
Ionisation: Turbo Spray (ESI negative for M684H001 and positive for M684H004))
Retention time: M684H001: approximately 8.4 min
M684H004: approximately 8.9 min
Mass transitions: M684H001: *m/z* 303→ 133 Quantification; m/z 303 → 105 Confirmatory
M684H004: *m/z* 291 → 105 Quantification; m/z 291 → 77 Confirmatory

The LOQ was 0.03 µg/L. A summary of the method validation data is given in Table B.5.2.4-2.

Table B.5.2.4-2: Summary of method validation data for determination of cinmethylin metabolites M684H001 and M684H004 in ground and surface water

Matrix	Analyte	LOQ (µg/L)	Recovery fortification level (µg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Groundwater	**M684H001** *m/z* 303→ 133	0.03	0.03	81 – 91 (88)	4.8 (5)	0.9 – 15 ng/mL (0.009 – 0.15 µg/L) (n = 6) r = 1.0000
			0.3	92 – 97 (95)	2.3 (5)	
	M684H001 *m/z* 303→ 105	0.03	0.03	79 – 90 (87)	5.3 (5)	As above r=0.9998
			0.3	92 – 97 (95)	2.5 (5)	
	M684H004 *m/z* 291 → 105	0.03	0.03	91– 98 (96)	2.9 (5)	0.9 – 15 ng/mL (0.009 – 0.15 µg/L) (n = 6) r = 0.9992
			0.3	92 – 97 (95)	1.9 (5)	
	M684H004 *m/z* 291 → 77	0.03	0.03	95 – 100 (98)	2.0 (5)	As above r=0.9991
			0.3	94 – 99 (97)	2.0 (5)	
Surface water	**M684H001** *m/z* 303→ 133	0.03	0.03	84 – 86 (85)	0.9 (5)	0.9 – 15 ng/mL (0.009 – 0.15 µg/L) (n = 6) r = 1.0000
			0.3	97 – 98 (98)	0.5 (5)	
	M684H001 *m/z* 303→ 105	0.03	0.03	84 – 85 (84)	0.6 (5)	As above r=0.9998
			0.3	97 – 100 (98)	1.4 (5)	
	M684H004 *m/z* 291 → 105	0.03	0.03	98 – 100 (98)	1.1 (5)	0.9 – 15 ng/mL (0.009 – 0.15 µg/L) (n = 6) r = 0.9992
			0.3	98 – 99 (98)	0.8 (5)	
	M684H004 *m/z* 291 → 77	0.03	0.03	96 – 106 (103)	5.3 (5)	As above r=0.9991
			0.3	95 – 98 (97)	1.6 (5)	

Specificity and Confirmation of Analyte Identity:

LC-MS/MS is a highly specific self-confirmatory technique therefore additional confirmation of identity is not required. The ion transitions monitored are appropriate. Analysis of unfortified control samples and reagent blanks demonstrated no significant interference (> 30% of the LOQ) at the retention times of interest.

Linearity:

Linearity of detector response was tested using six calibration standard concentrations covering a concentration range of 0.9 – 15 ng/mL (equivalent to 0.009 – 0.15µg/L in the samples). The calibration standards were prepared in acetonitrile/water (50/50, v/v). Correlation coefficients of ≥0.99 were obtained for both metabolites and both ion transitions.

Matrix Effects:

Matrix effects were tested preparing matrix-matched standards for each matrix. The matrix effects were negligible and solvent-based standards were used for quantification.

Accuracy and Precision:

Five individual replicates of samples of ground and surface water were fortified at the LOQ and 10x LOQ levels. Mean recoveries at both levels were within the acceptable range for both analytes and both ion transitions. The %RSD at each fortification level was within the acceptable level (<20 % RSD).

Storage stability:

Stability was confirmed for M684H001 and M684H004 in stock and calibration solutions for 30 days, when stored refrigerated at 4°C in the dark. Both analytes were stable for up to 8 days in ground water and 7 days in surface water when stored refrigerated at 4 °C in the dark.

Conclusion:

The method is fully validated in accordance with SANCO/825/00 rev. 8.1. for the determination of residues of the cinmethylin metabolites M684H001 and M684H004 via HPLC-MS/MS in surface and ground water with an LOQ of 0.03 µg/L.

Report:	**KCA 4.2./7, Joos S., Tussetschlaeger S., (2017a)**
Title	Independent laboratory validation of the methods L0366/01 and L0366/02 for the determination of BAS 684 H (Reg.No. 5925581 and 5925632) and metabolites M684H001 (Reg.No. 6055521) and M684H004 (Reg.No. 6055480) in surface and groundwater Report number: 2017/1223471 (Study ID: 738435)
Guidelines:	SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4, EPA 850.6100
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Method L0366/01 for the determination of the enantiomers of cinmethylin and method L0366/02 for the determination of cinmethylin metabolites M684H001 and M684H004 were both independently validated in ground and surface water in the same study.

Deviations from the primary method validation studies:

Significant matrix effects > 20% were observed for the cinmethylin enantiomers in both ground & surface water therefore matrix matched standards were used for the compounds. No matrix effects were observed for the metabolites, so these were analysed using solvent standards as in the primary method validation.

For the analysis of the enantiomers of cinmethylin an injection volume of 70µL instead of 10µL was used.

The LOQ was 0.03 µg/L for all analytes. A summary of the method validation data for method L0366/01 is given in Table B.5.2.4-3 and for L0366/02 in Table B.5.2.4-4:

Table B.5.2.4-3: Summary of independent laboratory validation data for determination of cinmethylin enantiomers in ground and surface water

Matrix	Analyte	LOQ (µg/L)	Recovery fortification level (µg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Groundwater	**Reg. 5925581 (1S,2R,4R)** *m/z* 275 → 153	0.03	0.03	62 – 88 (72)	14 (5)	0.25 – 25 ng/mL (equivalent to 0.0075 – 0.75 µg/L) r = 0.998
			0.3	67 – 79 (72)	6.9 (5)	
	Reg. 5925581 (1S,2R,4R) *m/z* 275 → 105	0.03	0.03	68 – 77 (72)	5.2 (5)	As above r=0.996
			0.3	69 – 79 (74)	5.3 (5)	
	Reg. 5925632 (1R,2S,4S) *m/z* 275 → 153	0.03	0.03	73 – 89 (77)	13 (5)	0.25 – 25 ng/mL (equivalent to 0.0075 – 0.75 µg/L) r =0.997
			0.3	74 – 85 (79)	7.2 (5)	
	Reg. 5925632 (1R,2S,4S) *m/z* 275 → 105	0.03	0.03	65 – 82 (75)	9.3 (5)	As above r=0.998
			0.3	70 – 82 (75)	6.6 (5)	
Surface water	**Reg. 5925581 (1S,2R,4R)** *m/z* 275 → 153	0.03	0.03	66 – 87 (75)	11 (5)	0.25 – 25 ng/mL (equivalent to 0.0075 – 0.75 µg/L) r = 0.996
			0.3	63 – 76 (72)	7.2 (5)	
	Reg. 5925581 (1S,2R,4R) *m/z* 275 → 105	0.03	0.03	69 – 87 (75)	9.5 (5)	As above r = 0.996
			0.3	64 – 80 (70)	8.4 (5)	
	Reg. 5925632 (1R,2S,4S) *m/z* 275 → 153	0.03	0.03	69 – 92 (82)	12 (5)	0.25 – 25 ng/mL (equivalent to 0.0075 – 0.75 µg/L) r =0.996
			0.3	67 – 76 (71)	4.9 (5)	
	Reg. 5925632 (1R,2S,4S) *m/z* 275 → 105	0.03	0.03	66 – 77 (73)	7.8 (5)	As above r=0.999
			0.3	67 – 74 (71)	3.8 (5)	

Table B.5.2.4-4: Summary of independent laboratory validation data for determination of cinmethylin metabolites M684H001 and M684H004 in ground and surface water

Matrix	Analyte	LOQ (µg/L)	Recovery fortification level (µg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Groundwater	**M684H001** *m/z* 303→ 133	0.03	0.03	85 – 95 (90)	4.0 (5)	0.9 – 15 ng/mL (0.009 – 0.15 µg/L) r =0.999
			0.3	83 – 90 (87)	3.2 (5)	
	M684H001 *m/z* 303→ 105	0.03	0.03	83 – 93 (90)	4.3 (5)	As above r=0.999
			0.3	83 – 91 (87)	3.3 (5)	
	M684H004 *m/z* 291 → 105	0.03	0.03	78– 86 (82)	4.9 (5)	0.9 – 15 ng/mL (0.009 – 0.15 µg/L) r =0.999
			0.3	86 – 92 (88)	3.6 (5)	
	M684H004 *m/z* 291 → 77	0.03	0.03	75 – 106 (91)	13 (5)	As above r=0.998
			0.3	80 – 89 (87)	5.0 (5)	
Surface water	**M684H001** *m/z* 303→ 133	0.03	0.03	94 – 101 (97)	3.1 (5)	0.9 – 15 ng/mL (0.009 – 0.15 µg/L) r =0.999
			0.3	92 – 108 (97)	6.3 (5)	
	M684H001 *m/z* 303→ 105	0.03	0.03	93 – 100 (96)	2.8 (5)	As above r=0.999
			0.3	91 – 110 (96)	8.2 (5)	
	M684H004 *m/z* 291 → 105	0.03	0.03	81 – 91 (87)	4.4 (5)	0.9 – 15 ng/mL (0.009 – 0.15 µg/L) r =0.998
			0.3	93 – 110 (99)	6.7 (5)	
	M684H004 *m/z* 291 → 77	0.03	0.03	74– 106 (90)	5.3 (5)	As above r=0.997
			0.3	86 – 108 (95)	8.8 (5)	

Linearity:

Linearity of detector response was tested using six calibration standard concentrations covering a concentration range of 0.25 – 25 ng/mL (equivalent to 0.0075 – 0.75µg/Lin the samples) for cinmethylin (enantiomers) and a range of 0.9 – 15 ng/mL (equivalent to 0.009 – 0.15µg/L in the samples) for M684H001 and M684H004 . The calibration standards were prepared in solvent with the exception of cinmethylin (enantiomers) where matrix matched standards were used. Correlation coefficients of ≥0.99 were obtained in all cases.

Accuracy and Precision:

Five individual replicates of samples of ground and surface water were fortified at the LOQ and 10x LOQ levels for each analyte. Mean recoveries at both levels were within the acceptable range for all analytes and ion transitions. The %RSD at each fortification level was within the acceptable level (<20 % RSD).

Conclusion:

The stated deviations from the primary method descriptions are not considered to significantly impact on the method performance or the study. It is noted that significant matrix effects were observed, this could be in part linked to the increased injection volume used (70 µL) compared with the primary method validation. In addition, in the example chromatograms submitted for the LOQ level and lowest calibration level the analyte peaks are not always adequately integrated/resolved from the matrix background. The applicant advised that matrix effects can vary between different types of groundwater and surface water because of the variation in the content of minerals, and that the higher injection will increase matrix-effects because of the higher content of interfering matrix compounds. Therefore, the applicant has concluded it is not unusual that matrix effects are different for the same matrix type. Given that the validation criteria are all met the justification is considered acceptable.

Both methods have been acceptably independently validated in accordance with SANCO/825/00 rev. 8.1. for the determination of residues of cinmethylin (enantiomers) and the metabolites M684H001 and M684H004 in surface and ground water with an LOQ of 0.03 µg/L.

B.5.2.5. Methods for residues in air

Title	Validation of Analytical Method L0371/01 for the determination of BAS 684 H (Reg.No. 900202) in air using LC-MS/MS Report number: 2017/1210714 (Study ID: 738434)
Guidelines:	SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4, EPA 850.6100
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Method L0371/01 was developed and validated for the determination of cinmethylin in air.

Sample preparation:

The content of the ORBO™ adsorbed tube is extracted with acetonitrile, followed by 2 aliquots of acetonitrile/water 80/20 (v/v). The extracts are combined and filtered then made up to 50 mL with acetonitrile prior to final determination by LC-MS/MS for which typical conditions are provided below:

Chromatographic system: Waters Acquity LC system
Analytical column: Betasil C18 (100 mm x 2.1 mm, Particle size 5 µm)
Target column temperature: 25 °C
Injection volume: 10 µL
Mobile phase A: Water/formic acid (1000/1, v/v)
Mobile phase B: Acetonitrile/formic acid (1000/1, v/v)
Flow rate: 600 µL/min
Gradient (including wash and equilibration):

Time (min)	Phase A (%)	Phase B (%)
0	70	30
0.1	40	60
2.5	40	60
5.5	20	80
5.6	0.1	99.9
7.0	0.1	99.9
7.1	70	30
10.0	70	30

Detection system: AB Sciex API 5000 Mass Spectrometer

Ionisation: Turbo Spray (ESI positive)
Retention time: Cinmethylin: approximately 4.6 min
Mass transitions: *m/z* 275→ 105 Quantification
m/z 275 → 153 Confirmation

For method validation the test item was spiked onto the front filter of an adsorbent tube, air with a humidity of 80% and a temperature of 35 °C (540 L per filter) was passed over the filter and the adsorbent tube was extracted as outlined above. The LOQ was 0.05 ng/L. A summary of the method validation data is given in Table B.5.2.5-1.

Table B.5.2.5-1: Summary of method validation data for determination of cinmethylin in air

Matrix	Analyte	LOQ (ng/L)	Recovery fortification level (ng/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Air, 35 °C RH 80%	**Cinmethylin** *m/z* 275→ 105	0.05	0.05	90 – 106 (99)	5.9 (5)	0.1 – 10 ng/mL (equivalent to 0.01 – 1 ng/L) (n = 7) r = 0.9997
			0.5	92 – 96 (95)	1.7 (5)	
	Cinmethylin *m/z* 275→ 153	0.05	0.05	93 – 106 (101)	6.8 (5)	As above r=0.9990
			0.5	92 – 98 (96)	2.7 (5)	

Specificity and Confirmation of Analyte Identity:

LC-MS/MS is a highly specific self-confirmatory technique therefore additional confirmation of identity is not required. The ion transitions monitored are appropriate. Analysis of unfortified control samples and reagent blanks demonstrated no significant interference (> 30% of the LOQ) at the retention time of interest.

Linearity:

Linearity of detector response was tested using seven calibration standard concentrations covering a concentration range of 0.1 – 10 ng/L (equivalent to 0.01 – 1 ng/L in air). Correlation coefficients of ≥0.99 were obtained for both ion transitions.

Matrix Effects:

Matrix effects were tested preparing matrix-matched standards for each matrix. The matrix effects were negligible and solvent-based standards were used for quantification.

Accuracy and Precision:

Five individual replicates were fortified at the LOQ and 10x LOQ levels by 'spiking' adsorption tubes at levels of 27 ng and 270 ng (as 540 L air passed through each tube). Mean recoveries at both levels were within the acceptable range for both ion transitions. The %RSD at each fortification level was within the acceptable level (<20 % RSD).

Breakthrough testing:

Three replicates were fortified at 100 x LOQ and tested for breakthrough by passing 540 L air through each filter and then analysing the front and back parts of the filter separately. Recoveries from the front parts were 94 – 98% and no cinmethylin was detected in the back part indicating that the capacity of the filter is adequate to collect residues of cinmethylin up to a concentration of 5 ng/L air.

Storage stability:

Stability was confirmed for cinmethylin in stock and calibration solutions for 49 days, when stored refrigerated at 4°C in the dark and for up to 8 days in the final sample solution when stored refrigerated at 4 °C in the dark.

Conclusion:

The method is fully validated in accordance with SANCO/825/00 rev. 8.1. for the determination of residues of the cinmethylin via HPLC-MS/MS in air with an LOQ of 0.05 ng/L.

B.5.2.6. Methods for residues in body fluids and tissues

Report:	**KCA 4.2./9 Ivanov E., Bruhn F., (2018a)**
Title	Validation of BASF analytical methods L0387/01 for the determination of BAS 684 H and its metabolite M684H011in body fluids Report number: 2017/1202143 (Study ID: 809766)
Guidelines:	SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4, ENV/MC/CHEM(98)17, OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007), EPA 860.1340
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Method L0385/01 can be used to determine residues of cinmethylin in tissues. See Section B.5.2.2 for the method description and validation.

Method L0387/01 was developed and validated for the determination of cinmethylin and the metabolite M684H011 in body fluids (porcine whole blood and urine).

Sample preparation:

Samples (10 mL) of urine are extracted by homogenisation with acetonitrile containing 1% formic acid. After addition of QuEChERS extraction salts kit, the mixture is shaken intensively and centrifuged for phase separation. The organic supernatant is cleaned up by dispersive SPE (d-SPE) using QuEChERS clean up-kit containing $MgSO_4$, PSA (Primary Secondary Amine Sorbent) and C18. An aliquot (0.1 mL) of the cleaned-up extract is diluted to 1 mL with acetonitrile/water 1/1 (v/v) before analysis.

Samples of blood (2 mL) are extracted by homogenisation with acetonitrile containing 1% formic acid after the addition of 10ml water. After the addition of magnesium sulphate and sodium chloride, the mixture is shaken intensively and then centrifuged. An aliquot (0.5 mL) of the supernatant is diluted further with water containing 0.1% formic acid prior to final determination by LC-MS/MS.

Typical LC-MS/MS conditions are provided below:

Chromatographic system: Agilent 1260 Binary LC system
Analytical column: Betasil C18 (100 mm x 2.1 mm, Particle size 5 µm)
Target column temperature: 40 °C
Injection volume: 10 µL
Mobile phase A: Acetonitrile containing 0.1% formic acid
Mobile phase B: Water containing 0.1% formic acid
Flow rate: 600 µL/min
Gradient (including wash and equilibration):

Time (min)	Phase A (%)	Phase B (%)
0	30	70
1.0	30	70
7.0	95	5
8.0	95	5
8.1	30	70
10.0	30	70

Detection system: AB Sciex API 5500 Mass Spectrometer
Ionisation: Turbo Spray (ESI positive for cinmethylin and negative for M684H011)
Retention time: Cinmethylin: approximately 6.5 min

M684H011: approximately 2.7 min
Mass transitions: Cinmethylin: *m/z* 275→ 153 Quantification; m/z 275 → 105 Confirmatory
M684H011: *m/z* 319 → 133 Quantification; m/z 319 → 105 Confirmatory

The LOQ was 0.01 mg/L for both analytes. A summary of the method validation data is given in Table B.5.2.6-1.

Table B.5.2.6-1: Summary of method validation data for determination of cinmethylin and the metabolite M684H011 in body fluids

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Urine	**Cinmethylin** *m/z* 275→ 153	0.01	0.01	87-96 (90)	4.0 (5)	0.3 – 25 ng/mL (0.003 – 0.25 mg/L) (n = 7) r = 0.9997
			0.1	92 – 97 (93)	3.1 (5)	
	Cinmethylin *m/z* 275→ 105	0.01	0.01	94 – 100 (95)	2.8 (5)	As above r=0.9994
			0.1	92 – 98 (95)	2.6 (5)	
	M684H011 *m/z* 319 → 133	0.01	0.01	77– 80 (78)	1.3 (5)	0.3 – 25 ng/mL (0.003 – 0.25 mg/L) (n = 7) r = 1.000
			0.1	77 – 88 (87)	0.6 (5)	
	M684H011 *m/z* 319 → 105	0.01	0.01	79 – 85 (81)	2.8 (5)	As above r=1.000
			0.1	88 – 90 (89)	1.0 (5)	
Whole blood	**Cinmethylin** *m/z* 275→ 153	0.01	0.01	88 – 108 (99)	7.7 (5)	0.3 – 25 ng/mL (0.003 – 0.25 mg/L) (n = 7) r = 0.9998
			0.1	96 – 102 (99)	2.1 (5)	
	Cinmethylin *m/z* 275→ 105	0.01	0.01	90 – 98 (93)	3.7 (5)	As above r=0.999
			0.1	94 – 103 (99)	3.7 (5)	
	M684H011 *m/z* 319 → 133	0.01	0.01	87– 95 (92)	3.4 (5)	0.3 – 25 ng/mL (0.003 – 0.25 mg/L) (n = 7) r = 0.9998
			0.1	90 – 93 (92)	1.7 (5)	
	M684H011 *m/z* 319 → 105	0.01	0.01	87 – 92 (90)	3.0 (5)	As above r=0.9999
			0.1	89 – 94 (92)	2.1 (5)	

Specificity and Confirmation of Analyte Identity:

LC-MS/MS is a highly specific self-confirmatory technique therefore additional confirmation of identity is not required. The ion transitions monitored are appropriate. Analysis of unfortified control samples and reagent blanks demonstrated no significant interference (> 30% of the LOQ) at the retention times of interest.

Linearity:

Linearity of detector response was tested using seven calibration standard concentrations covering a concentration range of 0.3 – 25 ng/mL (equivalent to 0.003 – 0.25 mg/Lin the samples). The calibration standards were prepared in acetonitrile/water 1/1 (v/v) for the determination of cinmethylin and in acetonitrile/0.1% formic acid 1/1 (v/v) for the determination of M684H011. Correlation coefficients of ≥0.99 were obtained for both analytes and both ion transitions.

Matrix Effects:

Matrix effects were tested preparing matrix-matched standards for each matrix. The matrix effects were negligible and solvent-based standards were used for quantification.

Accuracy and Precision:

Five individual replicates of samples of blood and urine were fortified at the LOQ and 10x LOQ levels. Mean recoveries at both levels were within the acceptable range for both analytes and both ion transitions. The %RSD at each fortification level was within the acceptable level (<20 % RSD).

Storage stability:

Stability was confirmed for cinmethylin and M684H011 in stock and calibration solutions for at least 29 days, when stored refrigerated (1 to 10°C) in the dark. Both analytes were stable for up to 7 days in blood sample extracts and up to 11 days in urine sample extracts when stored refrigerated (1 to 10°C) in the dark.

Conclusion:

The method is fully validated in accordance with SANCO/825/00 rev. 8.1. for the determination of residues of cinmethylin and the metabolite M684H011 via HPLC-MS/MS in urine and blood with an LOQ of 0.01 mg/L.

B.5.3. REFERENCES 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	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 4.1.1/1	Nemitz A.	2015 a	Determination of Cinmethylin in Technical Grade Active Ingredient (TGAI) by means of GC 2015/1174457 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF	None
KCA 4.1.1/2	Nemitz A.	2015 b	Validation of the analytical method APL0687/01: Determination of Cinmethylin in Technical Grade Active Ingredient (TGAI) by means of GC 2015/1174458 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/1	Ertunc T. et al.	2017 a	Validation of analytical method L0308/01 for the determination of BAS 684 H enantiomers in soil and sediment 2017/1004384 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/2	Wallace D.	2017 a	Large outdoor wind tunnel study to evaluate volatilisation, short range transport and deposition of volatilised BAS 684 H (applied as EC formulated product) as a function of distance from the treated area (0-20 m) 2017/1192649 RLP AgroScience GmbH, Neustadt/Weinstrasse, Germany Fed.Rep. yes Unpublished	No	Yes	Not applicable-study evelauted in this section.	BASF	
KCA 4.1.2/3	[redacted]	1984 a	Five week dietary feeding study of sd95481 technical in dogs CI-420-004 [redacted] no	Yes	No	Not applicable	BASF	None

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
			Unpublished					
KCA 4.1.2/4	[redacted]	1983 a	Subchronic feeding study of sd95481 in the rat. Volume I CI-425-001 [redacted] yes Unpublished	Yes	Yes	Data for first approval	BASF	None
KCA 4.1.2/5	[redacted]	1983 a	Subchronic feeding study of sd95481 in the mouse CI-425-002 [redacted] yes Unpublished	Yes	Yes	Data for first approval	BASF	None
KCA 4.1.2/6	[redacted]	1987 a	13 week dietary feeding study in beagle dogs of cinch herbicide technical CI-425-003 [redacted] yes Unpublished	Yes	Yes	Data for first approval	BASF	None
KCA 4.1.2/7	[redacted]	1985 a	A one year dietars feeding study in dogs - sd95481 technical CI-427-002 [redacted] yes Unpublished	Yes	Yes	Data for first approval	BASF	None
KCA 4.1.2/8	[redacted]	1988 a	One year dietary feeding study in beagle dogs of cinch herbicide CI-427-003 [redacted] yes Unpublished	Yes	Yes	Data for first approval	BASF	None
KCA 4.1.2/9	[redacted]	1988 b	Cinch herbicide: reversibility of toxicity in beagle dogs (a 12 month feeding with 6 months reversibility) CI-427-004 [redacted] yes Unpublished	Yes	Yes	Data for first approval	BASF	None
KCA 4.1.2/10	[redacted]	1985 a	A 2 year feeding study of sd95481 in rats (volume 1 of 8) CI-427-001 [redacted]	Yes	No	Not applicable	BASF	None

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
			no Unpublished					
KCA 4.1.2/11	[redacted]	1986 a	Oncogenicity study of sd95481 in the mouse CI-428-001 [redacted] no Unpublished	Yes	No	Not applicable	BASF	None
KCA 4.1.2/12	[redacted]	1984 a	Cinch herbicide sd95481 teratology study in sprague dawley rats CI-432-001 [redacted] no Unpublished	Yes	No	Not applicable	BASF	None
KCA 4.1.2/13	Catchpole G., Hidding B.	2017 a	BAS 684 H (Cinmethylin) - Validation of an analytical method for the analysis of BAS 684 H in Isopropanol using GC-FID (control procedure 14/0066_07) 2017/1032967 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/14	Daum A.	2017 a	Analytical report BAS 684 H (Cinmethylin) - Concentration control analyses in paraffin 2017/1145822 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/15	[redacted]	2015 a	BAS 684 H (Cinmethylin) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet 2015/1076329 [redacted] yes Unpublished	Yes	Yes	Data for first approval	BASF	None
KCA 4.1.2/16	[redacted].	2016 a	BAS 684 H (Cinmethylin) - Repeated-dose 28-day toxicity study in C57BL/6JRj mice - Administration via the diet 2014/1162710 [redacted]. yes Unpublished	Yes	Yes	Data for first approval	BASF	None

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
KCA 4.1.2/17	Catchpole G., Hidding B.	2017 b	BAS 684 H (Cinmethylin) - Validation of an analytical method for the analysis of BAS 684 H in Ground Kliba maintenance diet mouse/rat GLP meal using GC (control procedure 14/066_2) 2017/1123754 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/18	Grauer E., Hidding B.	2017 a	Validation of an analytical method for the analysis of BAS 684 H (Cinmethylin) in corn oil using GC (control procedure 14/0066_05-02) 2017/1067141 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/19	Catchpole G., Hidding B.	2018 a	BAS 684 H (Cinmethylin) - Stability analysis in acetone 2018/1013043 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/20	Grauert E., Hidding B.	2017 a	BAS 684 H (Cinmethylin) - Validation of an analytical method for analysis of BAS 684 H in 1% CMC (as sodium salt) in drinking water with Tween 80 (3 drops/1000 mL) using GC (control procedure 14/0066_06-02) 2017/1166508 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/21	Catchpole G., Hidding B.	2018	BAS 684 H - Validation of an analytical method for the analysis of BAS 684 H and metabolites in rat plasma using HPLC-MS (control procedure: 14/0066_1) 2018/1037312 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/22	Spangler C. et al.	2016 a	Validation of analytical method L0337/01 for the determination of BAS 684 H residues in plant matrices by LC-MS/MS 2016/1029129 BASF SE, Limburgerhof, Germany Fed.Rep.	No	Yes	Data for first approval	BASF	None

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
			yes Unpublished					
KCA 4.1.2/23	Spangler C.	2018 b	Amendment 1: Validation of analytical method L0337/01 for the determination of BAS 684 H residues in plant matrices by LC-MS/MS 2018/1044640 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/24	Castro M.	2018 a	Validation BASF Method L0337/02 for the determination of M684H005 (Reg.No.6067256) and M684H006 (Reg.No. 6067258) in citrus fruit, dry beans seed, sunflower seeds, lettuce heads, wheat grain, wheat (whole plant) and wheat straw by LC-MS/MS 2018/3000081 BASF SA, Guaratingueta, Brazil yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/25	Rabe U., Forieri I.	2017 a	Investigation of the extractability of BAS 684 H in samples from 14C plant metabolism studies 2017/1166468 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/26	[redacted]	1983 a	Acute toxicity of technical sd95481 to bluegill sunfish lepomis macrochirus CI-511-002 [redacted] no Unpublished	No	No	Not applicable	BASF	None
KCA 4.1.2/27	[redacted]	1983 b	Acute toxicity of technical sd95481 to rainbow trout salmo gairdneri CI-511-003 [redacted], no Unpublished	No	No	Not applicable	BASF	None
KCA 4.1.2/28	Forbis A. et al.	1983 c	Acute toxicity of sd95481 to daphnia magna CI-521-001	No	No	Not applicable	BASF	None

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
			ABC - Analytical Bio-Chemistry Laboratories Inc., Columbia MO, United States of America no Unpublished					
KCA 8.2.1/9	[redacted]	1983 a	Dynamic acute toxicity of sd95481 to bluegill sunfish lepomis macrochirus CI-512-001 [redacted] GLP: no Unpublished	No	No	Not applicable	BASF	None
KCA 4.1.2/29	[redacted]	1983 a	Uptake, depuration and bioconcentration of 14c sd95481 by bluegill sunfish lepomis macrochirus CI-690-004 [redacted] no Unpublished	No	No	Not applicable-study not used in risk assessment	BASF	
KCA 4.1.2/30	[redacted]	1990 a	WL95481 (Argold): An early life stage test with the fathead minnow (Pimephales promelas) RAFINESQUE CI-512-002 [redacted] yes Unpublished	Yes	Yes	Data for first approval	BASF	None
KCA 4.1.2/31	Pearson N., Stephenson R.R.	1987 a	WL95481: Acute toxicity to selenastrum capricornutum CI-521-005 Shell Research Ltd., Sittingbourne Kent ME9 8AG, United Kingdom yes Unpublished	No	Yes	Not applicable-study not used in risk assessment	BASF	
KCA 4.1.2/32	Pearson N., Stephenson R.R.	1987 b	WL95481: Acute toxicity to Gammarus pulex, Lymnaea stagnalis, Tubifex tubifex and Chironomus lugubris CI-521-006 Sittingbourne Research Centre, Sittingbourne Kent ME9 8AG, United Kingdom yes Unpublished	No	Yes	Data for first approval	BASF	None

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
KCA 4.1.2/33	Pearson N., Girling A.	1989 a	WL95481: Chronic toxicity to Daphnia magna CI-523-001 Sittingbourne Research Centre, Sittingbourne Kent ME9 8AG, United Kingdom yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/34	[redacted]	1988 a	Cineole alcohol: Acute toxicity to rainbow trout Salmo gairdneri and Daphnia magna CI-570-001 [redacted] yes Unpublished	Yes	Yes	Data for first approval	BASF	None
KCA 4.1.2/35	Lockard L.A. et al.	2016 a	Analytical method verification for the determination of BAS 684 H in avian diet 2016/7001370 Wildlife International Ltd., Easton MD, United States of America yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/36	Lockard L.A., Martin K.H.	2017 a	Amended final report - Analytical method verification for the determination of BAS 684 H in avian diet 2017/7017248 Wildlife International Ltd., Easton MD, United States of America yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/37	Grande A.	2017 a	Validation of BASF method L0378/01 for the determination of BAS 684 H and its metabolites M684H001 and M684H004 by LC/UV 2017/1156774 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 8.2.5.1/2	Rzodeczko H.	2017 b	BAS 684 H - Daphnia magna reproduction test 2017/1000684 Institute of Industrial Organic Chemistry, Pszczyna, Poland GLP: yes Unpublished	No	Yes	Data for first approval	BASF	None

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
KCA 8.2.7/3	Rzodeczko H.	2017 c	BAS 684 H - Water-sediment Myriophyllum spicatum toxicity test 2017/1000221 Institute of Industrial Organic Chemistry, Pszczyna, Poland GLP: yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 8.2.7/4	Rzodeczko H.	2018 a	BAS 684 H, water-sediment Elodea canadensis toxicity test 2017/1000222 Institute of Industrial Organic Chemistry, Pszczyna, Poland GLP: yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 8.2.7/5	Rzodeczko H.	2017 d	BAS 684 H - Water-sediment Egeria densa toxicity test 2017/1000224 Institute of Industrial Organic Chemistry, Pszczyna, Poland GLP: yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 8.2.1/3	[redacted]	2017 a	BAS 684 H - Carp, acute toxicity test 2016/1063240 [redacted] GLP: yes Unpublished	Yes	Yes	Data for first approval	BASF	None
KCP 10.2.1/1	[redacted]	2017 a	BAS 684 03 H - Common carp, acute toxicity test 2017/1106099 [redacted] GLP: yes Unpublished	Yes	Yes	Data for first approval	BASF	None
KCP 10.2.1/3	Turek T.	2017 a	BAS 684 03 H - Daphnia magna, acute immobilisation test 2017/1106098 Institute of Industrial Organic Chemistry, Pszczyna, Poland GLP: yes Unpublished	No	Yes	Data for first approval	BASF	None
KCP 10.2.1/4	Turek T.	2017 b	BAS 684 03 H - Pseudokirchneriella subcapitata SAG 61.81, growth inhibition test 2017/1106097 Institute of Industrial Organic Chemistry, Pszczyna, Poland	No	Yes	Data for first approval	BASF	None

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			GLP: yes Unpublished					
KCP 10.2.1/5	Rzodeczko H.	2017 a	BAS 684 03 H - Lemna gibba CPCC 310 growth inhibition test 2017/1013180 Institute of Industrial Organic Chemistry, Pszczyna, Poland GLP: yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 8.2.4.1/4	Turek T.	2018 a	Reg. No. 6055521 (Metabolite of BAS 684 H, M684H001) Daphnia magna, acute immobilisation test 2017/1069818 Institute of Industrial Organic Chemistry, Pszczyna, Poland GLP: yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 8.2.7/6	Rzodeczko H.	2017 e	Reg.No. 6055521 (metabolite of BAS 684 H, M684H001) - Lemna gibba CPCC 310 growth inhibition test 2016/1224989 Institute of Industrial Organic Chemistry, Pszczyna, Poland GLP: yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 8.2.7/8	Rzodeczko H.	2017 f	Reg.No. 6055480 (metabolite of BAS 684 H, M684H004) - Lemna gibba CPCC 310 growth inhibition test 2016/1224988 Institute of Industrial Organic Chemistry, Pszczyna, Poland GLP: yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/38	Friedemann A., Stroemel C.	2017 a	Effect of BAS 684 03 H on vegetative vigour of ten species of terrestrial plants under greenhouse conditions 2017/1134475 Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/39	Friedemann A., Stroemel C.	2018 a	Effect of BAS 684 03 H on seedling emergence and seedling growth of ten species of terrestrial plants under greenhouse conditions 2017/1134474	No	Yes	Data for first approval	BASF	None

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			Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep. yes Unpublished					
KCA 4.1.2/40	Andre M.	2017 a	Validation of BASF Method L0361/01 for the determination of pesticides in water by LC-MS/MS 2017/1065621 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed. Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCP 10.2.1/6	Janson G.-M.	2017 a	Effect of BAS 684 03 H on the growth of the aquatic plant Glyceria maxima 2017/1000861 BASF SE, Limburgerhof, Germany Fed.Rep. GLP: yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 8.2.3/1	[redacted]	2020a	BAS 684 H - Amphibian Metamorphosis Assay with African Clawed Frog (*Xenopus laevis*) [redacted] yes Unpublished	Yes	Yes	Data for first approval	BASF	None
KCA 8.2.3/2	[redacted]	2020	Zebrafish (*Danio rerio*) - Short term reproduction assay, Flow through conditions [redacted]. yes Unpublished	Yes	Yes	Data for first approval	BASF	None
KCA 4.1.2/41	Grande A.	2017 b	Validation of BASF Method L0382/01 for the determination of M684H003 in water and 20xAAP medium by GC-FID 2017/1156775 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 8.2.4.1/5	Turek T.	2018 b	Reg.No. 4539586 (Metabolite of BAS 684 H, M684H003) - Daphnia magna, acute Immobilisation test 2017/1069817 Institute of Industrial Organic Chemistry, Pszczyna, Poland GLP: yes	No	Yes	Data for first approval	BASF	None

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			Unpublished					
KCA 8.2.7/7	Turek T.	2018 c	Reg.No. 4539586 (Metabolite of BAS 684 H, M684H003) - Lemna gibba CPCC 310 growth inhibition test 2017/1032136 Institute of Industrial Organic Chemistry, Pszczyna, Poland GLP: yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/42	Catchpole G., Hidding B.	2017 c	BAS 684 H (Cinmethylin) - Validation of an analytical method for the analysis of BAS 684 H in test water using HPLC-MS (control procedure 14/0066_08-02) 2017/1047671 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/43	Kleebaum K.	2016 a	Repeated exposure of BAS 684 H to honey bee (Apis mellifera) larvae under laboratory conditions (in vitro) 2016/1044854 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 8.3.1.1.1/2	Amsel K.	2017 a	Acute toxicity of BAS 684 H to the bumblebee Bombus terrestris L. under laboratory conditions 2017/1140992 BioChem agrar GmbH, Gerichshain, Germany Fed.Rep. GLP: yes Unpublished	No	Yes	Data for first approval	BASF	None
KCP 10.3.1.3/1	Kleebaum K.	2017 a	Repeated exposure of honey bee (Apis mellifera) larvae to BAS 684 03 H under laboratory conditions (in vitro) 2017/1036677 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP: yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/44	Haerthe N.	2016 a	Acute toxicity of BAS 684 H (Cinmethylin) to Daphnia magna STRAUS in a 48 hour static test 2016/1001943	No	Yes	Data for first approval	BASF	None

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			BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished					
KCA 8.2.6.1/2	Kauf A.	2017 a	Effect of BAS 684 H (Reg.No.: 900202) on the growth of the green alga Pseudokirchneriella subcapitata 2016/1001944 BASF SE, Limburgerhof, Germany Fed.Rep. GLP: yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 8.2.7/2	Vlechev S.	2017 b	Effects of BAS 684 H on the growth of the aquatic plant Glyceria maxima 2015/1029520 BASF SE, Limburgerhof, Germany Fed.Rep. GLP: yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/45	Kauf A.	2017 a	Effect of BAS 684 H (Reg.No.: 900202) on the growth of the blue alga Anabaena flos-aquae 2016/1001945 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.1.2/46	Vlechev S.	2017 a	Effect of BAS 684 H on the growth of Lemna gibba 2015/1029521 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 2.5/1	Daum A.	2017a	Water solubility of Cinmethylin (BAS 684 H) pure active ingredient (PAI) 2017/1077867 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.2/1	Bodsch J.	2018 a	Independent laboratory validation of BASF method L0337/01 for the determination of BAS 684 H residues in plant matrices by LC-MS/MS 2017/1202457 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed. Rep. yes	No	Yes	Data for first approval	BASF	None

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			Unpublished					
KCA 4.2/2	Asekunowo J.	2018 a	Validation of BASF analytical method L0385/01 for the determination of BAS 684 H in animal matrices 2017/1202142 EAG Laboratories GmbH, Ulm, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.2/3	Ford K.	2018 a	Independent laboratory validation of BASF analytical method L0385/01 for the determination of BAS 684 H in animal matrices 2017/1202456 CEMAS - CEM Analytical Services Ltd., Workingham Berkshire RG41 2FD, United Kingdom yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.2/4	[redacted]	2018 a	Investigation of the extractability of BAS 684 H in liver from a 14C goat metabolism study (enforcement methods) 2017/1192630 [redacted] yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.2/5	Obermann M., Arndt S.	2018 a	Validation of Analytical Method L0366/01 for the Enantiomers Reg. No. 5925632 and Reg. No. 5925581 of BAS 684 H in Water by reversed-phase chiral LC-MS/MS 2017/1194948 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.2/6	Obermann M., Arndt S.	2018 c	Validation of analytical method L0366/02 for the determination of Metabolites M684H001 (Reg.No. 6055521) and M684H004 (Reg.No. 6055480) in drinking (ground) and surface-water by LC-MS/MS 2018/1011310 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.2/7	Joos S., Tussetschlaege	2017 a	Independent laboratory validation of the methods L0366/01 and L0366/02 for the determination of BAS 684 H (Reg.No. 5925581 and 5925632) and metabolites M684H001 (Reg.No. 6055521) and	No	Yes	Data for first approval	BASF	None

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	r S.		M684H004 (Reg.No. 6055480) in surface and groundwater 2017/1223471 EAG Laboratories GmbH, Ulm, Germany Fed.Rep. yes Unpublished					
KCA 4.2/8	Obermann M., Arndt S.	2018 b	Validation of Analytical Method L0371/01 for the determination of BAS 684 H (Reg.No. 900202) in air using LC-MS/MS 2017/1210714 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.2/9	Ivanov E., Bruhn F.	2018 a	Validation of BASF analytical methods L0387/01 for the determination of BAS 684 H and its metabolite M684H011in body fluids 2017/1202143 Eurofins Agroscience Services Chem GmbH, Hamburg, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.2/10	Spangler, C	2020	Validation of BASF analytical method L0337/03 for the determination of BAS 684 H (Reg. No. 900202) in honey by LC-MS/MS. 2020/2002775 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first approval	BASF	None
KCA 4.2/11	Link T., Walsch M.	2020	Independent Laboratory Validation of BASF analytical method L0337/03 for the determination of BAS 684 H in honey by LC-MS/MS. 2020/2004119 SGS Institut Fresenius, GmbH yes Unpublished	No	Yes	Data for first approval	BASF	None