Renewal Assessment Report

beta-cyfluthrin

Volume 3 – B.5 Methods of analysis

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Version history

When	What

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B.5 Methods of analysis

Introduction
Beta-cyfluthrin and also cyfluthrin are insecticides which belong to the pyrethroid group. They are basically the same molecule which possesses three stereochemical centres resulting in eight possible isomers. All eight isomers can be grouped as four diastereomeric pairs, each pair consisting of two enantiomers.
Both active substances differ in their relative amount of isomers. While cyfluthrin is a mixture of all four diastereomeric pairs I to IV, the product beta-cyfluthrin only consists of the more active isomers II and IV. Due to the fact that the beta-cyfluthrin isomers are contained in cyfluthrin, the analytical methods can be regarded as being valid for cyfluthrin and vice versa.

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

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

B.5.1.1.1 Methods for the determination of pure active substance in the active substance as manufactured

References:
Sonnenschein (2014), Determination of the diastereomeric purity of beta-cyfluthrin (AE 1430672 / FCR4545) in technical grade and pure active substance by high performance liquid chromatography (HPLC), M-475685-01-1, Bayer CropScience (BVL no 2632908)
Sonnenschein (2013), Validation of the analytical method AM038113FP1: Determination of the diastereomeric purity of beta-cyfluthrin / AE 1430672 (FCR4545) in technical grade and pure beta-cyfluthrin by high performance liquid chromatography (HPLC), M-474367-01-1, Bayer CropScience (BVL no 2632909)

The method is AM038113FP1 is used for the determination of the diastereomeric purity of beta-cyfluthrin. With this method the content of the cyfluthrin diastereomers diastereomer I (AE 1421341), diastereomer II (AE 1421342), diastereomer III (AE 1421343) and diastereomer IV (AE 1421344) can be determined in pure and technical grade beta-cyfluthrin.

Principle of the method AM038113FP1:
After homogenisation, the technical material is dissolved in methyltertbutylether and made up to volume with n-heptane. The diastereomeric purity of beta-cyfluthrin is determined by reverse phase high performance liquid chromatography (HPLC). Quantification is made by comparison of the peak area with an external standard.

Column:	LiChrospher Si 60, 250 mm x 4.0 mm, 5 µm
Mobile phase:	n-heptane/ methyltertbutylether 96:4 (v/v)
Detector wavelength:	235 nm

Findings:

Table B.5.1-1: Validation data for the determination of beta-cyfluthrin in the technical material

	Linearity (linear between), Corr. Coeff.	**Precision - repeatability (%RSD)**	**Interference**
Diastereomer I (HPLC)	1.4 mg/L – 83.1 mg/L 0.999848	2.2 (mean content 0.3 %)	demonstrated; no interferences with diastereomers II and IV
Diastereomer II (HPLC)	74.5 mg/L – 1624.0 mg/L 0.999259	2.0 (mean content 36.6 %)	
Diastereomer III (HPLC)	1.5 mg/L – 76.3 mg/L 0.998594	2.1 (mean content 1.4 %)	
Diastereomer IV (HPLC)	71.9 mg/L – 1540.6 mg/L 0.999263	1.6 (mean content 60.5 %)	

The specificity of the method was demonstrated by retention time match and by comparison of the UV-spectra with reference standard.

Interferences from several potential impurities including those specified in the original DAR, Vol. 4 Addendum 2 (with the exception of AE 1821465) were investigated at a concentration of 2 %. The impurities AE F112323, AE 1344197, AE F054157 and AE 0452478 showed chromatographic interference with different diastereomers. Since these impurities and the not investigated impurity AE 1821465 are all well below 1 g/kg in the technical material no interference is expected with the active substance (diastereomers II and IV).

For evaluation of this method for diastereomer I and III see Volume 4.

Conclusion:
The method is acceptably validated and allows the determination of beta-cyfluthrin and the diastereomeric purity in the technical material.

CIPAC method:
There is a CIPAC method (482/TC/M/-) available for the determination of diastereomeric purity of beta-cyfluthrin in technical grade active substance.

B.5.1.1.2 Methods for the determination of relevant impurities in the active substance as manufactured

Not relevant as there is no relevant impurity in the technical beta-cyfluthrin originating from Bayer CropScience AG manufacturer.

B.5.1.2 Methods for risk assessment

B.5.1.2.1 Analytical methods used in residue studies – Part A: Field trials

The considered residue definition for risk assessment includes cyfluthrin and other mixtures of constituent isomers (sum of isomers). The residue definition for risk assessment used in the original DAR (1996, ASB2010-10436), which was prepared by Germany, does not differ from the considered residue definition in this RAR.
All methods accepted in the DAR do not longer fulfil requirements of SANCO/825/00 rev. 8.1. Provided new methods are reported below.

Method 1, code/number 922

Data point: KCA 4.1.2/80

Author (year): Schöning (2005)

Title/report number: Analytical method 00922 for the determination of residues of beta-cyfluthrin in/on plant material by HPLC-MS/MS
Method No: 00922, Edition No.: M-244829-01-1, MET2006-93

The method 1 is used in following studies:

Table B.5.1-2: Field trials, which are using method 1 (code/number 922)

Data point:	Author (year):	Title/report number:
KCA 4.1.2 /81 KCA 6.3.1 /12	Schöning, R.; Reineke, A. (2011)	Determination of the residues of beta-cyfluthrin and Imidacloprid in/on sugar beet after seed treatment of Montur Forte FS 230 in the field in Germany and the Netherlands; ASB2012-4627

Principle of the method, validation results and conclusion:

The method is also accepted as monitoring method. For details see section B.5.2. Residue data obtained with this method are reliable.

Method 2, code/number 00086/DFG S19

Data point: KCA 4.2/89

Author (year): Weber, H., 2009

Title/report number: Validation of enforcement method DFG S 19 (L 00.00-34) (BCS method ID 00086/M088) for the determination of residues of cyfluthrin (AE F057122) in/on plant materials
Report No: Specht File Reference: G08-0201, EASSM No.: S09-00092
Edition No.: M-347371-01-1, ASB2014-2283

The method 2 is used in following studies:

Table B.5.1-3: Field trials, which are using method 2 (code/number 00086/DFG S 19)

Data point:	Author (year):	Title/report number:
KCA 6.3.3 /15	Lebrun (2013)	Magnitude of the residue of beta-cyfluthrin in potato (raw agricultural commodity) after two applications of Bulldock 25 EC - two decline curve trials and two trials in northern Europe (northern France, United Kingdom and Germany) – 2012, Report No.: 12SGS078, Edition Number: M-481204-01-1; ASB2014-6718
KCA 6.3.4 /28	Bousquet (2013)	Magnitude of the residue of beta-cyfluthrin in winter wheat (raw agricultural commodity) after two applications of Bulldock 25 EC - 4 harvest trials in Northern Europe (Northern France, United Kingdom and Germany) – 2012, Report No.: 12SGS096, Edition Number: M-481205-01-1; ASB2014-6714
KCA 6.6.2/04	Chevallier (2013)	Magnitude of the residue of beta-cyfluthrin in succeding crops after application of Bullock 25 EC in two trials in southern Europe (southern France and Spain) - 2012 Report No.: 12SGS117, Edition Number: M-481208-01-1, ASB2014-7884

Principle of the method, validation results and conclusion:

The method is also accepted as monitoring method. For details see section B.5.2. The only deviation is the use of negative ion chemical ionisation instead of electron impact ionisation for MS. Residue data obtained with this method are reliable.

Method 3, code/number 00255

Data point: KCA 4.1.2 /35, KCA 4.2 /77

Author (year): Ohs, P., 1992

Title/report number: Method for the gas chromatographic determination of residues of the insecticidal compounds beta-cyfluthrin and Cyfluthrin in plant materials and their processed products by online LC-GC-coupling
Report No.: MO-03-011300, Edition Number: M-109494-01-1, RIP9400739

The method 3 is used in following studies:

Table B.5.1-4: Field trials, which are using method 3 (code/number 00255)

Data point:	Author (year):	Title/report number:
KCA 6.3.3 /14	Heinemann, O.; Seym, M. (1998)	Determination of residues of Enduro 258 EC in/on potato following spray application in the field in France, ASB2014-7858
KCA 6.3.3 /13	Heinemann, O.; Seym, M. (1998)	Determination of residues of Enduro 258 EC in/on potato following spray application in the field in Germany, Great Britain and France, ASB2014-6706
KCA 6.3.3 /11	Seym, M. (1997)	Determination of residues of Bulldock 025 EC and Bulldock 025 SC on potato in Portugal and Spain, ASB2014-6702
KCA 6.3.3 /12	Heinemann, O.; Schöning, R. (2002)	Determination of residues of beta-cyfluthrin in/on Potato after spray application of Bulldock 025 SC in Greece (2001), RIP2003-272
KCA 6.3.4 /27	Heinemann, O.; Schöning, R. (2001)	Determination of residues of beta-cyfluthrin on wheat following spray treatment of Bulldock 025 EC in Italy, Spain and France - incl. Amendment 1 vom 13.11.2001, RIP2003-275

Principle of the method:

Samples with high water content and dry sample materials (after addition of water) are homogenized in acetone. The obtained acetone/water ratio is 2/1 (v/v). Extracted residues are partitioned into dichloromethane and the dichloromethane phase is evaporated to dryness. The dry residue of samples with high oil content is dissolved in acetonitrile and the solution is washed with hexane. The defatted acetonitrile solution is again evaporated to dryness. Finally, dry residues are dissolved in a mixture of methyl-tertiary butyl ether/hexane (7/93, v/v). For final determination of residues of beta-cyfluthrin a combination of normal phase liquid chromatography (Spherisorb SI column) and gas chromatography (Ultra 1 column) with electron capture detection is used.

Table B.5.1-5: Validation results of the method 3 for residues of beta-cyfluthrin in samples of plant origin

Reference	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses	Selectivity demonstrated?	Calibration valid?
Ohs (1992, RIP9400739)	peas (sseds)	0.01 0.10	98 115	3.8 9.2	6 5	yes	yes
	barley/oats/ wheat grain	0.01 0.04	89 107	9.0 10	6 9	yes	yes
	potato (tuber)	0.01 0.10	93 112	3.2 3.7	3 3	yes	yes
	sugar beet root	0.01 0.10	115 105	5.7 7.2	9 8	yes	yes

Conclusion

In accordance to SANCO/3029/99 rev 4 the method is successfully validated for crops with high water content and cereal grains. Residue data obtained with this method are reliable.

Method 4, code/number 01379

Data point: KCA 4.1.2 /82

Author (year): Uceda, L.(2013)

Title/report number: Analytical method 01379 for the determination of cyfluthrin in/on sugarbeet (leaf with root collar and body) by HPLCMS/MS – Cyfluthrin
Report No.: 01379, Edition Number: M-463052-01-1, ASB2014-7710

The method 4 is used in following studies:

Table B.5.1-6: Field trials, which are using method 4 (code/number 01379)

Data point:	Author (year):	Title/report number:
KCA 6.3.1 /11	Meilland-Berthier, I. 2013)	Determination of the residues of Cyfluthrin in/on sugarbeet after seed treatment with Montur Forte FS 230 in Germany, Northern France, United Kingdom, Spain, Italy, Greece and Southern France ASB2014-7883

Principle of the method:

Cyfluthrin is extracted from sugar beet (leaf with root collar) and sugar beet (body) with a mixture of acetone/water (2/1, v/v) using a blender. After filtration, the extract is partitioned against dichloromethane. The organic phase is concentrated to dryness, redissolved in dichloromethane and further cleaned-up on a Florisil column cartridge. The eluate is evaporated to dryness and dissolved in a mixture of acetonitrile/water (50/50; v/v). The extracts are diluted in a mixture of acetonitrile/water (1/2; v/v) by adding the internal standard. The sample concentration in final extracts is 0.04 g/mL. The concentration of cyfluthrin in final extracts is quantified by LC-MS/MS using a C18 column, positive/negative electrospray ionisation and two MRM transitions (m/z 451→191, m/z 451→127). m/z 451 corresponds to the [M+NH4] ion. Cyfluthrin results in two incompletely resolved LC peaks.

Table B.5.1-7: Validation results of the method 4 for residues of cyfluthrin in samples of plant origin

Reference	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses	Selectivity demonstrated?	Calibration valid?
Uceda (2013) ASB2014-7710 m/z 451→191	Sugarbeet leaf with root collar	0.01 0.10	78 79	13 2.5	5 5	yes	yes
	Sugarbeet body	0.01 0.10	87 84	2.6 7.5	5 5	yes	yes
Uceda (2013) ASB2014-7710 m/z 451→127	Sugarbeet leaf with root collar	0.01 0.10	interference 83	 11	5 5	yes	yes
	Sugarbeet body	0.01 0.10	95 84	9.7 9.4	5 5	yes	yes

Conclusion

In accordance to SANCO/3029/99 rev 4 the method is successfully validated for crops with high water content. Residue data obtained with this method are reliable.

Method 5

Data point: KCA 4.1.2 /87

Author (year): Schäufele, M., 2012

Title/report number: Bulldock 25 EC: Magnitude of residues of beta-Cyfluthrin in greenhouse tomato raw agricultural commodity and processed fractions after 2 applications of Bulldock 25 EC - 2 trials - in Northern Europe (Germany) in 2011, Report No.: M-481079-01-1, Edition Number: M-481079-01-1, ASB2014-7712

The method 5 is used in following studies:

Table B.5.1-8: Field trials, which are using method 5

Data point:	Author (year):	Title/report number:
KCA 4.1.2 /83	Schäufele, M. (2012)	Bulldock 25 EC and Bulldock 25 CS - Magnitude of residues of beta-cyfluthrin in greenhouse tomatoe raw agricultural commodity after 2 applications of Bulldock 25 EC or Bulldock 25 CS - 8 decline trials - in northern and southern Europe (the Nederlands, Germany, Northern France, Greece, Spain and Italy) in 2011, Report No.: JDV0077, Edition Number: M-481199-01-1; ASB2014-7711

Principle of the method:

Samples are homogenised in acetonitrile in the presence of solid NaCl. After centrifugation the supernatant is filtered through a C18 SPE cartridge. The filtrate is dried with sodium sulfate and evaporated near to dryness. The residue is reconstituted in acetonitrile/toluene (3/1, v/v) and filtered through a combination of an ENVI-Carb (upper) and NH2-SPE (lower) cartridge. To complete elution, further acetonitrile/toluene (3/1, v/v) is used. After evaporation of most solvent, the extract is reconstituted in toluene. The sample concentration in final extracts is 0.4 g/mL. The concentration of beta-cyfluthrin in final extracts is quantified by GC-MS using a DB-5 column, in negative chemical ionisation mode with selected ion monitoring of m/z = 207.

Table B.5.1-9: Validation results of the method 5 for residues of beta-cyfluthrin in samples of plant origin

Reference	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses	Selectivity demonstrated?	Calibration valid?
Schäufele (2012) ASB2014-7712	tomato fruit	0.01 0.10	99 81	14 6.8	5 5	yes	yes
	canned tomato	0.01 0.10	94 100	1.8 4.6	3 3	yes	yes
	tomato juice	0.01 0.10	90 87	8.2 8.7	3 3	yes	yes
	tomato puree	0.01 0.10	77 80	9.2 5.2	3 3	yes	yes

Conclusion

In accordance to SANCO/3029/99 rev 4 the method is successfully validated for crops with high water content. Residue data obtained with this method are reliable.

Method 6, code/number 00015

Data point: KCA 4.1.2 /31

Author (year): Seym, M., 1992

Title/report number: Modification M010 of method 00015: Method on the gaschromatographic determination of cyfluthrin residues in plant material, beer, and sugar with an electron capture detector (ECD), Report No.: 00015/M010, Edition Number: M-007892-02-1, Method Report No.: I640, Method Report No.: RA-318/92, RIP9500545

The method 6 is used in following studies:

Table B.5.1-10: Field trials, which are using method 6 (code/number 00015)

Data point:	Author (year):	Title/report number:
KCA 6.3.4 /08	Seym, M. (1993)	Determination of Residues of FCR 4545 125 SC in/on Common Oat, Spring Barley and Spring Wheat under Actual Use Conditions in the Federal Republic of Germany, RIP9500526, RIP9500587
KCA 6.3.4 /10	Seym, M. (1993)	Determination of residues of FCR 4545 125 SC in/on spring barley, spring wheat and common oat under actual use conditions in Germany, RIP9500530, RIP9500588

Principle of the method:

Beta-cyfluthrin is extracted from plant material with acetonitrile. The acetonitrile extract is defatted with hexane and evaporated to dryness. The dry residue is redissolved in methylene chloride and the solution is purified on a Florisil column. The eluate is evaporated to dryness and reconstituted in cyclohexane. The concentration of beta-cyfluthrin in final extracts is quantified by GC-ECD.

Table B.5.1-11: Validation results of the method 6 for residues of cyfluthrin in samples of plant origin

Reference	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses	Selectivity demonstrated?	Calibration valid?
Seym (1992) RIP9500545	wheat, oats, barley grain	0.02 0.20 1.00	94 98 97	8.4 8.5 9.7	6 6 6	yes	yes

Conclusion

In accordance to SANCO/3029/99 rev 4 the method is successfully validated for dry crops. Residue data obtained with this method are reliable.

Method 7, code/number 00223 (Mobay method 85823)

Data point: KCA 4.1.2 /08

Author (year): Harbin, A. M.; Minor, R. G.; Freeseman, P. L.; Pfankuche, L. K., 1983

Title/report number: A gas chromatographic method for Baythroid residue in crops; Supplements E001 - E032; Modifications M001 - M044 of method 00223 (1160 pages!)
Report No.: MO-03-011091, Edition Number: M-108507-01-1, ASB2009-9418, RIP9400735, ASB2016-1500

The method 7 is used in following studies:

Table B.5.1-12: Field trials, which are using method 7 (code/number 00223; Mobay method 85823)

Data point:	Author (year):	Title/report number:
KCA 6.6.2 /02	Leslie, W. L. (1988)	Baythroid R - residues in field rotational crops: field, Report No.: MR98429, Edition Number: M-067638-01-1, RIP9400845
KCA 6.5.3 /10	Leslie, W. L. (1988)	Baythroid - Magnitude of the residue on tomato processed products Report No.: 98399, Report includes Trial Nos.: RTX-F2060-83P Edition Number: M-136610-01-1, EPA MRID No.: 41001615, RIP9401061

Principle of the method:

Beta-cyfluthrin is extracted from plant material with methanol/water (original method), chloroform/acetone (appendix III), methanol/water + methanol + acetonitrile (modification M001), acetonitrile (modification M002) or acetone/hexane (modification M018). In the original method the raw extract is evaporated to the aqueous residue, partitioned between aqueous acetone and chloroform, evaporated to dryness, defatted with hexane/acetonitrile and finally purified on a Florisil column.
Alternative or additional clean-up procedures are described in modifications M001, M002, M006, M018, M022, M028, M036, and M038.
In the validation studies (supplements E001 – E027), the concentration of beta-cyfluthrin in final extracts is mostly quantified by GC with conductivity detectors (Coulsen™ detector or Hall™ detector). By contrast, most modifications use an ECD detector.
As a consequence of this diversity of procedures used, method 00223 (Mobay method 85823) cannot be considered as a unique method. Therefore, reporting of validation data is focused on concurrent recovery data described in field trial reports.

Table B.5.1-13: Validation results of the method 7 for residues of cyfluthrin in samples of plant origin

Reference	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses	Selectivity demonstrated?	Calibration valid?
Leslie (1988) RIP9400845	Corn, grain and straw	0.01 0.02 0.05	86 86 97	19 16 -	4 4 2	yes	yes
	Corn, green forage	0.01 0.02 0.05	89 84 114	- - -	4 3 2	yes	yes
Leslie (1988) RIP9401061	Tomato (fruit, juice, ketchup, purée, paste, pulp)	0.10 0.50	84 88	4 18	6 13	yes	not reported

Conclusion

In accordance to SANCO/3029/99 rev 4 the modifications of the method are not successfully validated. However, considering the total amount of validation for similar matrices and identical detection (GC-ECD) the data are acceptable. Residue data obtained with this method are reliable.

Provided analytical methods, which are not used in accepted residue trials

Table B.5.1-14: List of methods, which are provided but not required

Data point:	Author (year):	Title/report number:
KCA 4.1.2 /05	Dejonckheere, W.; Verstraeten, R.; Steurbaut, W.; Melkebeke, G.; Kips, R. H. (1982)	Permethrin and deltamethrin residues on lettuce Report No.: MO-02-007002, Edition Number: M-062839-01-1 RIP9400730
KCA 4.1.2 /06	Wagner, K. (1983)	Gas-chromatographic determination of cyfluthrin in rapseed Report No.: I478, Edition Number: M-059615-01-2, RIP9400732

B.5.1.2.2 Analytical methods used in residue studies – Part B: Animal feeding

The considered residue definition for risk assessment includes cyfluthrin and other mixtures of constituent isomers (sum of isomers). The residue definition for risk assessment used in the original DAR (1996, ASB2010-10436), which was prepared by Germany, does not differ from the considered residue definition in this RAR.
All methods accepted in the DAR do not longer fulfil requirements of SANCO/825/00 rev. 8.1. Provided new methods are reported below.

Method 1, code/numbers I476 / 85883 (method used in animal feeding studies)

Data point: KCA 4.1.2/52 (KCA 6.2.2/06, 4.2/14, 6.2.3/08, 6.4.2 /01)

Author (year): [redacted] (1983)

Title/report number: An analytical method for Baythroid in bovine and poultry tissues, milk and eggs, Mobay I476, Method 85883, RIP9400740

Method 1 is used in following studies:

Table B.5.1-15: Feeding studies, which are using method 1 (code/numbers I476 / 85883)

Data point:	Author (year):	Title/report number:
KCA 6.4.1/04	[redacted] (1983)	A 28 day Baythroid poultry feeding study Report MR86046, M-060241-02-1 RIP9400721
KCA 6.4.1/03	[redacted] (1983)	Residues of Baythroid in chicken tissues. Raw data (parent in chicken tissues), Report 86033, M-062920-01-1 RIP9400718
KCA 6.4.1/06	[redacted] (1983)	Residues of Baythroid in chicken eggs. Raw data (parent in eggs), Report 86034, M-136924-01-1 RIP9400719
KCA 6.4.2/05	[redacted] (1984)	Baythroid (TM) 28 day bovine feeding study (revised 23.01.1984), Report MR86045, M-055028-02-1 RIP9400720
KCA 6.4.2/03	[redacted] (1983)	Residue of Baythroid in bovine milk. Raw data (parent in milk), Report 86040, M-062087-01-1 RIP9400716
KCA 6.4.2/04	[redacted] (1983)	Residues of Baythroid in cattle tissues. Raw data (parent in tissues), MR86039, M-062229-01-1 RIP9400717
KCA 6.4.2/09	[redacted] (1985)	Baythroid: Identity of major components in cow liver (1st revision 05.08.1985), Raw data (parent in liver and kidney) Report 88970, M-053779-01-1, RIP9400724
KCA 6.4.2/08	[redacted] (1994)	Cyfluthrin - A 28 day dairy cattle feeding study Report 106628 , M-054521-01-1 RIP9500449
KCA 6.4.2/06	[redacted] (1985)	Residue cattle feeding study (28 days). Bovine Milk. Report 90386, M-054888-01-1 RIP9400726
KCA 6.4.2/07	[redacted] (1985)	28 day residue feeding study. Cattle Tissues. Report 90387, M-054668-01-1 RIP9400725

Principle of the method:

Milk is acidified with HCl an extracted with acetone/ chloroform (2/1, v/v). The organic phase is evaporated to dryness. The dry residue is redissolved in water/methanol/ethyl acetate (25/5/90, v/v/v). The ethyl acetate phase is dried and evaporated to dryness. The dry residue is reconstituted in equal volumes of hexane and acetonitrile. After mixing and phase separation, the acetonitrile phase is evaporated to dryness. The dry residue is reconstituted in acetone/hexane (1/1, v/v) and water is added. After phase separation, the hexane phase is dried and evaporated. The dry residue is reconstituted in hexane and further purified by chromatography on silica. The eluate is evaporated and dissolved in acetone.
Muscle, liver and kidney are homogenised with acetone/chloroform (2/1, v/v) in the presence of Hyflo Super-cel. The homogenate is filtrated and the extract is evaporated to dryness. The dry residue is redissolved in water/methanol/ethyl acetate (25/5/90, v/v/v). The ethyl acetate phase is dried and evaporated to dryness. The dry residue is reconstituted in equal volumes of hexane and acetonitrile. After mixing and phase separation, the acetonitrile phase is evaporated to dryness, reconstituted in hexane and further purified by chromatography on silica. The eluate is evaporated and dissolved in acetone.
Fat and skin are homogenised with hexane in the presence of Hyflo Super-cel and sodium sulfate.

After filtration residues are partitioned into acetonitrile. The acetonitrile phase is evaporated to dryness. The dry residue is redissolved in water/methanol/ethyl acetate (25/5/90, v/v/v). The ethyl acetate phase is dried and evaporated to dryness. The dry residue is reconstituted in equal volumes of hexane and acetonitrile. After mixing and phase separation, the acetonitrile phase is evaporated to dryness and reconstituted in acetone/hexane (1/1, v/v). Water is added. After phase separation, the hexane phase is dried and evaporated. The dry residue is reconstituted in hexane and further purified by chromatography on silica. The eluate is evaporated and dissolved in acetone.
Eggs are mixed with Florisil and the free flowing powder is filled into a column. Residues are extracted with hexane/chloroform (9/1, v/v). The eluate is evaporated to dryness. The dry residue is redissolved in hexane. The hexane solution is washed with 5 % NaCl in water. Residues are partitioned into acetonitrile. The acetonitrile phase is evaporated to dryness, reconstituted in hexane and further purified by chromatography on Florisil. The eluate is evaporated and dissolved in acetone.
The sample concentration in all final extracts was 5 g/mL. Final extracts were analysed for residues of cyfluthrin by GC-ECD using a packed column with 15 % UC W98 (silicone rubber), which did not allow the separation of peaks of diastereoisomeric pairs. Residue concentrations were calculated based on one point calibration corresponding to 0.10 mg/kg.

Conclusion

In accordance to SANCO/3029/99 rev 4 the method cannot be considered as valid. Valid studies with identical extraction methods and clean-up are not presented. Therefore, any sound estimation of LOQ is not possible and reported residue concentrations <0.10 mg/kg should be considered as ≤0.10 mg/kg.

Justification:

The extent of recovery trials (number of replicates per level) is too low. The linearity of calibration and the selectivity (chromatograms of control samples) are not or inadequately demonstrated. All recovery data are obtained by single level calibration using a standard corresponding to 0.10 mg/kg. Many control samples have shown too high signal intensities at the retention time of cyfluthrin.
It could be shown by Murphy (1985, RIP9400724) that the extraction efficiency strongly depends on experimental parameters. The exchange of an Omni Mixer (applied in Morse Laboratories) by a Tekmar Tissumizer resulted in ≥ 800 % recovery of cyfluthrin from kidney and liver samples with incurred residues.

Table B.5.1-16: Validation results of the method 1 for residues of cyfluthrin in samples of animal origin[1]

Reference	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses	Selectivity demonstrated?	Calibration valid?
[redacted] (1983) RIP9400740	bovine muscle	0.05	85	-	1	no	calibration data were not presented
	poultry muscle	0.05	94	-	1	no	
	poultry gizzard	0.05	74	-	1	no	
	bovine liver	0.05	76	-	1	no	
	poultry liver	0.05	76	-	1	no	
	bovine fat	0.05	73	-	2	no	
	poultry fat	0.05	80	-	1	no	
	poultry skin	0.05	88	-	1	no	
	bovine kidney	0.05	78	-	1	no	
	poultry eggs	0.05	73	6	3	no	
	bovine milk	0.02	**112**	11	3	no	

[redacted] (1985) RIP9400741	bovine liver	0.01 0.02 0.05 0.10	90 85 86 84	- - - 4	2 1 1 3	no[2]	calibration data were not presented
	bovine kidney	0.01 0.02 0.05 0.10	80 80 90 98	- - - 6	2 1 1 3	no[3]	
	bovine muscle	0.01 0.02 0.05 0.10	90 90 86 **112**	- - - **26**	2 1 1 3	no[3]	
	bovine fat	0.01 0.02 0.05 0.10	85 70 80 87	- - - -	2 1 1 2	no[4]	
[redacted] (1985) RIP9400742	bovine milk	0.01 0.02 0.05 0.10	92 86 **111** 92	- - 20 -	1 1 14 1	no[5]	calibration data were not presented
[redacted] (1994) RIP9500449	bovine muscle	0.01 0.02 0.05	87 89 86	-	2 1 1	no	calibration data were not presented
	bovine liver	0.01 0.02 0.05	91 87 86	-	2 1 1	no[3]	
	bovine kidney	0.01 0.02 0.05	78 78 90	-	2 1 1	no[3]	
	bovine milk	0.01 0.02 0.05 0.10	92 86 82 92	-	1 1 1 1	no	

[1]) Validation data summarised by [redacted] (1983, RIP9400740) are also reported by [redacted] (1983, MET9400017, KCA 4.1.2/67), [redacted] (1983, RIP9401251, KCA 4.1.2/65) and Shaw II, Chopade, Ayers and Gentile (1983, ASB2014-12196 and RIP9401252, KCA 4.1.2/66,)
[2]) One control sample has shown 70 % of mean intensity of samples fortified with 0.01 mg/kg.
[3]) All control samples have shown >30 % of mean intensity of samples fortified with 0.01 mg/kg.
[4]) All control samples have shown >100 % of intensity of sample fortified with 0.05 mg/kg.
[5]) One control samples has shown >150 % of intensity of sample fortified with 0.02 mg/kg.

Method 2, code/number I488/86217 (method used in animal feeding studies)

Data point: KCA 4.1.2/54

Author (year): [redacted] (1983)

Title/report number: An analytical method for quantitating Baythroid metabolite residues in animal tissues, Report No.: I488, Edition Number: M-066384-01-1 RIP9400874

Method 2 is used in following studies:

Table B.5.1-17: Feeding studies, which are using method 2 (code/numbers I488 / 86217)

Data point:	Author (year):	Title/report number:
KCA 6.4.1/05	[redacted] (1983)	Baythroid metabolites in chicken tissues (muscle, fat, skin, liver, and gizzard), Report No.: MR86658, Edition Number: M-068248-01-1 RIP9400723
KCA 6.4.2/10	[redacted]	Bovine residue feeding study (28 day): Analysis of Baythroid metabolite residues (FCR 1272 metabolite : COE 538/78; cow; USA) Report No.: 86218, Edition Number: M-068788-01-1 RIP9400722

Principle of the method:

The method allows the determination of the metabolites "acid Baythroid" (ά-[[[3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropyl]carbonyl]oxy]-4-fluoro-3-phenoxybenzeneacetic acid, "FPB acid" (4-fluoro-3-phenxoybenzoic acid), "FPB alc" (4-fluoro-3-phenoxybenzenemethanol) and FPB ald (4-fluoro-3-phenoxybenazldehyde).
Samples are homogenised with acetone/chloroform (2/1, v/v) in the presence of Hyflo Super-cel. The homogenate is filtrated and the extract is evaporated to dryness. The dry residue is redissolved in water/methanol/ethyl acetate (25/5/90, v/v/v). The ethyl acetate phase is dried and evaporated to dryness. The dry residue is reconstituted in methanol/chloroform (3/24, v/v) and purified by gel permeation chromatography. The eluate is divided into two parts.
For determination of FPB acid, FPB alc und FPBald, a first part is chromatographed on silica, oxidized with permanganate (to FPB acid), partitioned into chloroform, washed with methanol/water, partitioned into aqueous bicarbonate solution; acidified with HCl, partitioned into chloroform, evaporated to dryness, derivatised with diazomethane, chromatographed on Florisil, evaporated to dryness and re-dissolved in acetonitrile. Sample concentration in final extracts is 100 g/mL.
For determination of acid BAYTHROID, a second part of GPC eluate is chromatographed on florisil, evaporated to dryness, derivatised with diazomethane, chromatographed on Florisil, evaporated to dryness and re-dissolved in hexane. Sample concentration in final extracts is 100 g/mL.
Final extracts were analysed for residues of acid Baythroid methyl ester by GC with electrolytic conductivity detector using a packed column with OV 101(methyl silicone oil). FBB acid is quantified by HPLC with UV detection at 230 nm. Residue concentrations were calculated based on one point calibration corresponding to 0.10 mg/kg.

Conclusion

The analytical method was validated following the scientific and quality standards applying at the time it was done. The reported results are representative of the state of the art at that time. But it cannot be considered as valid in accordance to SANCO/3029/99 rev 4. Valid studies with identical extraction methods and clean-up are not presented. Therefore, any reliable estimation of LOQ is not possible and reported residue concentrations <0.10 mg/kg should be considered as ≤0.10 mg/kg. The comment made by Seym (1995, ASB2009-1209; KCA4.1.2/53) is not accepted. Despite of deficiencies the method is considered as "fit for purpose".

Table B.5.1-18: Validation results of the method 2 for residues of acid Baythroid in samples of animal origin[1]

Reference	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses	Selectivity demonstrated?	Calibration valid?
[redacted] (1983) RIP9400874	bovine liver	0.05	90	-	2	no	yes
	bovine kidney	0.05	78	-	1	no	yes

[redacted] (1983) RIP9400876	bovine liver	0.05	87	17	3	no	no
	bovine kidney	0.05	78	-	1	no	no
[redacted] (1983) RIP9400878	chicken liver	0.05	80	-	1	no	no
	chicken meat	0.05	76	-	1	no	no
	chicken fat	0.05	90	-	1	no	no
	chicken skin	0.05	82	-	1	no	no
	gizzard	0.05	86	-	1	no	no

1) Validation data presented by [redacted] (1983, RIP9400874) are taken from the study of [redacted] (1983, RIP9400877, KCA 4.1.2/70).

Table B.5.1-19: Validation results of the method 2 for residues of FPB acid in samples of animal origin[1]

Reference	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses	Selectivity demonstrated?	Calibration valid?
[redacted] (1983) RIP9400874	bovine liver	0.05	82	-	1	no	yes
	bovine kidney	0.05	78	-	1	no	yes
[redacted] (1983) RIP9400877	bovine liver	0.05	102	-	1	no	no
[redacted] (1983) RIP9400875	bovine kidney	0.05	76	-	1	no	no
[redacted] (1983) RIP9400876	bovine liver	0.05	80	-	1	no	no
	bovine kidney	0.05	82	-	1	no	no
[redacted] (1983) RIP9400878	chicken liver	0.05	94	-	1	no	no
	chicken meat	0.05	66	-	1	no	no
	chicken fat	0.05	84	-	1	no	no
	chicken skin	0.05	80	-	1	no	no
	gizzard	0.05	78	-	1	no	no

[1]Validation data presented by [redacted] (1983, RIP9400874) are taken from the study of [redacted] (1983, RIP9400877, KCA 4.1.2/70).

Table B.5.1-20: Validation results of the method 2 for residues of FPB alc in samples of animal origin[1]

Reference	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses	Selectivity demonstrated?	Calibration valid?
[redacted] (1983) RIP9400874	bovine liver	0.05	98	-	1	no	yes
	bovine kidney	0.05	85	-	1	no	yes
[redacted] (1983) RIP9400875	bovine kidney	0.05	116	-	1	no	no

[redacted] (1983) RIP9400876	bovine liver	0.05	96	-	1	no	no
	bovine kidney	0.05	84	-	1	no	no
[redacted] (1983) RIP9400878	chicken liver	0.05	80	-	1	no	no
	chicken meat	0.05	90	-	1	no	no
	chicken fat	0.05	94	-	1	no	no
	chicken skin	0.05	76	-	1	no	no
	gizzard	0.05	92	-	1	no	no

[1]Validation data presented by [redacted] (1983, RIP9400874) are taken from the study of [redacted]. (1983, RIP9400877, KCA 4.1.2/70).

Table B.5.1-21: Validation results of the method 2 for residues of FPB ald in samples of animal origin[1]

Reference	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses	Selectivity demonstrated?	Calibration valid?
[redacted] (1983) RIP9400874	bovine liver	0.05	72	-	1	no	yes
	bovine kidney	0.05	94	-	1	no	yes
[redacted] (1983) RIP9400877	bovine liver	0.05	100	-	1	no	no
[redacted] (1983) RIP9400875	bovine kidney	0.05	136	-	1	no	no
[redacted] (1983) RIP9400876	bovine liver	0.05	72	-	1	no	no
	bovine kidney	0.05	94	-	1	no	no
[redacted] 1983) RIP9400878	chicken liver	0.05	80	-	1	no	no
	chicken meat	0.05	94	-	1	no	no
	chicken fat	0.05	90	-	1	no	no
	chicken skin	0.05	80	-	1	no	no
	gizzard	0.05	112	-	1	no	no

[1]Validation data presented by [redacted] (1983, RIP9400874) are taken from the study of [redacted] (1983, RIP9400877, KCA 4.1.2/70).

Provided analytical methods, which are not used in accepted in residue studies (livestock)

Table B.5.1-22: List of methods, which are provided but not required

Data point:	Author (year):	Title/report number:
KCA 4.1.2 /55	Gronberg, R. R.; Pfankuche, L. K. (1986)	An analytical residue method for the determination of DCVA in bovine milk, Report No.: I657, Edition Number: M-066719-01-1 RIP9400879
KCA 4.1.2 /58	Maasfeld, W. (1989)	Method for the gas-chromatographic determination of residues of BAYOFLY in bovine tissues and milk, Report No.: 00553, Edition Number: M-012515-02-1, Method Report No.: RA-653 MET1999-99

KCA 4.1.2 /59	Schoening, R. (2001)	Amendment No.1: Supplement E001 of method 00553 for the determination of residues of cyfluthrin in/on animal materials Report No.: 00553/E001, Edition Number: M-006300-02-1, Method Report No.: MR-871/98, MET1999-995
KCA 4.1.2 /60	Schoening, R. (2001)	Amendment No.1: Supplement E002 of method 00553 for the determination of residues of cyfluthrin in/on animal materials Report No.: 00553/E002, Edition Number: M-015544-02-1, Method Report No.: MR-355/99, MET1999-993

B.5.1.2.3 Analytical methods used in residue studies – Part C: Storage stability

Method 1, code/number 00223 (Mobay method 85823)

Data point: KCA 4.1.2 /08

Author (year): Harbin, A. M.; Minor, R. G.; Freeseman, P. L.; Pfankuche, L. K., 1983

Title/report number: A gas chromatographic method for Baythroid residue in crops; Supplements E001 - E032; Modifications M001 - M044 of method 00223 (1160 pages!)
Report No.: MO-03-011091, Edition Number: M-108507-01-1, ASB2009-9418

The method 1 is used in following studies:

Table B.5.1-23: Storage stability studies, which are using method 1 (code/number 00223; Mobay method 85823)

Data point:	Author (year):	Title/report number:
KCA 6.1 /03	Wiedmann, J. L.; Amato, S. L.; Koch, D. A. (1992)	Storage stability of cyfluthrin in crops and processing fractions Report No.: 103821, Edition Number: M-136649-01-1, EPA MRID No.: 42710402, RIP9401055
KCA 6.1 /07	Wiedmann, J. L.; Amato, S. L.; Koch, D. A. (1994)	Storage stability of cyfluthrin in crops and processed products Report No.: 103821-1, Edition Number: M-051312-01-1, EPA MRID No.: 43510903, RIP9401056
KCA 6.1 /09	Lenz, C. A.; Lemke, V. J. (1996)	Addendum 3 - Storage stability of cyfluthrin in crops and processed products, Report No.: 103821-3, Report includes Trial Nos.: 103821 103821-1 and 103821-2, Edition Number: M-051281-01-1, EPA MRID No.: 45655803, ASB2009-1323
KCA 6.1 /11	Grace, T. J. (1989)	Freezer storage stability of cyfluthrin in hops Report No.: 99203, Edition Number: M-049817-01-1 , EPA MRID No.: 41150401, RIP9401052
KCA 6.1 /12	Delk, J. L. (1988)	Baythroid - Storage stability of residues in various frozen crops Report No.: 98334, Edition Number: M-049821-01-1, EPA MRID No.: 41001608, RIP9401051

Principle of the method:

Beta-cyfluthrin is extracted from plant material with methanol/water (original method), chloroform/acetone (appendix III), methanol/water + methanol + acetonitrile (modification M001), acetonitrile (modification M002) or acetone/hexane (modification M018). In the original method the raw extract is evaporated to the aqueous residue, partitioned between aqueous acetone and chloroform, evaporated to dryness, defatted with hexane/acetonitrile and finally purified on a Florisil column.
Alternative or additional clean-up procedures are described in modifications M001, M002, M006,

M018, M022, M028, M036, and M038
In the validation studies (supplements E001 – E027), the concentration of beta-cyfluthrin in final extracts is mostly quantified by GC with coulometric detector (Coulsen™ detector), or a conductivity detector (Hall™ detector). By contrast, most modifications use an ECD detector.
As a consequence of this diversity of procedures used, method 00223 (Mobay method 85823) cannot be considered as a unique method. Therefore, reporting of validation data is focused on concurrent recovery data described in storage stability reports.

Table B.5.1-24: Validation results of the method 1 for residues of cyfluthrin in storage stability samples

Reference	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses	Selectivity demonstrated?	Calibration valid?
Wiedmann, Amato, Koch (1992, RIP9401055) Wiedmann, Amato, Koch (1994, RIP9401056) Lenz, Lemke (1996, ASB2009-1323)	high water content	0.30 1.00	88 88	14 17	11 12	yes	not reported
	dry	0.30 1.00	90 97	16 19	11 12	yes	not reported
	acidic and high fat content	For both matrix types sufficient validation data are not reported. Due to a special clean-up with alumina columns, other studies cannot be considered.					
Grace (1989, RIP9401052)	hops	1.0 5.0 10.0	102 100 101	8 4 1	4 2 2	yes	not reported
Delk (1988, RIP9401051)	high water content	1.0	91	10	8	yes	yes

Conclusion

In accordance to SANCO/3029/99 rev 4 the modifications of the method are not successfully validated. However, considering the total amount of validation for similar matrices and identical detection (GC-ECD) the data are acceptable. Also several valid calibrations of the GC-ECD method using packed columns are reported. Storage stability data obtained with this method are reliable.

Methods which do not fulfil the requirements

Table B.5.1-25: List of methods, which do not fulfil requirements

Author(s) and year	Annex point/Report No	Reason
Nelson, T. R. (1979)	KCA 4.1.2 /39 Report No.: I641, RIP9400727	The study contains an insufficient number of validation data (n=1 on two levels)
Minor, R. G.; Freeseman, P. L. (1989)	KCA 4.1.2 /40, Report No.: 99631, RIP9401053	This study refers to the formation of metabolites and is conducted with radio-labelled cyfluthrin. It is the summary of five other studies. This report does not allow to evaluate validity.

Freeseman, P. L. (1992)	KCA 4.1.2 /41 Report No.: 102608 ASB2009-1208	This study refers to the formation of metabolites and is conducted with radio-labelled cyfluthrin. Radioactivity of samples stored up to 783 days is extracted with acetone/chloroform (2/1). Other samples stored longer are extracted with methanol to enhance extraction efficiency. However, stability is calculated on the extracted amaount of radioactivity. It is not possible to judge the change of solvent.
Harbin, A. M.; Gronberg, R. R.; Minor, R. M.; Bailey, S. R.; Freeseman, P. L.; Pfankuche, L. K. (1985)	KCA 4.1.2 /36 and 4.2/60 Report No.: 00033, Edition Number: M-009396-01-1 Method Report No: I577, RIP9400734	The study contains an insufficient number of validation data (n=2 on two levels)
Blass, W. (1988)	KCA 4.1.2 /37 and 4.2/61 Report No.: 00033/E001, Edition Number: M-009425-02-2 Method Report No: I577 RIP9401124	See KCA 4.1.2 /36
Blass, W. (1988)	KCA 4.1.2 /38 and 4.2 /62 Report No.: 00033/E002, Edition Number: M-045127-01-2 RIP9401125	Document is identical with KCA 4.1.2 /37 and 4.2/61, Report No.: 00033/E001, Edition Number: M-009425-02-2 Method Report No: I577 RIP9401124

B.5.1.2.4 Analytical methods used in environmental fate studies – Part A: Field dissipation

Method 1

Data point: KCA 4.1.2 /74

Author (year): Robinson, N. (2014)

Title/report number: beta-cyfluthrin - Field soil dissipation of beta-Cyfluthrin from a field trial carried out in Southern France
M-482355-01-1 ! 20120152 ! R-30598
ASB2014-7708

Principle of the method:

The soil sample is extracted by shaking with acetone. The combined soil extract is adjusted to a known volume and an aliquot is evaporated to low volume to remove the acetone. Methanol/water (1/1, v/v) is added to the sample and the sample partitioned into hexane. The combined hexane extract is evaporated to dryness and redissolved in hexane. The sample concentration in final extracts in 1 g/mL.
The concentration of beta-cyfluthrin in final extracts is quantified by GC-MS using a HP-5ms column, in negative chemical ionisation mode with selected ion monitoring of three ions. The most sensitive ion (m/z = 207) was used for residue calculation.

Table B.5.1-26: Validation results of the method for residues of beta-cyfluthrin in soil

Reference	Matrix	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses	Selectivity demonstrated?	Calibration valid?
Robinson (2014) ASB2014-7708	Fislis soil	0.01 0.10	87 83	5.0 1.7	5 5	yes	yes

Conclusion

Considering a post application soil concentration of 0.015 mg/kg on day 0, the method is barely acceptable.

Methods which do not fulfil the requirements

Table B.5.1-27: List of methods, which do not fulfil requirements

Author(s) and year	Annex point/Report No	Reason
Weeren and Pelz (1999) MET1999-1227	KCA 4.1.2 /43 Report No.: 00086/E050 Edition Number: M-009717-01-1	LOQ of method (0.05 mg/kg) is not sufficient and this GC-ECD method was not used in new field trials..
Wagner (1985) MET2005-58	KCA 4.1.2 /44 Report No.: I371, Edition Number: M-022599-01-2	LOQ of method (0.50 mg/kg) is not sufficient and this GC-ECD method was not used in new field trials...
Gronberg and Pfankuche (1983) MET2005-59	KCA 4.1.2 /46 Report No.: 85886, Edition Number: M-064739-01-1	LOQ of method (0.05 mg/kg) is not sufficient and this GC-ECD method was not used in new field trials..
Bachlechner (1990) MET9400013	KCA 4.1.2 /49 Report No.: 00195, Edition Number: M-017140-01-2	LOQ of method (0.0008 mg/kg) is sufficient, but this GC-ECD method was not used in new field trials.
Ishii, Y.; Ueyama (1983) RIP9400731	KCA 4.1.2 /07 Report No.: I471, Edition Number: M-059522-01-1	LOQ of method (0.10 mg/kg) is not sufficient and this GC-NPD method was not used in new field trials..
Brennecke (1984) RIP9400733	KCA 4.1.2 /03 Report No.: 00002, Edition Number: M-016057-03-1	The study describes a method for triadimenol and triadimefon.
Blass (1985) RIP9401113	KCA 4.1.2 /04 Report No.: MO-03-010452, Edition Number: M-105171-01-1	The study does not contain validation data for soil.
Anon (1983) ASB2009-1210	KCA 4.1.2 /48 Report No.: 84373, Edition Number: M-064743-01-1	LOQ of method (0.10 mg/kg) is not sufficient and this GC-ECD method was not used in new field trials..

Wagner (1982) ASB2009-1211	KCA 4.1.2 /45 Report No.: RA-430, Edition Number: M-065712-01-2	The study contains an insufficient number of supplementary validation data to a method , which was not supplied.
Wagner (1982) ASB2009-1212	KCA 4.1.2 /47 Report No.: 84342, Edition Number: M-065718-01-1	LOQ of method (0.05 mg/kg) is not sufficient and this GC-ECD method was not used in new field trials..

B.5.1.2.5 Analytical methods used in environmental fate studies – Part B: Other studies

Studies on analytical methods required for other environmental fate studies are neither provided nor identified by the RMS.

B.5.1.2.6 Analytical methods used in efficacy studies

Particular methods for the determination of beta-cyfluthrin in efficacy studies are neither provided nor identified by the RMS.

B.5.1.2.7 nalytical methods used in ecotoxicological studies – Part A: Terrestrial vertebrates

Particular methods for the determination of beta-cyfluthrin in feeding stuffs of birds or rats were not provided. Reported lowest endpoints are:

Birds: NOAEL of Mallard duck: 37.74 mg/kg bw/day
Rat: NOAEL: 3.3 mg/kg bw/day

In the ecotoxicological studies on terrestrial vertebrates feeding stuffs contained beta-cyfluthrin at levels >269 mg/kg for birds and 50 mg/kg for rats. Such high residue amounts in feeding stuffs do not provoke any analytical difficulties. The LOQ of available monitoring methods for food of plant and animal origin (see section B.5.2.1 and B.5.2.2) is about 10,000 times lower.

B.5.1.2.8 Analytical methods used in ecotoxicological studies – Part B: Aquatic organisms

Method 1, code/number 01174 (method used in aquatic toxicity test)

Data point: KCA 4.1.2 /75

Author (year): Braune, M.; Sandau, C. (2010)

Title/report number: Method 01174 for the determination of beta-Cyfluthrin FPB acid in test water from aquatic toxicity tests by HPLC-UV
M-361622-01-1 ! MR-09/133 ! P 604 097054 ! method 01174
ASB2014-6695

Principle of the method:

The method allows the detection of 4-fluoro-3-phenoxybenzoic acid (beta-cyfluthrin FPB). Test water is adjusted to pH 3 and analysed by direct injection of acidified water into a HPLC-UV system equipped with a Synergi Polar-RP 80 column. 4-fluoro-3-phenoxybenzoic acid is detected with an UV

detector operating at 220 nm.

Table B.5.1-28: Validation results of the method 01174 for residues of 4-fluoro-3-phenoxybenzoic acid (beta-Cyfluthrin FPB) in test water

Reference	Matrix	Fortification level [µg/L]	Average recovery [%]	RSD [%]	No. of analyses	Selectivity demonstrated?	Calibration valid?
Braune and Sandau (2010) ASB2014-6695	Test water	60 600	103 102	0.8 1.1	10 10	yes	yes

Conclusion

4-fluoro-3-phenoxybenzoic acid (beta-cyfluthrin FPB) is a metabolite occurring in surface water. All reported endpoints (LC_{50}, NOEC) in ecotoxicological studies are based on valid data if the above described method was used. Reported endpoints are:
Fish: LC_{50} of *Oncorhynchus mykiss*: 4.06 µg/L
Aquatic invertebrates: EC_{50} of *Daphnia magna*: 39.30 µg/L

Method 2, code/number D58731 (method used in aquatic toxicity test)

Data point: KCA 4.1.2 /76

Author (year): Kimmel, S. (2014)

Title/report number: Beta-cyfluthrin: Effect on the development of sediment dwelling larvae of Chironomus riparius in water-sediment systems with spiked sediment
Report No.: D58731, Edition Number: M-481037-01-1
ASB2014-7672

Principle of the method:

Water:
An aliquot of beta-cyfluthrin sample was diluted with the same amount acetonitrile and extracted using solid-phase extraction (Bakerbond C18, 1000 mg). The sample was loaded onto the preconditioned cartridge and subsequently dried by vacuum. The cartridge was eluted with 10 mL acetonitrile. The eluate was evaporated to dryness. The dry residue was dissolved in 2 mL test water/acetonitrile (v/v; 1/1). Sample concentration in final extracts is 0.075 L/mL (sample concentration factor of 0.0133) or 0.015 L/mL (sample concentration factor of 0.0667).
Sediment:
About 60 g of wet sediment corresponding to about 46 g of dry sediment were extracted twice by shaking with 150 mL acetonitrile. The combined organic phases were evaporated to dryness. The residue was dissolved in 10 mL of acetonitrile. The samples were further diluted into the calibration range with acetonitrile and with test water/acetonitrile (v/v; 1/1) in the first and second dilution step, respectively. Sample concentration in final extracts is 0.0122 g/mL (sample concentration factor of 378 and 46 g/10 mL) or 0.00175 g/mL (sample concentration factor of 2626 and 46 g/10 mL).
The concentration of beta-cyfluthrin in final extracts is quantified by LC-MS/MS using an Inertsile Ph-3 column, positive electrospray ionisation and one MRM transition (m/z 451→191).

Table B.5.1-29: Validation results of the method D58731 for residues of beta-cyfluthrin in water and sediment

Reference	Matrix	Fortification level [mg/kg] [µg/L]	Average recovery [%]	RSD [%]	No. of analyses	Selectivity demonstrated?	Calibration valid?
Kimmel (2014) ASB2014-7672	water	0.00532 0.199	93 95	4 4	6 6	yes	yes
	sediment	0.124 2.02	83 92	4 6	6 6	yes	yes

Conclusion

The following reported endpoints (EC_{50}, NOEC) in ecotoxicological studies of *Daphnia magna*, *Chironomus riparius* and *Scenedesmus subspicatus* are based on valid data if the above method was used. Reported endpoints are:
Aquatic invertebrates: EC_{50} of *Daphnia magna*: 0.025 µg/L
Sediment dweller: NOEC of *Chironomus riparius*: 0.4 µg/L
Algae: EC_{50} of *Scenedesmus subspicatus*: >10 µg/L

The above mentioned method has not been considered valid to check beta-cyfluthrin in test water corresponding to the lowest effect concentrations for fish, and for the aquatic invertebrates *Hyalella azteca and Americamysis bahia*.

Method 3, (method used in aquatic toxicity test)

Data point: KCA 8.2.5.2/01

Author (year): Schwader, A.L. (2013)

Title/report number: Beta-Cyfluthrin – Life-cycle toxicity test with mysids (*Americamysis bahia*); Appendix 3 – Analytical methodology
M-465880-01-1; Sponsor Project No. EBFRL028; Smither Viscient Study No. 13798.6307;

Principle of the method:

The method allows the detection of beta-cyfluthrin present in filtered seawater. Test water is adjusted to pH 3, extracted twice with ethyl acetate and reconstituted with 0.1 % peanut oil in acetone. The concentration of beta-cyfluthrin in final extracts is quantified by gas chromatography with mass selective detection in negative chemical ionisation mode equipped with a CP-Sil 8 CB column and monitoring m/z 207 for beta-cyfluthrin and m/z 213 for the internal standard d6-cyfluthrin.

Table B.5.1-30: Validation results of the method for residues of beta-cyfluthrin in seawater

Reference	Matrix	Fortification level [µg/L]	Average recovery [%]	RSD [%]	No. of analyses	Selectivity demonstrated?	Calibration valid?
Schwader (2013)	seawater	0.0001 0.0005 0.001	114 105 99	1.8 4.8 5.0	3 3 3	no	no

Conclusion

This analytical method was used for aquatic toxicity test with *Americamysis bahia.* Formally, the re-

ported endpoint (NOEC) of 0.00041 µg/L is covered by the LOQ of above described analytical method.
It should be noted, that the number of samples per fortification level (n=3) is not sufficient according to the requirements of SANCO/3029/99 rev 4. Furthermore, the calibration range (0.5–10 µg/L) seems to be not appropriate for the concentration ranges tested in the fortification experiment (0.0001–0.001 µg/L).
The LOQ of the analytical method is considered sufficient to check beta-cyfluthrin in test water corresponding to the lowest effect concentrations for fish (NOEC of *Oncorhynchus mykiss*: 0.0042 µg/L).

Method 4, (method used in aquatic toxicity test for *Hyaella azteca*)

Data point: KCA 4.1.2

Author (year): Dix, M.E. (2013)

Title/report number: Method validation for seven pyrethroids in freshwater by gas chromatography using mass selective detection with negative chemical ionization
M-536985-01-1 ! 13656.6125
ASB2016-1579

Principle of the method:

The method allows the detection of cyfluthrin present in freshwater. Samples are adjusted to pH 3, extracted twice with ethyl acetate and reconstituted with 0.1% peanut oil in acetone. The concentration of beta-cyfluthrin in final extracts is quantified by gas chromatography with mass selective detection in negative chemical ionisation mode equipped with a CP-Sil 8 CB column and monitoring m/z 207 for cyfluthrin and m/z 231 for the internal standard d6-cypermethrin.

Table B.5.1-31: Validation results of the method for residues of cyfluthrin in freshwater

Reference	Matrix	Fortification level [µg/L]	Average recovery [%]	RSD [%]	No. of analyses	Selectivity demonstrated?	Calibration valid?
Dix (2013) ASB2016-1579	freshwater	0.001 0.003 0.02 0.05	108 105 105 102	10.5 10.5 5.5 2.0	3 3 3 3	yes	no

Conclusion

This analytical method was used for an aquatic toxicity test with *Hyaella azteca*. The reported endpoint in the ecotoxicological study - the NOEC for *Hyaella azteca* of 0.000231 µg/L - is not covered by the LOQ of the above described analytical method.
Furthermore it should be noted, that the number of samples per fortification level (n=3) is not sufficient according to the requirements of SANCO/3029/99 rev 4. The calibration range (0.5–10 µg/L) seems to be not appropriate for the concentration ranges tested in the fortification experiment (0.001–0.05 µg/L).

B.5.1.2.9 Analytical methods used in ecotoxicological studies – Part C: Honey bees

Particular methods for the determination of beta-cyfluthrin in honey bees were not provided. Based on a LD_{50} of 0.012 µg/bee and an assumed mass of bee of 0.1 g, a LOQ of 0.12 mg/kg is required. In section B.5.2.2 analytical methods are described, which allow the determination of beta-cyfluthrin in matrices of animal origin with an LOQ of 0.01 mg/kg. Assuming that these methods are used, the es-

timation of LD_{50} seems reliable.

B.5.1.2.10 Analytical methods used in ecotoxicological studies – Part D: Other arthropod species

Particular methods for the determination of beta-cyfluthrin in other arthropod species were not provided. Acting on the assumption that the relevant ecotoxicological studies on other arthropod species did not need the analytical determination of applied amounts of beta-cyfluthrin, no analytical methods are required.

B.5.1.2.11 Analytical methods used in toxicological studies

Based on regulation (EU) No 283/2013 only studies conducted with non-isotope labelled residues are evaluated in this section. Most toxicological studies which contain analytical parts are conducted before adoption of SANCO/3029/99 rev.4 from 11/07/00. Such studies are accepted here when individual safety factors between typical LOQs (obtained at about 1985) and lowest reported concentrations are maintained. Such LOQs and (estimated) safety factors are:

Matrix	Typical LOQ of methods obtained at about 1985	Matrix	Safety factor	LOQs accepted without separate validation
Food of plant origin	0.10 mg/kg	Diets for rodents and dogs; corn oil; pads used for skin sensitisation tests	1000	100.00 mg/kg
Repeatedly successful calibrated concentration in solvents	10.00 µg/L	Aqueous solutions used as formulations (containing e.g. Cremophor); formulations based on PEG 400	100	1.00 mg/L
		Organic solutions administered by gavage	10	0.10 mg/L
		Air (assuming an air sample volume of 0.01 m^3 and a volume of final extract of 0.1 L)	1	0.10 mg/m^3
Blood/plasma/urine	0.10 mg/L	Blood/plasma/urine	50	5.00 mg/L

Considering either these LOQs acceptable without validation or considering the validation data provided in reports, analytical data in most toxicological studies reported in Vol.3 B.6 are reliable. However, reliability of analytical data is vague for the studies listed below (see Table B.5.1-32).

Table B.5.1-32: List of studies, which require low LOQs and do not contain sufficient validation data

Data point (cited in):	Author (year):	Title/report number:
Vol. 3 B.6.1	[redacted] (1982)	Comparative study of rats on absorption of FCR 1272 after single oral administration in polyethylene glycol 400 or cremophor EL/water as formulation vehicle; RIP9400865
Vol. 3 B.6.3	[redacted] (1984)	FCR 1272 (c.n.: Cyfluthrin): Study for subchronic inhalative toxicity to the rat for 13 weeks (exposure 63 x 6 hours) - incl. Addendum 1+2 TOX9401887

Vol. 3 B.6.6	[redacted] (1993)	FCR 1272: Determination of the FCR 1272 concentration in the plasma of rats following inhalative exposure; TOX9401913
Vol. 3 B.6.8	[redacted] (1988)	FCR 1272 (c.n. cyfluthrin, proposed): Study for sensory irritant potential in the mouse (RD50 determination); TOX9401869
Vol. 3 B.6.8	[redacted] (1989)	FCR 1272 (suggested common name: cyfluthrin): Study of the blood gases in rats; TOX9401870
Vol. 3 B.6.8	Pauluhn, J. (1996)	Risk assessment of pyrethroids following indoor use; TOX2001-880
KCA 4.1.2 /78 KCA 5.2.3 /03 Vol. 3 B.6.8	[redacted]1996)	Determination of Cyfluthrin (FCR 1272) in serum, fat and brain of rats after inhalation exposure or oral administration - Analytical part of study T7058167 Report No.: MR-365/95, Edition Number: M-044833-01-1, TOX2001-1767
KCA 4.1.2 /79 KCA 5.2.1 /14 Vol. 3 B.6.8	[redacted] [redacted] (1996)	Cyfluthrin: Concentration of the parent compound in blood plasma, brain and omental fat of rats following administration with the feed or by oral administration, Report No.: MR-625/95, Edition Number: M-044715-01-1, TOX2001-1768
Vol. 3 B.6.9	Ruddy, K.; Mair, S. J.; McInally, K., (1998)	Safety and tolerability study of FCR 1272 0.04 AE in healthy volunteers; TOX2001-879

Further analytical methods are provided, which may be applicable in toxicological studies. However, toxicological studies using these methods could not be identified (see Table B.5.1-33).

Table B.5.1-33: List of methods, which are provided but not required

Data point:	Author (year):	Title/report number:
KCA 4.1.2 /72	Heimann, K. G. (1994)	Cyfluthrin - Begruendung der Bestimmungsgrenze in der Luft Report No.: MO-02-012388, Edition Number: M-078663-01-1 MET9500022
KCA 4.1.2 /84	Frenzel, T.; Sochor, H.; Speer, K.; Uihlein, M. (2000)	Rapid multimethod for verification and determination of toxic pesticides in whole blood by means of capillary GC-MS Journal:Journal of Analytical Toxicology 24 (2000) 365-371, Report No.: 00561P, Edition Number: M-069346-01-1, MET2002-150
KCA 4.1.2 /86	Brennecke, R. (1998)	Independent laboratory validation of method EM F-05/98-0 "Rapid multimethod for verification and determination of toxic pesticides in whole blood by means of capillary GCMS" according to European guidelines Report No.: MR-918/98, Edition Number: M-005693-01-1 MET2000-3

B.5.1.2.12 Analytical methods used in tests of operator or worker exposure

Experimental tests of operator or worker exposure were not conducted.

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

Information on the active substance and further analytes

Name, code	beta-Cyfluthrin, FCR 1272 / AE F057122 / Baythroid
IUPAC	(RS)-α-cyano-4-fluoro-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-=2,2-dimethylcyclopropanecarboxylate
Formula	$C_{22}H_{18}Cl_2FNO_3$
Molecular Weight	434.29 g mol^{-1}, [M+NH4]+ = 451.09915 Da

New residue analytical methods for monitoring are provided. This is justified because most methods assessed in the previous DAR do not fulfil the requirements laid down in the most recent guidance document on pesticide residue methods (SANCO/825/00 rev.8.1).
The methods of analysis summarised below are intended to fully replace the methods assessed in the original review.

B.5.2.1 Analytical methods for the determination of residues in or on food and feed of plant origin

B.5.2.1.1 Acceptable methods/reports

Study 1

Data point:	KCA 4.2/89
Report:	Weber, H., 2009, Validation of enforcement method DFG S 19 (L 00.00-34) (BCS method ID 00086/M088) for the determination of residues of cyfluthrin (AE F057122) in/on plant materials, Report No: Specht File Reference: G08-0201, EASSM No.: S09-00092 Edition No.: M-347371-01-1, ASB2014-2283
Guideline(s):	Yes, SANCO/825/00 rev. 7
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods:

Fortified analyte(s):
cyfluthrin (containing 50 % beta-cyfluthrin and 50 % two other diastereoisomers)

Analyte(s) determined as:
cyfluthrin (containing 50 % beta-cyfluthrin and 50 % two other diastereoisomers)

Principle of the method:
The modular version of DFG S19 method was used. Cyfluthrin was extracted from lettuce (head), orange (fruit), dry bean (seed) and wheat (grain) specimens with acetone/water (2/1, v/v). Thereafter, ethyl acetate/cyclohexane (1/1, v/v) and sodium chloride were added to the extracts for liquid-liquid partition. An aliquot of the organic phase was evaporated to dryness. From oil seed rape (seeds), cyfluthrin was extracted with acetonitrile/acetone (9/1, v/v) in the presence of synthetic calcium silicate (trade name Calflo E) and Celite. The organic phase was filtered and evaporated to dryness.
For all matrices the evaporated extract was reconstituted in ethyl acetate/isooctane and cleaned up by gel permeation chromatography (GPC) on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane (1/1, v/v) as eluent. The collected extracts were further cleaned on a mini silicagel

column. The sample concentration in final extracts was 2.34 g/mL.
Final extracts were analysed for residues of cyfluthrin by gas chromatography using a DB-5MS capillary column with mass selective detection (GC-MSD). Three ions were monitored to fulfil the requirement for validation of this confirmatory method. Using GC-MSD, the ions 226, 206 and 199 were used for the determination of cyfluthrin in orange (fruit), oil seed rape (seed), dry bean (seed) and wheat (grain). For the gas chromatographic determination of cyfluthrin in lettuce (head), the ions 206, 163 and 165 were used due to interferences in the chromatograms of the lettuce extracts monitoring the ions 226 and 199. The detected ions resulted in four separate GC peaks with two peaks belonging to beta-cyfluthrin.

Results

Selectivity (specificity):
Signals from interferences >30 % of LOQ in blank samples were not detected.
Recovery (accuracy):
For accuracy of analytical results see Table B.5.2-1: .
Repeatability (precision):
For accuracy of analytical results see Table B.5.2-1: .
Limit of quantification (LOQ):
For each matrix type the lowest successfully validated level in Table B.5.2-1: is considered as limit of quantification.
Matrix effects:
Matrix effects were tested by evaluating the results with solvent standards and with matrix-matched standards. For cyfluthrin, signal suppression or enhancement below 10 % were observed for all matrices. Therefore solvent standards were used for the analysis.
Calibration (linearity):

- Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points): yes (8 levels)
- Accepted calibration range in concentration units: 10-2500 ng/mL
- Accepted calibration range in mass fraction units: 0.004-1.07 mg/kg
- Calibration conducted with matrix matched standards: no
- Sample chromatogram spiked at LOQ demonstrates sufficient sensitivity and signal-to-noise ratio?: yes

Confirmation
Signals obtained from three different fragment ions were used for quantification. All results were found to be valid (see Table B.5.2-1:). Therefore, a confirmatory method is not needed.

Conclusion

The analytical method DFG S 19 is suitable as enforcement method for beta-cyfluthrin and cyfluthrin in dry crops, commodities with high water content, commodities with high acid content and commodities with high oil content.
A confirmatory method for dry crops, commodities with high water content, commodities with high acid content and commodities with high oil content is provided by full validation of two additional fragment ions.
Blank values in chromatograms for quantification and confirmatory transitions, are generally below 30 % of LOQ in all matrices tested.

Table B.5.2-1: Validation of the method 00086/M088 (DFG S19) by Weber (2009) for residues in food of plant origin

Reference	Matrix	Detection method	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Weber (2009) (ASB2014-2283)	orange fruit	GC-MS, EI VF-5 ms m/z 226	0.01 0.10 1.00	92 101 86	8.0 3.3 15	5 5 5
	lettuce head	GC-MS, EI VF-5 ms m/z 163	0.01 0.10 1.00	101 76 74	7.7 10 6.4	5 5 5
	oil seed rape (seeds)	GC-MS, EI VF-5 ms m/z 226	0.01 0.10	89 86	9.9 7.3	5 5
	dry beans (seeds)		0.01 0.10	90 76	11 7.2	5 5
	wheat grain		0.01 0.10	83 80	14 8.0	5 5
	orange fruit	GC-MS, EI VF-5 ms m/z 206	0.01 0.10 1.00	98 100 86	6.0 3.6 14	5 5 5
	lettuce head		0.01 0.10 1.00	93 78 74	9.9 9.3 5.6	5 5 5
	oil seed rape (seeds)		0.01 0.10	90 86	10 7.9	5 5
	dry beans (seeds)		0.01 0.10	92 76	8.9 7.2	5 5
	wheat grain		0.01 0.10	89 80	8.8 9.7	5 5
	orange fruit	GC-MS, EI VF-5 ms m/z 199	0.01 0.10 1.00	102 102 87	5.9 5.3 14	5 5 5
	oil seed rape (seeds)		0.01 0.10	98 90	4.1 6.8	5 5
	dry beans (seeds)		0.01 0.10	100 79	12 9.8	5 5
	wheat grain		0.01 0.10	95 81	11 9.0	5 5
	lettuce head	GC-MS, EI VF-5 ms m/z 165	0.01 0.10 1.00	96 79 75	15 10 5.6	5 5 5

Study 2
(Independent laboratory validation of method 00086/M088 reported in study 1)

Data point: KCA 4.2/90

Report: Independent laboratory validation of the DFG method S19 (BCS method ID 00086/M088) for the determination of residues of cyfluthrin in plant materials, using GC/MS, Merdian (2009), Report No: PTRL Europe Study No.:P 1547 G, Edition No.: M-349219-01-1, ASB2014-2281

Guideline(s):	Yes, SANCO/825/00 rev. 7
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods:

Fortified analyte(s):
cyfluthrin (containing 50 % beta-cyfluthrin and 50 % two other diastereoisomers)

Analyte(s) determined as:
cyfluthrin (containing 50 % beta-cyfluthrin and 50 % two other diastereoisomers)

Principle of the method:
Extraction and clean-up were identical compared to the primary validation. However, the sample concentration in final extracts was 4.7 g/mL (lettuce, orange), 5.6 g/mL (oil seed rape), or 5.8 g/mL (wheat grain).
Final extracts were analysed for residues of cyfluthrin by gas chromatography using a VF-5ms capillary column with mass selective detection (GC-MSD). Three ions were monitored to fulfil the requirement for validation of this confirmatory method. Using GC-MSD, the ion 226 was used for quantification in all matrices. For confirmation m/z 206 and 199 were used for orange (fruit), oil seed rape (seed). Due to interferences in lettuce, the fragment ions m/z 206 and 165 were used. For confirmation of residues in wheat grain, the fragments m/z 199 and 163 were recorded. The detected ions resulted in four separate GC peaks with two peaks belonging to beta-cyfluthrin.

Results

Selectivity (specificity):
Signals from interferences >30 % of LOQ in blank samples were not detected except for m/z 199 in orange fruit and oil seed rape.
Recovery (accuracy):
For accuracy of analytical results see Table B.5.2-2:
Repeatability (precision):
For accuracy of analytical results see Table B.5.2-2:
Limit of quantification (LOQ):
For each matrix type the lowest successfully validated level in Table B.5.2-2: is considered as limit of quantification.
Matrix effects:
Matrix matched standards per sample matrix were prepared and injected to show the effects of matrix on GC/MS response. For cyfluthrin, signal enhancement below 10 % was observed for all matrices.
Calibration (linearity):

- Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points): yes (8 levels)
- Accepted calibration range in concentration units: 10-2500 ng/mL
- Accepted calibration range in mass fraction units: 0.002-0.53 mg/kg (letuce, orange)
  0.002-0.44 mg/kg (other)
- Calibration conducted with matrix matched standards: no
- Sample chromatogram spiked at LOQ demonstrates sufficient sensitivity and signal-to-noise ratio?: yes

Confirmation
See study 1

Conclusion

The study is considered as a successful independent laboratory validation of the method DFG S 19, which was first validated in study 1 by Weber (2009).

Table B.5.2-2: Validation of the method 00086/M088 (DFG S19) by Merdian (2009) for residues in food of plant origin

Reference	Matrix	Detection method	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Merdian (2009) (ASB2014-2281)	lettuce head	GC-MS, EI VF-5 ms m/z 226	0.01 0.10	101 99	2 10	5 5
	wheat grain		0.01 0.10	80 93	6 9	5 5
	orange fruit		0.01 0.10	107 82	7 18	4 5
	oil seed rape (seeds)		0.01 0.10	97 86	14 13	5 5
	lettuce head	GC-MS, EI VF-5 ms m/z 206	0.01 0.10	99 97	2 9	5 5
	orange fruit		0.01 0.10	99 78	10 17	4 5
	oil seed rape (seeds)		0.01 0.10	102 81	20 24	5 5
	wheat grain	GC-MS, EI VF-5 ms m/z 199	0.01 0.10	84 99	8 3	5 5
	orange fruit		0.01 0.10	interference 81	 19	 5
	oil seed rape (seeds)		0.01 0.10	Interference 87	 17	5 5
	lettuce head	GC-MS, EI VF-5 ms m/z 165	0.01 0.10	100 94	4 10	5 5
	wheat grain	GC-MS, EI VF-5 ms m/z 163	0.01 0.10	79 94	10 11	5 5

Study 3

Data point: KCA 4.2/98

Report: Beta-cyfluthrin: Validation of an analytical method for determination of beta-cyfluthrin in wheat grain, oil seed rape seeds, tomato and grapes, Airs (2013)
Report No.: GAW0002, Edition No.: M-481200-01-1, ASB2014-6696

Guideline(s): Yes, SANCO/825/00 rev. 7, SANCO/3029/99 rev. 4,

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods:

Fortified analyte(s):
beta-cyfluthrin

Analyte(s) determined as:
beta-cyfluthrin

Principle of the method:
The modular version of DFG S19 method is used. Tomato, grape and wheat grain samples are homogenised in acetone/water (2+1 v/v). After addition of solid NaCl; residues are partioned into ethyl acetate/cyclohexane (1/1 v/v). An aliquot of the extract is filtrated through sodium sulphate and evaporated to the aqueous reminder. The reminder is soluted in ethyl acetate/cyclohexane and dried with sodium sulphate/sodium chloride.
Rape seed samples are homogenised in acetone/acetonitrile (1/9 v/v) in the presence of Calflo E and Celite. An aliquot of the extract is filtrated through Calflo E. After addition of isooctane, the extract is evaporated to dryness and reconstituted in ethyl acetate/cyclohexane.
All extracts are purified by size exclusion chromatography. Isooctane is added to the obtained eluates, which are evaporated near to dryness. The reconstituted extracts are further purified by chromatography on silica (1.5 % water). Residues are eluted with toluene. Sample concentration in final extracts was 2.3 g/mL (tomato, grape and wheat grain), 5 g/mL (rape seed).
Final extracts were analysed for residues of beta-cyfluthrin by gas chromatography using a DB-5 capillary column with mass selective detection (GC-MSD). Three ions were monitored to fulfil the requirement for validation of this confirmatory method. Using GC-MSD, the ions 207, 209 and 171 were used for the determination. The detected ions resulted in one GC peak.

Results

Selectivity (specificity):
Signals from interferences >30 % of LOQ in blank samples were not detected.
Recovery (accuracy):
For accuracy of analytical results see Table B.5.2-3:
Repeatability (precision):
For repeatability of analytical results see Table B.5.2-3: .
Limit of quantification (LOQ):
For each matrix type the lowest successfully validated level in Table B.5.2-3: is considered as limit of quantification.
Matrix effects:
No significant enhancement or suppression of response was observed for beta-cyfluthrin in the final sample extracts.
Calibration (linearity):

- Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points): yes (10 levels)
- Accepted calibration range in concentration units: 5-500 ng/mL
- Accepted calibration range in mass fraction units: 0.001-0.1 mg/kg (rape seed)
  0.002-0.22 mg/kg (other)
- Calibration conducted with matrix matched standards: no
- Sample chromatogram spiked at LOQ demonstrates sufficient sensitivity and signal-to-noise ratio?: yes

Confirmation
Signals obtained from three different fragment ions were used for quantification. All results were found to be valid (see Table B.5.2-3:).
Therefore, a separate confirmatory method is not needed.

Conclusion

The suitability of the analytical method DFG S 19 was demonstrated again for beta-cyfluthrin in dry crops, commodities with high water content, commodities with high acid content and commodities with high oil content. A confirmatory method for dry crops, commodities with high water content, commodities with high acid content and commodities with high oil content is provided by full validation of two additional fragment ions.
Blank values in chromatograms for quantification and confirmatory transitions, are generally below 30 % of LOQ in all matrices tested. This demonstrates a sufficient selectivity of the method.

Table B.5.2-3: Validation of the method DFG S19 by Airs (2009) for residues in food of plant origin

Reference	Matrix	Detection method	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Airs (2013) ASB2014-6696	wheat grain	GC-MS, EI DB-5 column m/z 207	0.01 0.1	96 97	2.6 7.6	5 5
	oil seed rape		0.01 0.1	104 98	6.2 6.3	5 5
	tomato		0.01 0.1	96 96	18.7 6.3	5 5
	grapes		0.01 0.1	101 82	9.8 15.7	5 5
	wheat grain	GC-MS, EI DB-5 column m/z 209	0.01 0.1	95 97	3.2 7.8	5 5
	oil seed rape		0.01 0.1	105 99	5.1 4.8	5 5
	tomato		0.01 0.1	95 96	19.4 6.0	5 5
	grapes		0.01 0.1	99 81	10.2 15.6	5 5
	wheat grain	GC-MS, EI DB-5 column m/z 171	0.01 0.1	95 96	2.9 7.9	5 5
	oil seed rape		0.01 0.1	101 96	3.8 4.6	5 5
	tomato		0.01 0.1	95 96	19.2 6.1	5 5
	grapes		0.01 0.1	96 82	10.7 15.4	5 5

Study 4

Data point: KCA 4.1.2/81

Report: Analytical method 00922 for the determination of residues of beta-cyfluthrin in/on plant material by HPLC-MS/MS, Schöning (2005), Method No: 00922, Edition No.: M-244829-01-1, MET2006-93

Guideline(s): Yes, OPPTS 860.1340, SANCO/3029/99 rev. 4,

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods:

Fortified analyte(s):
beta-cyfluthrin

Analyte(s) determined as:
beta-cyfluthrin

Principle of the method:
Residues of beta-cyfluthrin are extracted from cereals (grain, green material, straw) and from sugar beet (body and leaf) with a mixture of acetone/water (2/1; v/v). After filtration, the extract is made up to volume with acetone/water. An aliquot of the extracts is partitioned against dichloromethane.
Rape (seed) samples are extracted with acetonitrile and after filtration the acetonitrile extract is washed with hexane. After evaporation to dryness, the residue is reconstituted in dichloromethane.
The dichloromethane extracts are filtered through a Florisil™ cartridge. The filtrate is evaporated to dryness and dissolved in an internal standard solution (containing 15.8 µg/L cyfluthrin-methyl-d6) in acetonitrile/water (1/1, v/v + 5 mmol ammonium acetate/L). Sample concentration in final extracts is 0.2 g/mL.
The concentration of beta cyfluthrin in final extracts is quantified by LC-MS/MS using a C18 column, positive electrospray ionisation and one MRM transitions (m/z 451→191). The internal standard cyfluthrin-methyl-d6 is quantified by the MRM transitions m/z 457→197.

Results

Selectivity (specificity):
Signals from interferences >30 % of LOQ in blank samples were not detected.
Recovery (accuracy):
For accuracy of analytical results see Table B.5.2-4.
Repeatability (precision):
For repeatability of analytical results see Table B.5.2-4.
Limit of quantification (LOQ):
For each matrix type the lowest successfully validated level in Table B.5.2-4 is considered as limit of quantification.
Matrix effects:
There was no need to consider matrix effects because of the use of stable isotope labelled standards.
Calibration (linearity):

- Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points): yes (9 levels)
- Accepted calibration range in concentration units: 0.5-250 ng/mL
- Accepted calibration range in mass fraction units: 0.003-1.25 mg/kg
- Calibration conducted with matrix matched standards: no
- Sample chromatogram spiked at LOQ demonstrates sufficient sensitivity and signal-to-noise ratio?: yes

Confirmation
The study does not contain validation data from confirmatory transitions or additional fragment ions.

Conclusion

The analytical method No. 00922 is applicable for beta-cyfluthrin in dry crops, commodities with high water content, and commodities with high oil content. Blank values in chromatograms are generally below 30 % of LOQ in all matrices tested. This demonstrates a sufficient selectivity of the method.
The method is not validated by an independent laboratory. Therefore, the method is considered as additional confirmatory method and is applicable for pre-registration studies.

Table B.5.2-4: Validation of the method No: 00922 by Schöning (2005) for residues in food of plant origin

Reference	Matrix	Detection method	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Schöning (2005), MET2006-93	oil seed rape (seeds)	LC-MS/MS C18 column, ESI+ m/z 451→191	0.01 0.10	90 89	2.7 1.6	5 5
	cereal straw		0.01 0.10	92 79	4.9 2.3	5 5
	cereal green material		0.01 0.10	88 88	5.5 2.3	5 5
	cereal grain		0.01 0.10	92 97	2.2 3.7	5 5
	sugar beet leafs		0.01 0.10	94 97	11 4.9	5 5
	sugar beet body		0.01 0.10	81 88	3.3 4.5	5 5

B.5.2.1.2 Extraction efficiency of analytical methods used for samples of plant origin

Within a metabolism study of seed treated sugar beets (Bongartz, 2013; ASB2014-7886), extraction efficiency tests were conducted. Sugar beet roots (10 x application rate of normal seed treatment) were harvested at maturity and extracted with acetone/water (2/1, v/v) according to the conditions of the residue methods 00922, S19 (00086/M088) and 00255. The extraction rate amounted to 51.5 % (0.027 mg/kg). The results are compared to the HPLC method BETACYFLU3LS used in the metabolism study (see Table B.5.2-5).
In both cases, beta-cyfluthrin was the main component in the extractable portion of TRR. Both solvent systems extracted a similar amount of beta-cyfluthrin (0.019 and 0.022 mg/kg, respectively) from beet roots. Even considering the higher total radioactive residue in the beet roots used in the study of monitoring solvents (0.053 mg/kg compared to 0.048 mg/kg), the extraction efficiency of acetone/water (2/1, v/v) is excellent (105 %).

Table B.5.2-5: Extractability of [fluorophenyl-UL-^{14}C]beta-cyfluthrin from sugar beet roots with solvents used in monitoring methods compared to the extractability in the metabolism study

	extraction used in the metabolism investigations		extraction according to residue methods[1]	
	% of TRR	mg/kg	% of TRR	mg/kg
total extractable	63.5	0.030	51.5	0.027
amount of parent compound in the extract	40.1	0.019	41.1	0.022
solids	36.5	0.017	48.5	0.026
sum of TRR		0.048		0.053
extraction efficiency	(0.022/0.019) × (0.048/0.053) = 105 %			

[1)] The extraction using the solvents from monitoring methods was conducted with sugar beets from the same trail. However, not identical crops wwere used 3 months after the inverstigation in the metabolism study.

In a second study on metabolism of ^{14}C-labelled cyfluthrin in tomatoes (Wagner and Neitzel, 1986; RIP9400821) pure acetone was used to rinse the radioactive residue from tomato fruits and leaves. At

harvest, 86 % of TRR was rinsed from tomato fruits and 97 % of TRR was rinsed from tomato leaves. Most of remaining radioactivity was extracted in a second step with dichloromethane.
In the study of Airs (2014, ASB2014-7715), the extraction with pure acetone followed by extraction with dichloromethane (= metabolism study) was compared with the extraction of the monitoring methods (DFG S19). For the comparison, samples from different matrix groups with incurred residues had been used.
In the first test barley grain, lettuce and oil seed rape pods with incurred residues were extracted with acetone/water (barley grain, lettuce) or acetone/acetonitrile (oil seed rape pods), which is the procedure of DFG S19 (GAW0002) method. In the second trial the solvents acetone and dichloromethane from the metabolism study (Wagner and Neitzel, 1986, RIP9400821) were applied. All extracts were cleaned-up by gel permeation chromatography and silica gel chromatography. Residues of beta-cyfluthrin were quantified by gas chromatography with mass spectrometric detection (GC-MS).
Treated samples with incurred residues were analysed along with untreated samples fortified with beta-cyfluthrin which acted as procedural recovery specimens. Recoveries were performed at fortification levels of 0.01 (LOQ) and 0.1 mg/kg.
Procedural recovery values were within the acceptable range of 70 to 110 %, with the exception of the recoveries performed by the metabolism method for the high oil crop (see Table B.5.2-6).
The extracted amount of beta-cyfluthrin from samples with incurred residues was either similar with both extraction methods (see lettuce), or better with the solvents of the monitoring method (barley grain; oil seed rape pods).
The results demonstrate that the extraction efficiency of the DFG S19 (GAW0002) method is equivalent to or greater than that obtained using the metabolism extraction method thus demonstrating that this methodology is suitable for use for the determination of beta-cyfluthrin in crops.

Table B.5.2-6: Extractability of beta-cyfluthrin from three matrix types (barley grain, lettuce and oil seed rape pods)

	extraction with acetone and dichloromethane (used in metabolism study)		**extraction in accordance to DFG S19 (used in monitoring methods)**	
	mg/kg detected	Procedural recovery	mg/kg detected	Procedural recovery
Barley grain	0.075	90 %	0.103	88 %
lettuce	0.35	75 %	0.36	80 %
oil seed rape pods	<LOQ	26 %	0.022	100 %

B.5.2.1.3 Methods which do not fulfill the requirements

All analytical methods for residues in plant materials reported in the DAR of cyfluthrin (Germany 1996, ASB2010-10436) do not fulfil the requirements of SANCO/825/00 rev. 8.1.

Table B.5.2-7: List of methods, which do not fulfil requirements

Author(s) and year	Annex point/Report No	Reason
Specht and Thier (1987) MET9400012	KCA 4.1.2/01 + 4.2/83 Report No.: 00086	First description of DFG S19 (original version; contains no data for cyfluthrin. (method described in DAR 1996)
Specht, Pelz, Gilsbach (1995) ASB2014-12216	KCA 4.2/84 Report No.: 00086 mod.extraction	Description of modified version of DFG S19; contains no data reported for cyfluthrin
Winkelmann (1989) ASB2014-12217	KCA 4.2/87 00086/M003/E003, Study protocal BAY-8806	only one recovery experiment at 0.01 mg/kg level (method described in DAR 1996)

Specht (1989) ASB2009-9416	KCA 4.1.2/02 Report No.: MO-04-002929, Edition Number: M-001160-01-1	Supplements E018, E019, E023, E024, E038 - E040, Modification M016 to method 00086 (DFG S19) only two recovery experiment at 0.01 mg/kg level for two matrices (sugar beet leaf and sugar beet root) and one experiment at 0.257 mg/kg level (sugar beet leaf) (method described in DAR 1996)
Specht (1989) MET9500021	KCA 4.1.2/73 00086/M016	only two recovery experiments at 0.01 mg/kg and one fortification experiment a t 0.5 mg/kg for each matrix investigated (method described in DAR 1996)
Nolting, Siebers and Köhle (1991) MET9400014	KCA 4.2/01 Report No.: MO-99-003969	no calibration shown, no recovery values reported; LOQ not sufficient (method described in DAR 1996)

B.5.2.1.4 Additional studies/reports provided by the applicant, which are not needed

Table B.5.2-8: List of additional methods for plant materials, which are not considered to be essential

Author(s) and year	Annex point/Report No.	Reason
Wagner (1981) RIP9400729	KCA 4.2/10 Report No.: I385, Edition Number: M-022605-01-2 Method Report No.: RA-998/81	The study describes the GC-ECD **method I385**, which is working with defatting with hexane of acetonitrile extracts, phoshate precipitation and subsequent chromatography on silica. GC analysis is done with packed columns.
Brennecke (1984) RIP9400733	KCA 4.2/21 Report No.: 00002 Edition Number: M-016057-03-1	The study describes the **method 00002**, which was developed for triadimol and triadimefon. Samples are extracted with aceotne/water (2/1; v/v). Extracts are partitioned into dichloromethane, cleaned by chromatography on silica and finally by gel permeation chromatography. Final determination is done by GC-NPD with packed columns.
Blass (1985) RIP9401113	KCA 4.1.2/04 Report No.: MO-03-010452, Edition Number: M-105171-01-1	The study describes the modification M007 of method 00002. Main modifications refer to the apllicability of the method for cyfluthrin.
Blass (1985) ASB2014-12188	KCA 4.2/31 00002/M007/E011	The study describes supplement E011 of modification 007 of method 00002 (validation data for cyfluthrin).
Blass (1985) ASB2014-12173	KCA 4.2/22 00002/M007/E012	The study describes supplement E012 of modification 007 of method 00002 (validation data for cyfluthrin).
Blass (1985) ASB2014-12176	KCA 4.2/23 00002/M007/E013	The study describes supplement E013 of modification 007 of method 00002 (validation data for cyfluthrin).
Blass (1985) ASB2014-12180	KCA 4.2/24 00002/M007/E014	The study describes supplement E014 of modification 007 of method 00002 (validation data for cyfluthrin).
Blass (1985) ASB2014-12181	KCA 4.2/25 00002/M007/E015	The study describes supplement E015 of modification 007 of method 00002 (validation data for cyfluthrin).
Blass (1985) ASB2014-12186	KCA 4.2/30 00002/M007/E016	The study describes supplement E016 of modification 007 of method 00002 (validation data for cyfluthrin).
Blass (1985) ASB2014-12182	KCA 4.2/26 00002/M007/E017	The study describes supplement E017 of modification 007 of method 00002 (validation data for cyfluthrin).

Blass (1985) ASB2014-12183	KCA 4.2/27 00002/M007/E018	The study describes supplement E018 of modification 007 of method 00002 (validation data for cyfluthrin).
Blass (1985) ASB2014-12184	KCA 4.2/28 00002/M007/E019	The study describes supplement E019 of modification 007 of method 00002 (validation data for cyfluthrin).
Blass (1985) ASB2014-12185	KCA 4.2/29 00002/M007/E020	The study describes supplement E020 of modification 007 of method 00002 (validation data for cyfluthrin).
Wagner (1980) RIP9400728	KCA 4.2/32 Report No.: 00010, Edition Number: M-007384-02-1	The study describes the GC-ECD **method 0010/I356**, which is working with phosphate precipitation, silica gel chromatography and packed GC columns. (old method No: I356)
Burger (1988) RIP9401109	KCA 4.2/41 00010/M015	The study describes modification M015 of method 00010.
Burger (1988) RIP9401110	KCA 4.2/42 00010/M016	The study describes modification M016 of method 00010.
Burger (1988) RIP9401111	KCA 4.2/43 00010/M017	The study describes modification M017 of method 00010.
Burger (1988) RIP9401112	KCA 4.2/44 00010/M018	The study describes modification M018 of method 00010.
Burger (1988) RIP9500538	KCA 4.2/08 00010/M019	The study describes modification M019 of method 00010.
Burger (1988) RIP9500539	KCA 4.2/09 00010/M020	The study describes modification M020 of method 00010.
Burger (1988) ASB2009-9157	KCA 4.1.2/61 MO-04-002884	The study describes modifications M019 and M020 of method 00010.
Wagner (1980) MET2005-48	KCA 4.2/07 MO-03-011062	The study describes supplements E001 - E023 and modifications M001, M002, M004 - M018 of method 00010
Blass (1985) RIP9401083	KCA 4.2/18 00010/E011	The study describes supplement E011 of method 00010.
Blass (1987) RIP9401084	KCA 4.2/37 00010/E012	The study describes supplement E012 of method 00010.
Blass (1985) RIP9401085	KCA 4.2/19 00010/E013	The study describes supplement E013 of method 00010.
Blass (1983) RIP9401086	KCA 4.2/17 00010/E014	The study describes supplement E014 of method 00010.
Blass (1985) RIP9401087	KCA 4.2/33 00010/E015	The study describes supplement E015 of method 00010.
Blass (1985) RIP9401088	KCA 4.2/34 00010/E016	The study describes supplement E016 of method 00010.
Blass (1987) RIP9401089	KCA 4.2/38 00010/E017	The study describes supplement E017 of method 00010.
Blass (1983) RIP9401090	KCA 4.2/16 00010/E018	The study describes supplement E018 of method 00010.
Blass (1983) RIP9401091	KCA 4.2/20 00010/E019	The study describes supplement E019 of method 00010.
Blass (1987) RIP9401092	KCA 4.2/39 00010/E020	The study describes supplement E020 of method 00010.
Blass (1987) RIP9401093	KCA 4.2/36 00010/E021	The study describes supplement E021 of method 00010.

Blass (1987) RIP9401094	KCA 4.2/35 00010/E022	The study describes supplement E022 of method 00010.
Blass (1988) RIP9401095	KCA 4.2/40 00010/E023	The study describes supplement E023 of method 00010.
Blass (1987) RIP9400737	KCA 4.1.2/23 + 4.2/59 Report No.: 00015, Edition Number: M-007653-01-2	The study describes the GC-ECD **method 0015**, which is working with partition of extracts into dichloromethane and subsequent chromatography on silica, florisil and C18. GC analysis is done with packed columns. (old method No: I640)
Blass (1989) RIP9401240	KCA 4.2/71 00015/M003	The study describes modification M003 of method 00015.
Blass (1988) RIP9401241	KCA 4.2/72 00015/M004	The study describes modification M004 of method 00015.
Blass (1988) RIP9401239	KCA 4.1.2/25 MO-04-008304	The study describes modifications M002 - M004 of method 00015.
Ohs (1989) RIP9401242	KCA 4.1.2/26 00015/M005	The study describes modification M005 of method 00015.
Blass (1989) RIP9500543	KCA 4.1.2/27 + 4.2/73 00015/M006,	The study describes modification M006 of method 00015.
Blass (1989) RIP9401243	KCA 4.1.2/28 + 4.2/74 00015/M007	The study describes modification M007 of method 00015.
Blass (1991) RIP9401244	KCA 4.1.2/29 + 4.2/76 MO-04-009012	The study describes modification M009 of method 00015.
Seym (1992) RIP9500545	KCA 4.1.2/31 00015/M010	The study describes modification M010 of method 00015.
Seym (1993) RIP9401245	KCA 4.2 /80 00015/M012	The study describes modification M012 of method 00015.
Seym (1993) RIP9401246	KCA 4.1.2/33 MO-04-008318	The study describes modifications M012, M013 of method 00015.
Blass (1987) RIP9401230	KCA 4.2/63 00015/E001	The study describes supplement E001 of method 00015.
Blass (1988) RIP9401231	KCA 4.2/64 00015/E002	The study describes supplement E002 of method 00015.
Blass (1988) RIP9401232	KCA 4.2/65 00015/E003	The study describes supplement E003 of method 00015.
Blass (1988) RIP9401233	KCA 4.2/66 00015/E004	The study describes supplement E004 of method 00015.
Blass (1988) RIP9401234	KCA 4.2/67 00015/E005	The study describes supplement E005 of method 00015.
Blass (1988) RIP9401235	KCA 4.2/68 00015/E006	The study describes supplement E006 of method 00015.
Ohs (1989) RIP9401236	KCA 4.2/75 00015/E007	The study describes supplement E007 of method 00015.
Blass (1988) RIP9401237	KCA 4.2/69 00015/E009	The study describes supplement E009 of method 00015.
Blass (1988) RIP9401238	KCA 4.2/70 00015/E011	The study describes supplement E011 of method 00015.
Blass (1987) ASB2009-9155	KCA 4.1.2/24 MO-04-008285	The study describes supplements E001-E009, E011 of method 00015.

Ohs (1989) RIP9500542	KCA 4.1.2/62 00015/E010	The study describes supplement E010 of method 00015.
Ohs (1991) RIP9500547	KCA 4.1.2/30 00015/M009/E012	The study describes supplement E012 to modification M009 of method 00015.
Seym (1992) RIP9500548	KCA 4.1.2/32 + 4.2/78 MO-04-008315	The study describes supplement E015 of modification M010 of method 00015
Seym (1992) RIP9500549	KCA 4.2/79 00015/M010/E016	The study describes supplement E016 of modification M010 of method 00015
Seym (1996) ASB2014-12214	KCA 4.2 /81 00015/M013/E017	The study describes supplement E017 of modification M013 of method 00015
Seym (1997) ASB2014-12215	KCA 4.2 /82 00015/M013/E018	The study describes supplement E018 of modification M013 of method 00015
Harbin, Gronberg, Minor, Bailey, Freeseman and Pfankuche (1985) RIP9400734	KCA 4.1.2/36 + 4.2/60 Report No.: 00033, Edition Number: M-009396-01-1 Method Report No.: I577	The study describes the GC-ECD **method 0033**, which allows the determination of three metabolites, but not of the parent compound. The method is not sufficiently validated. (old method No: I577)
Blass (1988) RIP9401124	KCA 4.1.2/37 + 4.2/61 00033/E001	The study describes supplement E001 of method 00033.
Blass (1988) RIP9401125	KCA 4.1.2/38 + 4.2/62 00033/E002	The study describes supplement E002 of method 00033.
Blass (1985) RIP9400736	KCA 4.1.2/09 + 4.2 /45 Report No.: 00047, Edition Number: M-008277-01-2	The study describes the GC-ECD **method 0047**, which is working with partition of extracts into dichloromethane and subsequent chromatography on florisil. GC analysis is done with packed columns. (old method No: I598)
Blass (1985) RIP9401228	KCA 4.1.2/21 + 4.2 /53 00047/M001	The study describes modification M001 of method 00047.
Blass (1986) RIP9401217	KCA 4.1.2/10 + 4.2 /55 00047/E001	The study describes supplement E001 of method 00047.
Blass (1986) RIP9401218	KCA 4.1.2/11 + 4.2 /56 00047/E002	The study describes supplement E002 of method 00047.
Blass (1986) RIP9401219	KCA 4.1.2/12 + 4.2 /46 00047/E003	The study describes supplement E003 of method 00047.
Blass (1986) RIP9401220	KCA 4.1.2/13 + 4.2 /47 00047/E004	The study describes supplement E004 of method 00047.
Blass (1986) RIP9401221	KCA 4.1.2/14 + 4.2 /48 00047/E005	The study describes supplement E005 of method 00047.
Blass (1986) RIP9401222	KCA 4.1.2/15 + 4.2 /57 00047/E006	The study describes supplement E006 of method 00047.
Blass (1985) RIP9401223	KCA 4.1.2/16 + 4.2 /49 00047/E007	The study describes supplement E007 of method 00047.
Blass (1986) RIP9401224	KCA 4.1.2/17 + 4.2 /50 00047/E008	The study describes supplement E008 of method 00047.
Blass (1986) RIP9401225	KCA 4.1.2/18 + 4.2 /51 00047/E009	The study describes supplement E009 of method 00047.
Blass (1987) RIP9401226	KCA 4.1.2/19 + 4.2 /58 00047/E010	The study describes supplement E010 of method 00047.
Blass (1986) RIP9401227	KCA 4.1.2/20 + 4.2 /52 00047/E011	The study describes supplement E011 of method 00047.

Blass (1985) RIP9401229	KCA 4.1.2/22 + 4.2 /54 00047/M001/E012	The study describes supplement E012 of modification M001 of method 00047.
Specht and Thier (1990) ASB2009-9416	KCA 4.2/83 Report No.: 00086 Edition Number: M-006227-03-2	The study presents additional validation data obtained with **method DFG S19**. (old method No: 00086)
Specht (1989) RIP9401247	KCA 4.2/85 00086/E002	The study describes supplement E002 of method 00086 (DFG S19).
Specht and Thier (1989) RIP9400738	KCA 4.1.2/34 MO-03-011361	The study presents additional validation data obtained with method DFG S19.
Specht (1989) RIP9401248	KCA 4.2/86 00086/M003	The study describes modification M003 of method 00086 (DFG S19).
Specht (1991) RIP9401250	KCA 4.2/88 00086/M015	The study describes modification M015 of method 00086 (DFG S19).
Ohs (1992) RIP9400739	KCA 4.2/77 Report No.: 00255, Edition Number: M-009335-02-1 Method Report No.: RA-321/91	The study describes the LC-GC-ECD **method 0255**, which requires not commonly available equipment.

B.5.2.2 Analytical methods for the determination of residues in or on food and feed of animal origin

B.5.2.2.1 Acceptable methods/reports

Study 1

Data point:	KCA 4.2/91
Report:	Enforcement method 00086/M045 for the determination of residues of cyfluthrin in materials of animal origin – validation of DFG method S19 (extended version), Steinhauer (2002) Company: Bayer CropScience Report No.: 00086/M045, Edition No.: M-052341-01-1, MET2005-856
Guideline(s):	Yes (Guidance document SANCO/825/00 rev. 6 of 20/06/00)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods:

Fortified analyte(s):
cyfluthrin (containing 50 % beta-cyfluthrin and 50 % two other diastereoisomers)

Analyte(s) determined as:
cyfluthrin (containing 50 % beta-cyfluthrin and 50 % two other diastereoisomers)

Principle of the method:
The modular version of DFG S19 method was used. Cyfluthrin was extracted from milk, egg, and meat with acetone/water (2/1, v/v). Thereafter, ethyl acetate/cyclohexane (1/1, v/v) and sodium chloride were added to the extracts for liquid-liquid partition. An aliquot of the organic phase was evaporated to dryness. Animal fat was extracted with acetonitrile/acetone (9/1, v/v) in the presence of synthetic calcium silicate (trade name Calflo E) and Celite. The organic phase was filtered and evaporated to dryness.
For all matrices the evaporated extract was reconstituted in ethyl acetate/isooctane and cleaned up by gel permeation chromatography (GPC) on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane (1/1, v/v) as eluent. The collected extracts were further cleaned on a mini silicagel column. The sample concentration in final extracts of milk, meat and eggs was 2.34 g/mL. Fat extracts contained residues of 1.2 g sample per mL.
Final extracts were analysed for residues of cyfluthrin by gas chromatography using a DB-1 capillary column with electron capture detector. The detected ions resulted in four separate GC peaks with two peaks belonging to beta-cyfluthrin.

Results

Selectivity (specificity):
Signals from interferences >30 % of LOQ in blank samples were not detected.
Recovery (accuracy):
For accuracy of analytical results see Table B.5.2-9.
Repeatability (precision):
For repeatability of analytical results see Table B.5.2-9.

Limit of quantification (LOQ):
For each matrix type the lowest successfully validated level in Table B.5.2-9 is considered as limit of quantification.
Matrix effects:
Matrix effects are not quantified in this study.
Calibration (linearity):

- Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points): yes (10 levels)
- Accepted calibration range in concentration units: 11.5-2300 ng/mL
- Accepted calibration range in mass fraction units: 0.010-1.90 mg/kg (fat)
  0.005-1.00 mg/kg (other)
- Calibration conducted with matrix matched standards: no
- Sample chromatogram spiked at LOQ demonstrates sufficient sensitivity and signal-to-noise ratio?: yes

Confirmation
The study does not contain sufficient validation data obtained with another GC column or an additional detection system.

Conclusion

The analytical enforcement method 00086/M045 (DFG S19) as validated by Steinhauer (2002) is suitable as enforcement method for cyfluthrin/beta-cyfluthrin in milk, meat, fat and eggs. A confirmatory method is not sufficiently validated.
Blank values in chromatograms for quantification and confirmatory transitions, are generally below 30 % of LOQ in all matrices tested. This demonstrates a sufficient selectivity of the method.

Table B.5.2-9: Validation of the enforcement method 00086/M045 (DFG S19) by Steinhauer (2002) for residues in food of animal origin

Reference	Matrix	Detection method	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Steinhauer (2002) MET2005-856	Milk	GC-ECD, DB-1 capillary column	0.01 0.10	83 85	14 2.4	5 5
	Egg		0.01 0.10	93 83	7.4 2.2	5 5
	Meat		0.01 0.10	77 74	3.2 5.9	5 5
	Fat		0.02 0.20	76 82	4.2 4.5	5 5

Study 2

Data point: KCA 4.2/97

Report: Merdian (2009), Validation of the DFG method S19-based on Bayer CropScience method 00086/M045 for the determination of residues of cyfluthrin in liver and kidney matrices, using GC/ECD,
Bayer CropScience, Report No: PTRL Report No.: P 1752 G
Edition No.: M-348265-01-1 (R-27984), ASB2014-2284

Guideline(s): Yes (EC Guidance document on residue analytical methods, SANCO/825/00 rev. 7 17/03/04 and OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods:

Fortified analyte(s):
cyfluthrin (containing 50 % beta-cyfluthrin and 50 % two other diastereoisomers)

Analyte(s) determined as:
cyfluthrin (containing 50 % beta-cyfluthrin and 50 % two other diastereoisomers)

Principle of the method:
The modular version of DFG S19 method was used. Cyfluthrin was extracted from liver and kidney with acetone/water (2/1, v/v). Thereafter, ethyl acetate/cyclohexane (1/1, v/v) and sodium chloride were added to the extracts for liquid-liquid partition. An aliquot of the organic phase was evaporated to dryness. The evaporated raw extract of both matrices was reconstituted in ethyl acetate/isooctane and cleaned up by gel permeation chromatography (GPC) on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane (1/1, v/v) as eluent. The collected extracts were further cleaned on a mini silicagel column. The sample concentration in final extracts of milk, meat and eggs was 2.92 g/mL.
Final extracts were analysed for residues of cyfluthrin by gas chromatography using a ZB-Multi Residue-1 capillary column with electron capture detector. The detected ions resulted in four separate GC peaks with two peaks belonging to beta-cyfluthrin. For confirmation, final extracts were analysed by GC-ECD using a DB-1701 capillary column.

Results

Selectivity (specificity):
Signals from interferences >30 % of LOQ in blank samples were not detected.
Recovery (accuracy):
For accuracy of analytical results see Table B.5.2-10.
Repeatability (precision):
For repeatability of analytical results see Table B.5.2-10.
Limit of quantification (LOQ):
For each matrix type the lowest successfully validated level in Table B.5.2-10 is considered as limit of quantification.
Matrix effects:
No matrix effects were observed when fortified sample extracts were injected as demonstrated by acceptable recoveries based on calibration solutions in solvent. Additionally, matrix matched standards per sample matrix were prepared and injected to show the effects of matrix on response. For cyfluthrin, signal enhancement below 10 % was observed for liver and kidney.
Calibration (linearity):

- Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points): yes (7 levels)
- Accepted calibration range in concentration units: 5-500 ng/mL
- Accepted calibration range in mass fraction units: 0.002-0.172 mg/kg
- Calibration conducted with matrix matched standards: no
- Sample chromatogram spiked at LOQ demonstrates sufficient sensitivity and signal-to-noise ratio?: yes

Confirmation
Signals obtained from two different GC capillary columns were used for quantification. All results were found to be valid (see Table B.5.2-10).

Conclusion

The analytical enforcement method 00086/M045 (DFG S19) as validated by Merdian (2009, ASB2014-2284) is suitable as enforcement method for cyfluthrin/beta-cyfluthrin in kidney and liver. A confirmatory method using a capillary column of different polarity is sufficiently validated.
Blank values in chromatograms for quantification and confirmatory transitions, are generally below 30 % of LOQ in all matrices tested. This demonstrates a sufficient selectivity of the method.

Table B.5.2-10: Validation of the enforcement method 00086/M045 (DFG S19) by Merdian (2009) for residues in kidney and liver

Reference	Matrix	Detection method	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Merdian (2009) ASB2014-2284	Liver	GC-ECD, ZB-Multi Residue-1 capillary column	0.01 0.1	91 83	14 16	6 6
	Kidney		0.01 0.1	91 88	16 13	6 5
	Liver	GC-ECD, VF-1701 MS capillary column	0.01 0.1	82 92	13 14	6 6
	Kidney		0.01 0.1	75 93	12 12	6 5

Study 3

Data point: KCA 4.2/92

Report: Reichert (2002), Independent Laboratory Validation of DFG Method S19 (Extended Revision) for the Determination of Residues of Cyfluthrin in Materials of Animal Origin (Bayer CropScience Enforcement Method 00086/M045),
Company: Bayer CropScience Study No: IF-02/00023853 (R-19692), Edition No.: M-075850-01-1, MET2005-640

Guideline(s): Yes (Guidance document SANCO/825/00 rev.6 of June 20, 2000)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods:

Fortified analyte(s):
cyfluthrin (containing 50 % beta-cyfluthrin and 50 % two other diastereoisomers)

Analyte(s) determined as:
cyfluthrin (containing 50 % beta-cyfluthrin and 50 % two other diastereoisomers)

Principle of the method:
The modular version of DFG S19 method was used. Cyfluthrin was extracted from milk and meat with acetone/water (2/1, v/v). Thereafter, ethyl acetate/cyclohexane (1/1, v/v) and sodium chloride were added to the extracts for liquid-liquid partition. An aliquot of the organic phase was evaporated to dryness.
For both matrices the evaporated extract was cleaned-up by gel permeation chromatography (GPC) on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane (1/1, v/v) as eluant. The collected extracts were further cleaned on a mini silicagel column. The sample concentration in final

extracts of milk and meat s was 2.25 g/mL.
Final extracts were analysed for residues of cyfluthrin by gas chromatography using a DB-1 capillary column with electron capture detector. The detected ions resulted in four separate GC peaks with two peaks belonging to beta-cyfluthrin. For confirmation final extracts were analysed by GC-ECD using a DB-1701 capillary column.

Results

Selectivity (specificity):
Signals from interferences >30 % of LOQ in blank samples were not detected.
Recovery (accuracy):
For accuracy of analytical results see Table B.5.2-11.
Repeatability (precision):
For repeatability of analytical results see Table B.5.2-11.
Limit of quantification (LOQ):
For each matrix type the lowest successfully validated level in Table B.5.2-11 is considered as limit of quantification.
Matrix effects:
Matrix effects are not quantified in this study.
Calibration (linearity):

- Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points): yes
- Accepted calibration range in concentration units: 10-300 ng/mL
- Accepted calibration range in mass fraction units: 0.004-0.133 mg/kg
- Calibration conducted with matrix matched standards: no
- Sample chromatogram spiked at LOQ demonstrates sufficient sensitivity and signal-to-noise ratio?: yes

Confirmation
Signals obtained from two different GC capillary columns were used for quantification. All results were found to be valid (see Table B.5.2-11).

Conclusion

The study is considered as a successful independent laboratory validation of the method 00086/M045 (DFG S19) described by Steinhauer (2002, MET2005-856).

Table B.5.2-11: Independent validation of the enforcement method 00086/M045 (DFG S19) by Reichert (2002) for residues in food of animal origin

Reference	Matrix	Detection method	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Reichert (2002) MET2005-640	milk	GC-ECD, DB-1 capillary column	0.01 0.10	96 87	5 9	5 5
	meat		0.01 0.10	93 87	2 4	5 5
	milk	GC-ECD, DB-1701 capillary column	0.01 0.10	89 85	7 7	5 5
	meat		0.01 0.10	91 79	6 1	5 5

Study 4

Data point:	KCA 4.2/94
Report:	Meyer, M. and Zietz, E., 2010, Independent Laboratory Validation of DFG Method S19 (Bayer CropScience Method 00086/M045) for the determination of residues of cyfluthrin in foodstuffs of animal origin using GC/ECD, Bayer CropScience Study No: P613097535, Edition No.: M-399396-01-1 ASB2014-2282
Guideline(s):	Yes (EU-guidance documents on residue analytical methods: SANCO/825/00-rev 7, 17 March 2004 and SANCO 3029/99 rev.4 11 July 2000; OECD document ENV/JM/MONO(2007)17, 13 August 2007)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods:

Fortified analyte(s):
cyfluthrin (containing 50 % beta-cyfluthrin and 50 % two other diastereoisomers)

Analyte(s) determined as:
cyfluthrin (containing 50 % beta-cyfluthrin and 50 % two other diastereoisomers)

Principle of the method:
The modular version of DFG S19 method was used. Cyfluthrin was extracted from egg, liver and kidney with acetone/water (2/1, v/v). Thereafter, ethyl acetate/cyclohexane (1/1, v/v) and sodium chloride were added to the extracts for liquid-liquid partition. An aliquot of the organic phase was evaporated to dryness. Animal fat was extracted with acetonitrile/acetone (9/1, v/v) in the presence of synthetic calcium silicate (trade name Calflo E) and Celite. The organic phase was filtered and evaporated to dryness.
For all matrices the evaporated extract was reconstituted in ethyl acetate/isooctane and cleaned-up by gel permeation chromatography (GPC) on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane (1/1, v/v) as eluent. The collected extracts were further cleaned on a mini silicagel column. The sample concentration in final extracts of eggs and fat was 2.3 g/mL. Extracts of liver and kidney contained residues of 2.9 g sample per mL at LOQ and 1.48 g/mL at 10 times LOQ.
Final extracts were analysed for residues of cyfluthrin by gas chromatography using a DB-1 capillary column with electron capture detector. The detected ions resulted in four separate GC peaks with two peaks belonging to beta-cyfluthrin. For confirmation final extracts were analysed by GC-ECD using a DB-1701 capillary column.

Results

Selectivity (specificity):
Signals from interferences >30 % of LOQ in blank samples were not detected.
Recovery (accuracy):
For accuracy of analytical results see Table B.5.2-12.
Repeatability (precision):
For repeatability of analytical results see Table B.5.2-12.
Limit of quantification (LOQ):
For each matrix type the lowest successfully validated level in Table B.5.2-12 is considered as limit of quantification.
Matrix effects:
Significant matrix effects (>20 %) were not observed.

Calibration (linearity):

- Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points): yes (8 level)
- Accepted calibration range in concentration units: 11-300 ng/mL
- Accepted calibration range in mass fraction units: 0.005-0.13 mg/kg (egg, fat)
  0.004-0.20 mg/kg (liver, kidney)
- Calibration conducted with matrix matched standards: no
- Sample chromatogram spiked at LOQ demonstrates sufficient sensitivity and signal-to-noise ratio?: yes

Confirmation
Signals obtained from two different GC capillary columns were used for quantification. All results were found to be valid (see Table B.5.2-12).

Conclusion

The study is considered as a successful independent laboratory validation of the method 00086/M045 (DFG S19) described by Steinhauer (2002, MET2005-856) and Merdian (2009, ASB2014-2284).

Table B.5.2-12: Independent validation of the enforcement method 00086/M045 (DFG S19) by Meyer and Zietz (2010) for residues in food of animal origin

Reference	Matrix	Detection method	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Meyer and Zietz (2010) ASB2014-2282	egg	GC-ECD, DB-1 capillary column	0.01 0.1	80 82	7.4 4.7	5 5
	fat		0.01 0.1	81 73	9.1 8.6	5 5
	liver		0.01 0.1	79 86	10.4 7.4	5 5
	kidney		0.01 0.1	95 89	13 4.5	5 5
	egg	GC-ECD, DB-1701 capillary column	0.01 0.1	97 89	5.2 8.7	5 5
	fat		0.01 0.1	88 73	6.1 8.5	5 5
	liver		0.01 0.1	81 84	10.4 5.9	5 5
	kidney		0.01 0.1	92 90	6.3 7.0	5 5

B.5.2.2.2 Extraction Efficiency of analytical methods used for samples of animal origin

Studies which confirm a sufficient extraction efficiency of the solvents used in the analytical method DFG S19, i.e. acetone/water (2/1, v/v) and acetonitrile/acetone (9/1, v/v), are not available.
In animal metabolism studies with laying hen (Chopade, McCann and Gentile, 1983, RIP9400869) and lactating cow (Shaw, Ayers and McCann, 1983, RIP9400870), radioactive residues of oral dosing of cyfluthrin in tissues and eggs were extracted using acetone/chloroform (2/1, v/v). Residues in fat were extracted in both studies with hexane. From muscle, fat, liver and kidney of the lactating cow, between 93 and 100 % of radioactivity were extracted with acetone/chloroform or hexane. More than 86 % of extracted radioactivity was identified as parent cyfluthrin. The only exception was kidney,

where cyfluthrin accounted only for 56 % of extracted radioactivity.
From kidney and liver of laying hens about 60 % of radioactivity was extracted with acetone/chloroform (2/1, v/v). From other tissues and eggs 75-83 % of the dosed radioactivity was extracted. However, in this case cyfluthrin accounted for only 15-48 % of extracted radioactivity in kidney, liver, skin and muscle. A higher portion of cyfluthrin was found in the extracts of fat and eggs, where 75 % and 90 % of the extracted radioactivity was identified as cyfluthrin. Summarising the hen metabolism study, it could be shown that parent cyfluthrin (i.e. the regulated residue) is sufficiently extractable from tissues of hens, although considering certain limitations for kidney and liver extraction.

B.5.2.2.3 Methods which do not fulfill the requirements

All analytical methods for residues in animal matrices reported in the DAR of cyfluthrin (Germany 1996, ASB2010-10436) do not fulfil the requirements of SANCO/825/00 rev. 8.1.

Table B.5.2-13: List of methods, which do not fulfil requirements

Author(s) and year	Annex point/Report No.	Reason
Shaw, Chopade, Ayeres and Gentile (1983) RIP9400740	KCA 4.1.2/52 + 4.2/14 Report No.: I476, Edition Number: M-066143-01-1 Method Report No.: MR-303/95	validation at one level with insufficient number of replicates

B.5.2.2.4 Additional studies/reports provided by the applicant, which are not needed

Table B.5.2-14: List of additional methods for animal materials, which are not considered to be essential

Author(s) and year	Annex point/Report No.	Reason
Eben, Fuchs, Kurz, Wuensche, Flucke (1987) TOX9401851	KCA 4.2/13 Report No.: 15849, Edition Number: M-038063-01-1	The study describes the metabolism of non-radiolabelled cyfluthrin in chicken. It does not allow to estimate extraction efficiency.

B.5.2.3 Analytical methods for the determination of residues in soil

B.5.2.3.1 Acceptable methods/reports

Study 1

Data point: KCA 4.2 / KCA 4.1.2/43

Report: Validation of DFG method S 19 with modified extraction for the determination of residues of cyfluthrin in soil; Weeren and Pelz, 1999, Specht & Partner No.: BAY-9906V, Az. M7706/99, MET1999-1227

Guideline(s): No (no guidelines available in 1999)

Deviations:

GLP: Yes

Acceptability: Yes

Materials and methods:

Fortified analyte(s):
cyfluthrin (containing 50 % beta-cyfluthrin and 50 % two other diastereoisomers)

Analyte(s) determined as:
cyfluthrin (containing 50 % beta-cyfluthrin and 50 % two other diastereoisomers)

Sample material:
Untreated loamy sand (standard soil 2.2, LUFA Speyer, Germany) was used in method validation. Analytical details of the soil are published at:
http://www.lufa-speyer.de/images/stories/StandardSoil.pdf

Principle of the method:
The modular version of DFG S19 method was used. Cyfluthrin was extracted from soil with acetone/water (2/1, v/v). Thereafter, ethyl acetate/cyclohexane (1/1, v/v) and sodium chloride were added to the extracts for liquid-liquid partition. An aliquot of the organic phase was evaporated to dryness.
The evaporated extract was reconstituted in ethyl acetate/isooctane and cleaned-up by gel permeation chromatography (GPC) on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane (1/1, v/v) as eluent. The sample concentration in final extracts was 2.3 g/mL.
Final extracts were analysed for residues of cyfluthrin by gas chromatography using a XTI-5 capillary column with electron capture detector. The detected ions resulted in four separate GC peaks with two peaks belonging to beta-cyfluthrin.

Results

Selectivity (specificity):
Signals from interferences >30 % of LOQ in blank samples were not detected.
Recovery (accuracy):
For accuracy of analytical results see Table B.5.2-15.
Repeatability (precision):
For repeatability of analytical results see Table B.5.2-15.
Limit of quantification (LOQ):
For each matrix type the lowest successfully validated level in Table B.5.2-15 is considered as limit of quantification.
Matrix effects:
Matrix effects are not discussed in the report.
Calibration (linearity):

- Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points): yes (9 levels)
- Accepted calibration range in concentration units: 20.1-4010 ng/mL
- Accepted calibration range in mass fraction units: 0.009-1.74 mg/kg
- Calibration conducted with matrix matched standards: no
- Sample chromatogram spiked at LOQ demonstrates sufficient sensitivity and signal-to-noise ratio?: yes

Confirmation
One final extract of each concentration level was measured in addition by GC-MS using m/z 206 for quantification. Because of the low number of experiments, this confirmatory method is not acceptable.

Conclusion

The analytical method DFG S19 validated by Weeren and Pelz (1999, MET1999-1227) is suitable as enforcement method for cyfluthrin/beta-cyfluthrin in soil.

Blank values in chromatograms for quantification and confirmatory transitions, are generally below 30 % of LOQ in all matrices tested. This demonstrates a sufficient selectivity of the method.
An additional confirmatory method for soil is not provided.

Table B.5.2-15: Validation of the method DFG 19 by Weeren and Pelz (1999) for residues in soil

Reference	Matrix	Detection method	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Weeren and Pelz (1999) MET1999-1227	standard soil 2.2	GC-ECD, XTI-5 capillary column	0.05 0.50	89 93	3.3 2.3	5 5

Study 2

Data point: KCA 4.1.2 /74

Report: Robinson, N., 2014, beta-cyfluthrin - Field soil dissipation of beta-Cyfluthrin from a field trial carried out in Southern France; M-482355-01-1 ! 20120152 ! R-30598 ASB2014-7708

Guideline(s): EPA OPPTS 850.6100; SANCO/3029/99 rev.4, July 11, 2000; SANCO/825/00 rev. 8.1, November 16, 2010

Deviations: no

GLP: Yes

Acceptability: Yes

Materials and methods:

Fortified analyte(s):
beta-cyfluthrin

Analyte(s) determined as:
beta-cyfluthrin

Sample material
A fully characterised silty clay loam (according to USDA) from Filis /France) was used.

Principle of the method:
The soil sample is extracted by shaking with acetone. The combined soil extract is adjusted to a known volume and an aliquot is evaporated to low volume to remove the acetone. Methanol/water (1/1, v/v) is added to the sample and the sample partitioned into hexane. The combined hexane extract is evaporated to dryness and redissolved in hexane. The sample concentration in final extracts in 1 g/mL.
The concentration of beta-cyfluthrin in final extracts is quantified by GC-MS using a HP-5ms column, in negative chemical ionisation mode with selected ion monitoring of three ions. The most sensitive ion (m/z = 207) was used for residue calculation.

Results

Selectivity (specificity):
Signals from interferences >30 % of LOQ in blank samples were not detected.
Recovery (accuracy):
For accuracy of analytical results see Table B.5.2-16.
Repeatability (precision):

For repeatability of analytical results see Table B.5.2-16.
Limit of quantification (LOQ):
For each matrix type the lowest successfully validated level in Table B.5.2-16 is considered as limit of quantification.
Matrix effects:
Significant enhancements by matrix effects are observed and considered by validation with matrix matched standards.
Calibration (linearity):

- Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points): yes (8 levels)
- Accepted calibration range in concentration units: 2.5-500 ng/mL
- Accepted calibration range in mass fraction units: 0.0025-0.50 mg/kg
- Calibration conducted with matrix matched standards: yes
- Sample chromatogram spiked at LOQ demonstrates sufficient sensitivity and signal-to-noise ratio?: yes

Confirmation
Final extracts of each concentration level were measured in addition by NCI GC-MS using m/z 209 and 171 for confirmation. For results see (see Table B.5.2-16).

Conclusion

The NCI-GC-MS method validated by Robinson (2014, ASB2014-7708) is suitable as enforcement method for cyfluthrin/beta-cyfluthrin in soil.
Blank values in chromatograms for quantification and confirmatory transitions, are generally below 30 % of LOQ in all matrices tested. This demonstrates a sufficient selectivity of the method.
An additional confirmatory method for soil is not required.

Table B.5.2-16: Validation of the NCI-GC-MS method by Robinson (2014) for residues in soil

Reference	Matrix	Detection method	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	No. of analyses
Robinson (2014) ASB2014-7708	silty clay loam from Filis /France)	m/z 207[a]	0.01 0.10	87 83	5.0 1.7	5 5
		m/z 209 [a]	0.01 0.10	88 83	4.5 1.6	5 5
		m/z 171 [a]	0.01 0.10	86 83	6.3 1.8	5 5

[a]) NCI-GC-MS, HP-5ms capillary column

B.5.2.3.2 Methods which do not fulfill the requirements

Table B.5.2-17: List of methods for soil, which do not fulfil requirements

Author(s) and year	Annex point/Report No.	Reason
Bachlechner (1990) MET9400013	KCA4.1.2/49 Report 00195 Bayer RA-498/90	no calibration data reported (study evaluated in DAR 1996)
Nolting, Siebers and Köhle (1991) MET9400014	DFG S23	no calibration shown, no recovery values reported; LOQ not sufficient (study evaluated in DAR 1996)
Yoshida, Yoshimoto and Takase (1981).	KCA 4.2/02 NR1197	no calibration shown; insufficient number of recovery tests, selectivity not demonstrated

MET2005-57		
Brennecke (1984) RIP9400733	KCA 4.1.2/03 + 4.2/21 Report No.: 00002 Edition Number: M-016057-03-1	The study describes the **method 00002**, which was developed for triadimol and triadimefon. Samples are extracted with aceotne/water (2/1; v/v). Extracts are partitioned into dichloromethane, cleaned by chromatography on silica and finally by gel permeation chromatography. Final determination is done by GC-NPD with packed columns.
Blass (1985) RIP9401113	KCA 4.1.2/04 Report No.: MO-03-010452, Edition Number: M-105171-01-1	The study describes the modification M007 of method 00002. Main modifications refer to the apllicability of the method for cyfluthrin.
Blass (1985) ASB2014-12188	KCA 4.2/31 00002/M007/E011	The study describes supplement E011 of modification 007 of method 00002, but not data for soil.
Blass (1985) ASB2014-12173	KCA 4.2/22 00002/M007/E012	The study describes supplement E012 of modification 007 of method 00002, but not data for soil.
Blass (1985) ASB2014-12176	KCA 4.2/23 00002/M007/E013	The study describes supplement E013 of modification 007 of method 00002, but not data for soil.
Blass (1985) ASB2014-12180	KCA 4.2/24 00002/M007/E014	The study describes supplement E014 of modification 007 of method 00002, but not data for soil.
Blass (1985) ASB2014-12181	KCA 4.2/25 00002/M007/E015	The study describes supplement E015 of modification 007 of method 00002, but not data for soil.
Blass (1985) ASB2014-12186	KCA 4.2/30 00002/M007/E016	The study describes supplement E016 of modification 007 of method 00002, but not data for soil.
Blass (1985) ASB2014-12182	KCA 4.2/26 00002/M007/E017	The study describes supplement E017 of modification 007 of method 00002, but not data for soil.
Blass (1985) ASB2014-12183	KCA 4.2/27 00002/M007/E018	The study describes supplement E018 of modification 007 of method 00002, but not data for soil.
Blass (1985) ASB2014-12184	KCA 4.2/28 00002/M007/E019	The study describes supplement E019 of modification 007 of method 00002, but not data for soil.
Blass (1985) ASB2014-12185	KCA 4.2/29 00002/M007/E020	The study describes supplement E020 of modification 007 of method 00002, but not data for soil.

B.5.2.4 Analytical methods for the determination of residues in drinking/surface water

B.5.2.4.1 Acceptable methods/reports

Study 1

Data point: KCA 4.2/03 (KCA 4.1.2/50)

Report: Method for gas chromatographic determination of cyfluthrin in drinking water, Koenig, T. (1992)
Report No.: 00271, Edition Number: M-012493-02-1
Method Report No.: RA-337/92; MET9400015

Guideline(s): No (no guidelines available)

Deviations:

GLP: No

Acceptability: Yes

Conclusion:

This study was evaluated and accepted in the DAR 1996. Considering requirements of SANCO/825/00 rev. 8.1, the method is acceptable for analysis of cyfluthrin/beta-cyfluthrin in drinking water with minor deficiencies (higher level was rested with 4 replicates only). A confirmatory technique and an ILV were not provided.

Study 2

Data point:	KCA 4.2/95
Report:	Analytical method 01342 for the determination of beta-Cyfluthrin in drinking and surface water by HPLC-MS/MS, method 01342, Braune, 2012 Method report no. MR-12/053, Report No: 01342, Edition No.: M-436448-01-1, ASB2014-7713
Guideline(s):	Yes, EC Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1 and European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements, SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes/

Materials and methods:

Fortified analyte(s):
beta-cyfluthrin

Analyte(s) determined as:
beta-cyfluthrin

Principle of the method:
Water samples are diluted with acetonitrile (1/1; v/v). Residues of beta-cyfluthrin are extracted by SPE using a C18 cartridge. The cartridge is dried by vacuum and residues are eluted with acetonitrile. The eluate is evaporated to dryness and reconstituted in acetonitrile/water (1/1; v/v). Sample concentration in final extracts is 0.1 L/mL. The concentration of beta-cyfluthrin in final extracts is quantified by LC-MS/MS using an Ascentis Express C18 column, positive electrospray ionisation and two MRM transitions (m/z 451→191, m/z 451→127).

Results

Selectivity (specificity):
Signals from interferences >30 % of LOQ in blank samples were not detected.
Recovery (accuracy):
For accuracy of analytical results see Table B.5.2-18.
Repeatability (precision):
For repeatability of analytical results see Table B.5.2-18.
Limit of quantification (LOQ):
For each matrix type the lowest successfully validated level in Table B.5.2-18 is considered as limit of quantification.

Matrix effects:
The MS/MS detection is affected by the matrix. Peak area suppression for the 1st and 2nd mass transition up to 30 % compared to the peak area in solvent was observed for beta-cyfluthrin. Matrix effects were eliminated by using matrix-matched standard solutions.
Calibration (linearity):

- Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points): yes (6 levels)
- Accepted calibration range in concentration units: 0.25-25 ng/mL
- Accepted calibration range in mass fraction units: 0.0025-0.25 µg/L
- Calibration conducted with matrix matched standards: yes
- Sample chromatogram spiked at LOQ demonstrates sufficient sensitivity and signal-to-noise ratio?: yes

Confirmation
Signals obtained from two different MRM transitions were used for quantification. Both results were found to be valid (see Table B.5.2-18).

Conclusion

The analytical method 01342 as validated by Braune (2012, ASB2014-7713) is suitable as enforcement method for beta-cyfluthrin in drinking water. A confirmatory method for drinking is provided by full validation of a second MS/MS transition.
Blank values in chromatograms for quantification and confirmatory transitions, are generally below 30 % of LOQ in all matrices tested. This demonstrates a sufficient selectivity of the method.

Table B.5.2-18: Validation of the method 01342 by Braune (2012) for residues in surface water

Reference	Matrix	Detection method	Fortification level [µg/L]	Average recovery [%]	RSD [%]	No. of analyses
Braune (2012) ASB2014-7713	River Rhine	LC-MS/MS, C18 column, ESI, m/z 451→191	0.01 0.10	96 95	2.5 5.9	5 5
	River Rhine	LC-MS/MS, C18 column, ESI, m/z 451→127	0.01 0.10	96 95	2.9 6.1	5 5

Study 3

Data point: KCA 4.2/96

Report: Bomke, 2013, Independent Laboratory Validation (ILV) of the analytical method 01342 for the determination of beta-cyfluthrin in drinking and surface water using HPLC-MS/MS.
Bayer CropScience Report No: MR-13/024, Edition No.: M-457906-01-1, ASB2014-7714

Guideline(s): Yes, EC Guidance Document SANCO/825/00 rev. 8.1; U.S. EPA Guideline, OPPTS 860.1340 Residue Analytical Method of August 1996

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods:

Fortified analyte(s):
beta-cyfluthrin

Analyte(s) determined as:
beta-cyfluthrin

Principle of the method:
Water samples are diluted with acetonitrile (1/1; v/v). Residues of beta-cyfluthrin are extracted by SPE using a C18 cartridge. The cartridge is dried by vacuum and residues are eluted with acetonitrile. The eluate is evaporated to dryness and reconstituted in acetonitrile/water (1/1; v/v). Sample concentration in final extracts is 0.1 L/mL. The concentration of beta-cyfluthrin in final extracts is quantified by LC-MS/MS using an Ascentis Express C18 column, positive electrospray ionisation and two MRM transitions (m/z 451→191, m/z 451→127).

Results

Selectivity (specificity):
Signals from interferences >30 % of LOQ in blank samples were not detected.
Recovery (accuracy):
For accuracy of analytical results see Table B.5.2-19.
Repeatability (precision):
For repeatability of analytical results see Table B.5.2-19.
Limit of quantification (LOQ):
For each matrix type the lowest successfully validated level in Table B.5.2-19 is considered as limit of quantification.
Matrix effects:
The MS/MS detection is affected by the matrix. Peak area suppression for the 1st and 2nd mass transition up to 30 % compared to the peak area in solvent was observed for beta-cyfluthrin. Matrix effects were eliminated by using matrix-matched standard solutions.
Calibration (linearity):

- Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points): yes (9 levels)
- Accepted calibration range in concentration units: 0.25-25 ng/mL
- Accepted calibration range in mass fraction units: 0.0025-0.25 µg/L
- Calibration conducted with matrix matched standards: yes
- Sample chromatogram spiked at LOQ demonstrates sufficient sensitivity and signal-to-noise ratio?: yes

Confirmation
Signals obtained from two different MRM transitions were used for quantification. Both results were found to be valid (see Table B.5.2-19.

Conclusion

The study is considered as a successful independent laboratory validation of the method 01342, which was first validated in Study 2 by Braune (2012, ASB2014-7713).

Table B.5.2-19: Independent validation of the method 01342 by Bomke (2013, ASB2014-7714) for residues in surface water

Reference	Matrix	Detection method	Fortification level [µg/L]	Average recovery [%]	RSD [%]	No. of analyses
Braune (2012) ASB2014-7713	River Saône	LC-MS/MS, C18 column, ESI, m/z 451→191	0.01 0.10	102 98	5.0 1.2	5 5
	River Saône	LC-MS/MS, C18 column, ESI, m/z 451→127	0.01 0.10	97 98	6.4 1.6	5 5

B.5.2.4.2 Methods which do not fulfill the requirements

Table B.5.2-20: List of methods, which do not fulfil requirements

Author(s) and year	Annex point/Report No.	Reason
Nolting, Siebers and Köhle (1991) MET9400014	KCA 4.2/01 Report No.: MO-99-003969	no calibration shown, no recovery values reported; LOQ not sufficient
Sommer (1999) MET1999-1224	KCA 4.2/04 Report No.: 00587	LOQ not sufficient

B.5.2.5 Analytical methods for the analysis in air

B.5.2.5.1 Acceptable methods/reports

Study 1

Data point: KCA 4.2 / KCA 4.1.2/77

Report: beta-Cyfluthrin: Analytical method for determination in air, Method P 2474G. Bacher, 2013
Edition No. M-462363-01-1, ASB2014-7709

Guideline(s): Yes, EC Guidance document on residue analytical methods, SANCO/825/00 rev. 8.1

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods:

Fortified analyte(s):
beta-cyfluthrin

Analyte(s) determined as:
beta-cyfluthrin

Principle of the method:
Air is sucked through XAD adsorption tubes with two layers (A and B) at about 1.0 L/min for 6 hours (total air sampling volume about 0.36 m^3). The adsorption material is extracted with ethyl acetate. The sample concentration in final extracts was 0.036 m^3/mL. Extracts are analysed by gas chromatography with tandem mass spectrometric detection (GC-MS/MS) using an Agilent VF-5ms capillary column and electron impact ionisation. Two parent-daughter ion transitions (m/z 163→127; m/z 226→206) are monitored. The diastereomers of beta-cyfluthrin (isomer II and isomer IV) are quantified separately.

Results

Selectivity (specificity):
Signals from interferences >30 % of LOQ in blank samples were not detected.
Recovery (accuracy):
For accuracy of analytical results see Table B.5.2-21. All recovery data refer to the amount of beta-cyfluthrin found in layer A. The breakthrough from layer A to layer B of XAD absorption tubes was <4 % during 6 h of sampling period with warm humid air.
Repeatability (precision):
For repeatability of analytical results see Table B.5.2-21. All repeatability data refer to the amount of beta-cyfluthrin found in layer A.
Limit of quantification (LOQ):
For each matrix type the lowest successfully validated level in Table B.5.2-21 is considered as limit of quantification.
Matrix effects:
Matrix effects are not discussed in the report.
Calibration (linearity):

- Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points): yes (5 levels)
- Accepted calibration range in concentration units: 0.75-50 ng/mL
- Accepted calibration range in mass fraction units: 0.021-1.39 µg/m^3
- Calibration conducted with matrix matched standards: no
- Sample chromatogram spiked at LOQ demonstrates sufficient sensitivity and signal-to-noise ratio?: yes

Confirmation
Signals obtained from two different MRM transitions were used for quantification. Both results were found to be valid (see Table B.5.2-21).

Conclusion

The analytical method P 2474 as validated by Bacher (2013, ASB2014-7709) is suitable as enforcement method for beta-cyfluthrin in air. A confirmatory method for air is provided by full validation of a second MS/MS transition.
Blank values in chromatograms for quantification and confirmatory transitions, are generally below 30 % of LOQ in all matrices tested. This demonstrates a sufficient selectivity of the method.

Table B.5.2-21: Validation of the method P 2474 by Bacher (2013) for residues in air

Reference	Matrix	Detection method	Analyyte	Fortification level [µg/m³]	Average recovery [%]	RSD [%]	No. of analyses
Bacher (2013) ASB2014-7709	air, 35 °C, 88 % relative humidity	GC-MS/MS, VF-5ms column, EI, m/z 163→127	Isomer II	0.069 0.690	101 90	5 12	5 5
			Isomer IV	0.069 0.690	105 92	12 11	5 5
		GC-MS/MS, VF-5ms column, EI, m/z 226→206	Isomer II	0.069 0.690	109 89	9 12	5 5
			Isomer IV	0.069 0.690	105 92	9 12	5 5

B.5.2.5.2 Methods which do not fulfill the requirements

Table B.5.2-22: List of methods, which do not fulfil requirements

Author(s) and year	Report No	Reason
Riegner (1993) MET9400016	KCA 4.2/05 Report No.: 00309 Bayer RA-791/92	The sensitivity of the method (LOQ = 0.73 µg/m³) is not sufficient.
Hellpointer (1993) MET2000-1	KCA 4.2/06 Report No.: 00309C	The sensitivity of the method (LOQ = 0.73 µg/m³) is not sufficient.

B.5.2.6 Analytical methods for the analysis in body fluids and tissues

B.5.2.6.1 Acceptable methods/reports

Study 1

Data point: KCA 4.2/94

Report: Krebber and Braune, 2009, Analytical method 01127 for the determination of cyfluthrin and deltamethrin in blood by HPLC-MS/MS.
Bayer CropScience Report No.: MR-08/176, Edition No.: M-348630-01-1, ASB2014-2286

Guideline(s): Yes, Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 7

Deviations: No

GLP: Yes/

Acceptability: Yes

Materials and methods:

Fortified analyte(s):
Cyfluthrin and deltamethrin

Analyte(s) determined as:
Cyfluthrin and deltamethrin

Principle of the method:
Blood is extracted and deproteinised by mixing with acetonitrile. After centrifugation, the supernatant is diluted with water. The sample concentration in final extracts is 0.05 mL/mL.
The concentration of beta-cyfluthrin in final extracts is quantified by LC-MS/MS using an Ascentis Express C18 column, positive electrospray ionisation and two MRM transitions (m/z 451→191, m/z 451→127).

Results

Selectivity (specificity):
Signals from interferences >30 % of LOQ in blank samples were not detected.
Recovery (accuracy):
For accuracy of analytical results see Table B.5.2-23.
Repeatability (precision):
For repeatability of analytical results see Table B.5.2-23.
Limit of quantification (LOQ):
For each matrix type the lowest successfully validated level in Table B.5.2-23 is considered as limit of quantification.
Matrix effects:
The MS/MS detection of cyfluthrin was affected by the matrix. The peak areas of the quantification and confirmatory ion in a matrix matched sample containing 25 µg/L of cyfluthrin were about 25 % lower than the corresponding peak areas in deionised water. Therefore, quantification was performed using matrix-matched standards.
Calibration (linearity):

- Does the calibration consist of at least 3 levels (duplicated points) or 5 levels (single points): yes (5 level)
- Accepted calibration range in concentration units: 2-50 ng/mL
- Accepted calibration range in mass fraction units: 0.04-1 mg/L
- Calibration conducted with matrix matched standards: yes
- Sample chromatogram spiked at LOQ demonstrates sufficient sensitivity and signal-to-noise ratio?: yes

Confirmation
Signals obtained from two different MRM transitions were used for quantification. Both results were found to be valid (see Table B.5.2-23).

Conclusion

The analytical method 01127 as validated by Krebber and Braune (2009, ASB2014-2286) is suitable as enforcement method for cyfluthrin/beta-cyfluthrin in blood. A confirmatory method for blood is provided by full validation of a second MS/MS transition/two additional fragment ions.
Blank values in chromatograms for quantification and confirmatory transitions, are generally below 30 % of LOQ in all matrices tested. This demonstrates a sufficient selectivity of the method.

Table B.5.2-23: Validation of the method 01127 by Krebber and Braune (2009) for residues in blood

Reference	Matrix	Detection method	Fortification level [mg/L]	Average recovery [%]	RSD [%]	No. of analyses
Krebber and Braune (2009) ASB2014-2286	Blood	LC-MS/MS, C18 column, ESI, m/z 451→191	0.05 0.5	106 105	4.6 1.8	5 5
	Blood	LC-MS/MS, C18 column, ESI, m/z 451→127	0.05 0.5	101 105	6.5 3.7	5 5

Study 2

Data point: KCA 4.2/97

Report: Merdian (2009), Validation of the DFG method S19-based on Bayer CropScience method 00086/M045 for the determination of residues of cyfluthrin in liver and kidney matrices, using GC/ECD,
Bayer CropScience, Report No: PTRL Report No.: P 1752 G
Edition No.: M-348265-01-1 (R-27984), ASB2014-2284

Guideline(s): Yes (EC Guidance document on residue analytical methods, SANCO/825/00 rev. 7 17/03/04 and OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/Mono (2007) 17, 2007-08-13)

Deviations: No

GLP: Yes

Acceptability: Yes

A full description of the method and all finding obtained during validation are reported in Section B.5.2.2.1. (see Study 2).
The analytical enforcement method 00086/M045 (DFG S19) as validated by Merdian (2009, ASB2014-2284) is suitable as enforcement method for cyfluthrin/beta-cyfluthrin in kidney and liver. A confirmatory method using a capillary column of different polarity is sufficiently validated.

B.5.2.6.2 Methods which do not fulfill the requirements

Table B.5.2-24: List of methods, which do not fulfil requirements

Author(s) and year	Report No	Reason
Shaw, Chopade, Ayeres and Gentile (1983)	Notebook reference 82-R99, 82-R103, 82-R224,83-R03 and 83-R101 MET9400017	validation at one level with insufficient number of replicates

B.5.3 References relied on

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not BVL registration number	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.1 /10	Sonnenschein, L.	2014	Determination of the diastereomeric purity of beta-Cyfluthrin (AE 1430672 / FCR4545) in technical grade and pure active substance by high performance liquid chromatography (HPLC) Allessa Chemie GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: AM038113FP1, Edition Number: M-475685-01-1 Date: 2014-01-27 GLP/GEP: no, unpublished 2632908	N	Y	new study according to current requirements	Bayer CropScience
KCA 4.1.1 /11	Sonnenschein, L.	2013	Validation of the analytical method AM038113FP1: Determination of the diastereomeric purity of Beta-Cyfluthrin / AE 1430672 (FCR4545) in technical grade and pure Beta-Cyfluthrin by high performance liquid chromatography (HPLC) Allessa GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: B066/2013, Edition Number: M-474367-01-1 Date: 2013-11-28 GLP/GEP: yes, unpublished confidential 2632909	N	Y	new study according to current requirements	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not BVL registration number	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2	Dix, M. E.	2013	Method validation for seven pyrethroids in freshwater by gas chromatography using mass selective detection with negative chemical ionization Bayer CropScience M-536985-01-1 ! 13656.6125 GLP: no unpublished BVL-2968006	N	Y		Bayer CropScience
KCA 4.2	Specht, W.; Thier, H. P.	1989	Organochlorine and organophosphorus compounds as well as nitrogen containing and other plant protectants - Gas chromatographic determination after clean-up by gel chromatography and at a mini-silica gel column Publisher:Deutsche Forschungsgemeinschaft / VCH, Location:Weinheim, Journal:Manual of Pesticide Residue Analysis, Volume:I, Pages:383 - 400, Year:1987, Report No.: 00086, Edition Number: M-006227-03-2 GLP/GEP: no, published **...also filed: KCA 4.2 /83**	N	N		

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not BVL registration number	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.2	Harbin, A. M.; Minor, R. G.; Freeseman, P. L.; Pfankuche, L. K.	1983	A gas chromatographic method for Baythroid residue in crops; Supplements E001 - E032; Modifications M001 - M044 of method 00223 Mobay Chemical Corporation, Kansas City, MO, USA Report No.: MO-03-011091, Edition Number: M-108507-01-1 Date: 1983-06-14 GLP/GEP: no, unpublished ASB2009-9418 BVL-1707011 (MET)	N	N		Bayer CropScience
KCA 4.2	Seym, M.	1992	Modification M010 of method 00015: Method on the gaschromatographic determination of cyfluthrin residues in plant material, beer, and sugar with an electron capture detector (ECD) Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: 00015/M010, Edition Number: M-007892-02-1 Method Report No.: I640 Method Report No.: RA-318/92 Date: 1992-06-04 GLP/GEP: no, unpublished BVL-1707059 (MET)	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not BVL registration number	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.2	Ohs, P.	1992	Method for the gas chromatographic determination of residues of the insecticidal compounds Beta-Cyfluthrin and Cyfluthrin in plant materials and their processed products by online LC-GC-coupling; Supplements E003 - E005 of method 00255 Bayer AG, Leverkusen, Germany Report No.: MO-03-011300, Edition Number: M-109494-01-1 Date: 1992-10-01 GLP/GEP: no, unpublished BVL-1707067 (MET) –	N	N		Bayer CropScience
KCA 4.2	Wiedmann, J. L.; Amato, S. L.; Koch, D. A.	1992	Storage stability of cyfluthrin in crops and processing fractions Miles Inc., Agriculture Division, Kansas City, MO, USA Bayer CropScience, Report No.: 103821, Edition Number: M-136649-01-1 EPA MRID No.: 42710402 Date: 1992-12-18 GLP/GEP: yes, unpublished **...also filed: KCA 6.1 /03** BVL-1707081 (MET)	N	Y		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not BVL registration number	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2 /43	Weeren, R. D.; Pelz, S.	1999	Supplement E050 to method 00086: Validation of DFG method S 19 with modified extraction for the determination of residues of cyfluthrin in soil Dr. Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Germany Bayer CropScience, Report No.: 00086/E050, Edition Number: M-009717-01-1 Method Report No.: Az.M7706/99 Date: 1999-07-27 GLP/GEP: yes, unpublished	N	Y		Bayer CropScience
KCA 4.1.2 /44	Wagner, K.	1985	Gas chromatographic determination of FCR 1272 in soil Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: I371, Edition Number: M-022599-01-2 Method Report No.: RA-28/81 Date: 1985-01-09 GLP/GEP: no, unpublished	N	N		Bayer CropScience
KCA 4.1.2 /45	Wagner, K.	1982	Modification to method I371 : Gas chromatic determination of FCR 1272 in soil Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: RA-430, Edition Number: M-065712-01-2 Method Report No.: RA-430 Date: 1982-04-15 GLP/GEP: no, unpublished	N	N		Bayer CropScience

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Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not BVL registration number	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2 /46	Gronberg, R. R.; Pfankuche, L. K.	1983	An analytical residue method for Baythroid and its major metabolites in soil Mobay Chemical Corporation, Kansas City, MO, USA Bayer CropScience, Report No.: 85886, Edition Number: M-064739-01-1 EPA MRID No.: 00143146 Date: 1983-06-15 GLP/GEP: no, unpublished	N	N		Bayer CropScience
KCA 4.1.2 /47	Wagner, K.	1983	Supplement to method I371 : Gas chromatic determination of FCR 1272 in soil Mobay Chemical Corporation, Kansas City, MO, USA Bayer CropScience, Report No.: 84342, Edition Number: M-065718-01-1 Date: 1983-07-21 GLP/GEP: no, unpublished	N	N		Bayer CropScience
KCA 4.1.2 /50	Koenig, T.	1992	Method for gas chromatographic determination of cyfluthrin in drinking water Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: 00271, Edition Number: M-012493-02-1 Method Report No.: RA-337/92 Date: 1992-06-12 GLP/GEP: no, unpublished **...also filed: KCA 4.2 /03**	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not BVL registration number	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.2	Seym, M.	1995	Limit of determination (LOD) for cyfluthrin in eggs Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: MR-303/95, Edition Number: M-066177-01-2 Date: 1995-03-07 GLP/GEP: no, unpublished ASB2009-1209 BVL-1707115 (MET)	N	N		Bayer CropScience
KCA 4.2	Shaw, H. R.; Gronberg, R. R.; Harbin, A. M.; Ayers, J. E.; Pfankuche, L. K.; Freeseman, P. L.	1983	An analytical method for quantitating Baythroid metabolite residues in animal tissues Mobay Chemical Corporation, Kansas City, MO, USA Bayer CropScience, Report No.: I488, Edition Number: M-066384-01-1 Date: 1983-11-14 GLP/GEP: no, unpublished BVL-1707117 (MET)		N		Bayer CropScience
KCA 4.2	[redacted]	1983	Metabolism of Baythroid in a dairy cow [redacted] Bayer CropScience, Report No.: MR86043, Edition Number: M-052654-01-1 Date: 1983-09-27 GLP/GEP: yes, unpublished **...also filed: KCA 4.2 /11** **...also filed: KCA 5.1 /05** **...also filed: KCA 5.1.1 /06** BVL-1707123 (MET)	Y	Y		Bayer CropScience

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Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not BVL registration number	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.2	[redacted]	1983	The distribution and metabolism of Baythroid in laying hens [redacted] Bayer CropScience, Report No.: MR-86044, Edition Number: M-054113-01-1 Date: 1983-09-20 GLP/GEP: no, unpublished ...also filed: KCA 4.2 /12 ...also filed: KCA 5.1.1 /07 BVL-1707125 (MET)	Y	N		Bayer CropScience
KCA 4.1.2 /63	Anon.	1985	Recovery of Baythroid from bovine tissues Mobay Chemical Corporation, Stanley, KS, USA Bayer CropScience, Report No.: 90388, Edition Number: M-066351-01-1 Date: 1985-09-25 GLP/GEP: no, unpublished RIP9400741 ASB2014-12191, BVL-2620318	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not BVL registration number	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2 /64	Anon.	1985	Recovery of Baythroid from bovine milk Mobay Chemical Corporation, Kansas City, MO, USA Bayer CropScience, Report No.: 90392, Edition Number: M-066349-01-1 Date: 1985-09-25 GLP/GEP: no, unpublished RIP9400742 ASB2014-12191; BVL-2610775	N	N		Bayer CropScience
KCA 4.1.2 /65	Anon.	1983	Recovery of Baythroid from bovine tissues Mobay Chemical Corporation, Kansas City, MO, USA Bayer CropScience, Report No.: 85981, Edition Number: M-066345-02-1 Date: 1983-09-07 GLP/GEP: no, unpublished **...also filed: KCA 6.2.3 /02** BVL-2619841 RIP9401251	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not BVL registration number	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2 /66	Anon.	1983	Recovery of Baythroid from chicken eggs Mobay Chemical Corporation, Kansas City, MO, USA Bayer CropScience, Report No.: 85982, Edition Number: M-066333-02-1 Date: 1983-09-07 GLP/GEP: no, unpublished **...also filed: KCA 6.2.2 /02** BVL-2620319 RIP9401252 ASB2014-12196	N	N		Bayer CropScience
KCA 6.2.2	Anon.	1983	Recovery of Baythroid from chicken tissues Mobay Chemical Corporation, Kansas City, MO, USA Bayer CropScience, Report No.: 85983, Edition Number: M-066320-02-1 Date: 1983-09-07 GLP/GEP: no, unpublished **...also filed: KCA 6.2.2 /03** BVL-2620320	N	N		Bayer CropScience
KCA 4.1.2 /68	Anon.	1985	Recovery of Baythtroid from cattle tissue Mobay Chemical Corporation, Kansas City, MO, USA Bayer CropScience, Report No.: 87217, Edition Number: M-066488-01-1 Date: 1985-02-18 GLP/GEP: no, unpublished **...also filed: KCA 6.2.3 /05** BVL-2619843 RIP9400875	N	N		Bayer CropScience

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Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not BVL registration number	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.2.3	Anon.	1984	Recovery of Baythroid, and its metabolites from bovine kidney and liver tissues Mobay Chemical Corporation, Kansas City, MO, USA Bayer CropScience, Report No.: 86220, Edition Number: M-066406-01-1 Date: 1984-01-03 GLP/GEP: no, unpublished **...also filed: KCA 6.2.3 /06** BVL-2619849 RIP9400876	N	N		Bayer CropScience
KCA 4.1.2 /70	Anon.	1985	Recovery of Baythroid from cattle tissue Mobay Chemical Corporation, Kansas City, MO, USA Bayer CropScience, Report No.: 87216, Edition Number: M-066471-01-1 Date: 1985-02-20 GLP/GEP: no, unpublished **...also filed: KCA 6.2.3 /07** BVL-2619867 RIP9400877	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not BVL registration number	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2 /71	Anon.	1984	Recovery report of Baytroid metabolites from chicken tissues Mobay Chemical Corporation, Kansas City, MO, USA Bayer CropScience, Report No.: 84631, Edition Number: M-066399-01-1 Date: 1984-02-03 GLP/GEP: no, unpublished **...also filed: KCA 6.2.2 /05** BVL-2620339 RIP9400878	N	N		Bayer CropScience
KCA 4.1.2 /74	Robinson, N.	2014	Beta-cyfluthrin - Field soil dissipation of beta-cyfluthrin from a field trial carried out in southern France Innovative Enviromental Service Ltd, Witterswil, Switzerland Irvita Plant Protection, Report No.: 20120152, Edition Number: M-482355-01-1 Date: 2014-04-01 GLP/GEP: yes, unpublished **...also filed: KCA 7.1.2.2.1 /16**	N	Y	data not submitted on EU Level	Irvita Plant Protection

Annex point / reference number	**Author(s)**	**Year**	**Title** **Source** ***(where different from company)*** **Company name, Report No., Date, GLP status** ***(where relevant)*****, published or not** **BVL registration number**	**Verte-brate study Y/N**	**Data protection claimed Y/N**	**Justification if data protection is claimed**	**Owner**
KCA 4.1.2 /75	Braune, M. Sandau, C.	2010	Method 01174 for the determination of beta-cyfluthrin FPB acid in test water from aquatic toxicity tests by HPLC-UV BCS-Irvita, Report No.: 01174, Edition Number: M-361622-01-1 Method Report No.: 01174 Method Report No.: MR-09/133 Date: 2010-01-06 GLP/GEP: no, unpublished BVL-2632911 ASB2014-6695	N	Y	to fulfill data requirement for beta-cyfluthrin	BCS-Irvita
KCA 4.1.2 /76	Kimmel, S.	2014	Beta-cyfluthrin: Effect on the development of sediment-dwelling larvae of Chironomus riparius in water-sediment systems with spiked sediment Harlan Laboratories Ltd., Itingen, Switzerland BCS-Irvita, Report No.: D58731, Edition Number: M-481037-01-1 Date: 2014-03-21 GLP/GEP: yes, unpublished **...also filed: KCA 8.2.5.3 /02** **...also filed: KCA 8.2.5.4 /03**	N	Y	to fulfill data requirement for beta-cyfluthrin	BCS-Irvita

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not BVL registration number	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2 /77	Bacher, R.	2013	Beta-cyfluthrin: Analytical method for determination in air PTRL Europe, Ulm, Germany Bayer CropScience, Report No.: P 2474 G, Edition Number: M-462363-01-1 Method Report No.: P 2474 G Date: 2013-06-28 GLP/GEP: yes, unpublished **...also filed: KCA 4.2 /99** ASB2014-7709 BVL-2632913	N	Y	new study according to current requirements	BCS-Irvita
KCA 4.1.2 /78	[redacted]	1996	Determination of Cyfluthrin (FCR 1272) in serum, fat and brain of rats after inhalation exposure or oral administration - Analytical part of study T7058167 [redacted] Bayer CropScience, Report No.: MR-365/95, Edition Number: M-044833-01-1 Date: 1996-01-30 GLP/GEP: yes, unpublished **...also filed: KCA 5.2.3 /03** BVL-2632914 (TOX)	Y	Y	data not submitted on EU Level	Bayer Crop Science, owner Bayer CropScience, license Irvita Plant Protection B.V.Science

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KCA 4.1.2 /79	[redacted]	1996	Cyfluthrin: Concentration of the parent compound in blood plasma, brain and omental fat of rats following administration with the feed or by oral administration [redacted] Bayer CropScience, Report No.: MR-625/95, Edition Number: M-044715-01-1 Date: 1996-03-15 GLP/GEP: yes, unpublished **...also filed: KCA 5.2.1 /14** BVL-2632915 (TOX)	Y	Y	data not submitted on EU Level	Bayer Crop Science, owner Bayer CropScience, license Irvita Plant Protection B.V.Science
KCA 4.1.2 /80	Schoening, R.	2005	Analytical method 00922 for the determination of residues of Beta-Cyfluthrin in/on plant material by HPLC-MS/MS Report No.: 00922, Edition Number: M-244829-01-1 Method Report No.: MR-190/04 Date: 2005-01-18 GLP/GEP: yes, unpublished **...also filed: KCA 4.2 /100** BVL-2632916	N	Y	data requirement under Reg. 1107/2009 (method in support of the new submitted residue trials)	BCS-Irvita

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KCA 4.1.2 /81	Schoening, R.; Reineke, A.	2011	Determination of the residues of beta-cyfluthrin and imidacloprid in/on sugar beet after seed treatment of Montur Forte FS 230 in the field in Germany and the Netherlands Bayer CropScience, Report No.: 09-2044, Report includes Trial Nos.: 09-2044-01 09-2044-02 Edition Number: M-404508-01-1 Date: 2011-03-25 GLP/GEP: yes, unpublished **...also filed: KCA 6.3.1 /12** BVL-2632917 ASB2012-4627	N	Y	data requirement under Reg. 1107/2009	Bayer CropScience
KCA 4.1.2 /82	Uceda, L.	2013	Analytical method 01379 for the determination of cyfluthrin in/on sugarbeet (leaf with root collar and body) by HPLC-MS/MS - Cyfluthrin Bayer S.A.S., Bayer CropScience, Lyon, France Bayer CropScience, Report No.: 01379, Edition Number: M-463052-01-1 Date: 2013-08-29 GLP/GEP: yes, unpublished ASB2014-7710 BVL-2632918	N	Y	data requirement under Reg. 1107/2009 (method in support of the new submitted residue trials)	Bayer CropScience

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KCA 4.1.2 /83	Schaeufle, M.	2012	Bulldock 25 EC and Bulldock 25 CS - Magnitude of residues of beta-cyfluthrin in greenhouse tomatoe raw agricultural commodity after 2 applications of Bulldock 25 EC or Bulldock 25 CS - 8 decline trials - in northern and southern Europe (the Nederlands, Germany, Northern France, Greece, Spain and Italy) in 2011 Huntington Life Sciences; Eye research centre, Suffolk, United Kingdom Irvita Plant Protection, Report No.: JDV0077, Edition Number: M-481199-01-1 Date: 2012-06-11 GLP/GEP: yes, unpublished **...also filed: KCA 6.3.2 /12** BVL-2632919 ASB2014-7711	N	Y	data not submitted on EU Level	Irvita Plant Protection
KCA 4.1.2 /85	Krebber, R.	2009	Analytical method 01127 for the determination of cyfluthrin and deltamethrin in blood by HPLC-MS/MS Bayer CropScience, Report No.: MR-08/176, Edition Number: M-348630-01-1 Method Report No.: MR-08/176 Date: 2009-06-03 GLP/GEP: yes, unpublished **...also filed: KCA 4.2 /94** BVL-2632921 ASB2014-2286 (TOX)	N	Y	Not available for last Annex I listing; required method improved according to the latest science	Bayer CropScience

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KCA 4.1.2 /87	Schaeufle, M.	2012	Bulldock 25 EC - Magnitude of residues of beta-cyfluthrin in greenhouse tomato raw agricultural commodity and processed fractions after 2 applications of Bulldock 25 EC - 2 trials - In northern europe (Germany) in 2011 Huntington Life Sciences; Eye research centre, Suffolk, United Kingdom Irvita Plant Protection, Report No.: M-481079-01-1, Edition Number: M-481079-01-1 Date: 2012-06-22 GLP/GEP: no, unpublished **...also filed: KCA 6.5.3 /22** BVL-3139633 ASB2014-7712	N	Y		Irvita Plant Protection
KCA 4.2 /03	Koenig, T.	1992	Method for gas chromatographic determination of cyfluthrin in drinking water Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: 00271, Edition Number: M-012493-02-1 Method Report No.: RA-337/92 Date: 1992-06-12 GLP/GEP: no, unpublished MET9400015	N	N		Bayer CropScience

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KCA 4.2 /11	[redacted]	1983	Metabolism of Baythtroid in a dairy cow [redacted] Bayer CropScience, Report No.: MR86043, Edition Number: M-052654-01-1 Date: 1983-09-27 GLP/GEP: yes, unpublished ...also filed: KCA 4.1.2 /56 RIP9400870	Y	N		Bayer CropScience
KCA 4.2 /12	[redacted]	1983	The distribution and metabolism of Baythroid in laying hens [redacted] Bayer CropScience, Report No.: MR-86044, Edition Number: M-054113-01-1 Date: 1983-09-20 GLP/GEP: no, unpublished ...also filed: KCA 4.1.2 /57 RIP9400869	Y	N		Bayer CropScience

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KCA 4.2 /89	Weber, H.	2004	Validation of enforcement method DFG S 19 (L 00.00-34) (BCS method ID 00086/M088) for the determination of residues of cyfluthrin (AE F057122) in/on plant materials Dr. Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Germany BCS-Irvita, Report No.: 00086/M088, Edition Number: M-347371-01-1 Date: 2004-08-05 GLP/GEP: yes, unpublished 2632924; ASB2014-2283	N	Y	new residue methods have been developed and validated after Annex I inclusion	BCS-Irvita
KCA 4.2 /90	Merdian, H.	2009	Independent laboratory validation of DFG method S19 (BCS method-ID 00086M088) for the determination of residues of cyfluthrin in plant materials, using GC/MS Dr. Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Germany BCS-Irvita, Report No.: P612097520, Edition Number: M-349219-01-1 Date: 2009-06-10 GLP/GEP: yes, unpublished 2632925; ASB2014-2281	N	Y	new residue methods have been developed and validated after Annex I inclusion	BCS-Irvita

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KCA 4.2 /91	Steinhauer, S.	2002	Enforcement method 00086/M045 for the determination of residues of cyfluthrin in materials of animal origin - Validation of DFG method S 19 (extended revision) Dr. Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Germany Report No.: 00086/M045, Edition Number: M-052341-01-1 Method Report No.: BAY-0206V Date: 2002-09-02 GLP/GEP: yes, unpublished MET2005-856	N	Y	new residue methods have been developed and validated after Annex I inclusion	Bayer CropScience; owner Bayer CropScience, license Irvita Plant Protection B.V.
KCA 4.2 /92	Reichert, N.	2002	Independent laboratory validation of DFG method S19 (extended revision) for the determination of residues of cyfluthrin in materials of animal origin (Bayer CropScience enforcement method 00086/M045) Institut Fresenius Chem.und Biolog. Lab. AG, Taunusstein, Germany Report No.: IF-02/00023853, Edition Number: M-075850-01-1 Method Report No.: IF-02/00023853 Date: 2002-12-20 GLP/GEP: yes, unpublished MET2005-640	N	Y	new residue methods have been developed and validated after Annex I inclusion	Bayer CropScience; owner Bayer CropScience, license Irvita Plant Protection B.V.

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KCA 4.2 /93	Meyer, M.; Zietz, E.	2010	Independent laboratory validation of DFG M method S19 (Bayer CropScience method 00086/M045) for the determination of residues of cyfluthrin in foodstuffs of animal origin using GC/ECD SGS Institut Fresenius GmbH, Taunusstein, Germany Bayer CropScience, Report No.: IF-09/01516201, Edition Number: M-399396-01-1 Date: 2010-12-15 GLP/GEP: yes, unpublished 2632928; ASB2014-2282	N	Y	new residue methods have been developed and validated after Annex I inclusion	Bayer CropScience
KCA 4.2 /94	Krebber, R.	2009	Analytical method 01127 for the determination of cyfluthrin and deltamethrin in blood by HPLC-MS/MS Bayer CropScience, Report No.: MR-08/176, Edition Number: M-348630-01-1 Method Report No.: MR-08/176 Date: 2009-06-03 GLP/GEP: yes, unpublished 2632929; ASB2014-2286	N	Y	Not available for last Annex I listing; required method improved according to the latest science	Bayer CropScience
KCA 4.2 /95	Braune, M.	2012	Analytical method 01342 for the determination of beta-cyfluthrin in drinking and surface water by HPLC-MS/MS BCS-Irvita, Report No.: MR-12/053, Edition Number: M-436448-01-1 Method Report No.: MR-12/053 Date: 2012-08-13 GLP/GEP: yes, unpublished 2632930; ASB2014-7713	N	Y	new study according to current requirements	BCS-Irvita

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Black writing indicates a Supplementary Dossier Study

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KCA 4.2 /96	Bomke, S.	2013	Independent laboratory validation (ILV) of the analytical method 01342 for the determination of beta-cyfluthrin in drinking and surface water using HPLC-MS/MS Bayer CropScience, Report No.: MR-13/024, Edition Number: M-457906-01-1 Date: 2013-06-21 GLP/GEP: yes, unpublished 2632931; ASB2014-7714	N	Y	new method developed after Annex I inclusion	BCS-Irvita
KCA 4.2 /97	Merdian, H.	2009	Validation of the DFG method S19-based on Bayer CropScience method 00086/M045 for the determination of residues of cyfluthrin in liver and kidney matrices, using GC/ECD PTRL Europe GmbH, Ulm, Germany BCS-Irvita, Report No.: P 1752 G, Edition Number: M-348265-01-1 Date: 2009-05-28 GLP/GEP: yes, unpublished 2632925; ASB2014-2284	N	Y	new residue methods have been developed and validated after Annex I inclusion	BCS-Irvita

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KCA 4.2 /98	Airs, D.	2013	Beta-cyfluthrin: Validation of an analytical method for the determination of beta-cyfluthrin in wheat grain, oil seed rape seeds, tomatoe and grapes Huntington Life Sciences; Eye research centre, Suffolk, United Kingdom Irvita Plant Protection, Report No.: GAW0002, Edition Number: M-481200-01-1 Date: 2013-06-10 GLP/GEP: yes, unpublished 2632933, ASB2014-6696	N	Y	new method developed after Annex i inclusion	Irvita Plant Protection
KCA 4.2 /99	Bacher, R.	2013	Beta-cyfluthrin: Analytical method for determination in air PTRL Europe, Ulm, Germany Bayer CropScience, Report No.: P 2474 G, Edition Number: M-462363-01-1 Method Report No.: P 2474 G Date: 2013-06-28 GLP/GEP: yes, unpublished 2632934; ASB2014-7709	N	Y	new study according to current requirements	BCS-Irvita
KCA 4.2 /100	Schoening, R.	2005	Analytical method 00922 for the determination of residues of Beta-Cyfluthrin in/on plant material by HPLC-MS/MS Report No.: 00922, Edition Number: M-244829-01-1 Method Report No.: MR-190/04 Date: 2005-01-18 GLP/GEP: yes, unpublished MET2006-93	N	Y	data requirement under Reg. 1107/2009 (method in support of the new submitted residue trials)	BCS-Irvita

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KCA 4.2 /101	Airs, D.	2014	Beta-cyfluthrin: Determination of beta-cyfluthrin residues in three crop sample types (high water, high oil and dry matrices) using two different extraction methods in order to demonstrate extraction efficiency Huntingdon Life Sciences, Suffolk, England BCS-Irvita, Report No.: R-33373, Edition Number: M-483395-01-1 Date: 2014-04-07 GLP/GEP: yes, unpublished 2632936, ASB2014-7715	N	Y	data not submitted on EU Level	BCS-Irvita
KCA 4.2	Weeren, R. D.; Pelz, S.	1999	Supplement E050 to method 00086: Validation of DFG method S 19 with modified extraction for the determination of residues of cyfluthrin in soil Dr. Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Germany Bayer CropScience, Report No.: 00086/E050, Edition Number: M-009717-01-1 Method Report No.: Az.M7706/99 Date: 1999-07-27 GLP/GEP: yes, unpublished MET1999-1227	N	Y		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not BVL registration number	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.2	Robinson, N.	2014	Beta-cyfluthrin - Field soil dissipation of beta-cyfluthrin from a field trial carried out in southern France Innovative Enviromental Service Ltd, Witterswil, Switzerland Irvita Plant Protection, Report No.: 20120152, Edition Number: M-482355-01-1 Date: 2014-04-01 GLP/GEP: yes, unpublished 2832233; ASB2014/7708	N	Y	data not submitted on EU Level	Irvita Plant Protection
KCA 8.2.5.2/01	Schwader, A.L.	2013	Beta-Cyfluthrin – Life-cycle toxicity test with mysids (*Americamysis bahia*); Appendix 3 – Analytical methodology M-465880-01-1; Sponsor Project No. EBFRL028; Smither Viscient Study No. 13798.6307 GLP: yes unpublished	N	Y		BCS/Irvita