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PIRIMICARB

Volume 3 – B.5 (AS)

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Co-Rapporteur Member State: Sweden

Version History

When	What
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Table of contents

B.5. METHODS OF ANALYSIS 4

B.5.1. METHODS USED FOR THE GENERATION OF PRE-AUTHORISATION DATA 4

B.5.1.1. Methods for the analysis of the active substance as manufactured 4
B.5.1.2. Methods for risk assessment 6

B.5.2. METHODS FOR POST-APPROVAL CONTROL AND MONITORING PURPOSES 36

B.5.3. REFERENCES RELIED ON 70

B.5. METHODS OF ANALYSIS

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

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

a) Active Substance

Method SA-52/1: Determination of pirimicarb in the technical material

An analytical method has been developed for the determination of the pure active substance, pirimicarb, in the active substance as manufactured. This method has not previously been reviewed at EU level and is provided in support of this assessment.

Report:	**Kirchner, J. (2011), Pirimicarb - Determination of PP62 in technical material Syngenta File No PP62_10070**

Previous evaluation	None. Submitted for the purposes of renewal

Report:	**Kirchner, J. (2011), Pirimicarb - Determination of PP62 in technical material Syngenta File No PP62_10070**

Previous evaluation	None. Submitted for the purposes of renewal

The active substance pirimicarb is determined in pirimicarb technical material using capillary gas chromatography and an external standard. The method uses a 10m fused silica column and with a DB-1 stationary phase with FID detection. Quantification is achieved by comparison of the peak area to that of a reference solution.

Test Solution Preparation

100.0 ±20.0 mg of pirimicarb technical substance was weighed out and 10.0 mL internal standard solution (docosane in chloroform, 4 mg/mL) added. Samples were sonicated for 1 min. to dissolve and analysed by GC-FID (test solution contains ~ 10 mg/mL pirimicarb).

Confirmation of Identity

The IR spectrum of the test substance was compared with that of the reference substance.

Instrument Conditions

Chromatograph:	Agilent 6890
Detector:	FID, output voltage = 1 V
Integrator:	Atlas/Windows 2000 (ThermoElectron)
Column:	fused silica, 10 m length, 0.18 mm i/d/ (capillary)
Stationary phase:	DB-1
Film thickness:	0.4 µm
Column temperature:	150°C (±10°C) for 0 minutes Program 1: 20°C/minute to 240°C Hold 240°C for 0 minutes Program 2: 60°C/minute to 280°C Hold 280°C for 1 minute
Detector temperature:	275°C FID
Injector temperature:	250°C
Injector liner:	Deactivated straight split liner with approx. 1cm deactivated glass wool (critical for good peak shape).
Carrier gas:	hydrogen, linear velocity 101 cm/second
Flow rate:	1.8 mL/minute

Mode:	Constant flow
Split flow:	90 mL/minute
Split ratio:	50:1
Make-up gas:	nitrogen, flow rate 20 ml/minute
Size of sample:	1 µl of reference
Run Time:	approx. 6.2 minutes
Internal Standard:	4 mg/ml docosane in chloroform

Components	**Retention time [minutes]**
Pirimicarb	2.4
Internal Standard	4.1

Summary of Validation Data

LOQ (%w/w)	**Recovery fortification level (%w/w)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**	**Specificity**
Not required for the active in the TGAI			% Found (average): 97.8% N=6 %RSD: 1.19 Acceptable Horowitz: 1.34	5.1-15 mg/mL (50-150% of nominal content) N=6 R^2=0.9907	Yes – retention time match with reference standard No interference ≥3.0%. Confirmation of identity by GC-FID retention time matches with analytical standard and IR spectra.

Specificity: Solutions of the cab-o-sil, internal standard, the pirimicarb analytical master standard and a solution of the analytical impurity standards were injected in duplicate, followed by blank solvent (chloroform) injections. No significant interference between the components was observed.

Linearity: The method linearity was determined by analysing six single injections over a range of 50% to 150% of the specified sample concentration. The linearity was considered acceptable since the correlation coefficient was ≥0.99.

Precision: The method precision was determined by calculating the standard deviation from the mean value based on the analysis of at least five weights of the Technical sample. The method precision was considered acceptable since the relative standard deviation was less than the modified Horwitz value.

The method is satisfactorily validated in accordance with SANCO/3030/99 rev. 4.

Applicability of existing CIPAC methods

There is no CIPAC method for the determination of pirimicarb in pirimicarb technical material.

b) Significant and Relevant Impurities
No relevant impurities are stated for pirimicarb.

See individual volume 4 documents of pirimicarb RAR for methods for significant impurities.

B.5.1.2. Methods for risk assessment

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

Report:	**Harradine, K J and Patel, A (1996), Desmethyl pirimicarb: Residue Levels in Soil from a Laboratory Soil Degradation Study carried out in the UK during 1995-96, Report Number: PP62_10070**

Previous evaluation	In DAR (2003) for original approval

The rate of degradation of the pirimicarb metabolite R34836 (desmethyl pirimicarb) was studied in three different solids stored in the dark for a period of up to 100 days at a constant temperature and soil humidity. An HPLC-MS-MS method was used to study the rate of degradation of R34836 under these storage conditions. This method was later written up as RAM 274/02 which was evaluated during the first Annex 1 review of pirimicarb and considered acceptable for the determination of desmethyl pirimicarb in soil samples with an LOQ of 0.01 mg/kg. The method was re-evaluated in the context of the renewal submission (see section B.5.2.1.2. for further details).

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

No specific methods for the support of efficacy studies were developed.

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

Report:	**Bruce, S. (2017), Pirimicarb - Oral (Gavage) Proof of Exposure Study in Mice, Report Number: AE77RC.DRF000M.BTL**

Previous evaluation	None. Submitted for the purposes of renewal

Three male CD-1 mice per time point were dosed with pirimicarb at 43 mg/kg in corn oil at a dose volume of 10 mL/kg by a single oral gavage dose. Proof of exposure was demonstrated by detectable plasma concentrations of pirimicarb metabolite R31805 in male CD-1 mice at 1 and 4 hours post dose.

Method Summary
Immediately following collection of each blood sample, 20 µL of whole blood was accurately measured into a polypropylene tube containing 60 µL of 1% (v/v) formic acid in acetonitrile (1:3 whole blood:acidified acetonitrile). Acidified whole blood samples were mixed gently by inversion and frozen on dry ice prior to storage. The remaining blood samples were maintained on wet ice until centrifugation. The remaining blood samples were not diluted. The remaining blood samples were then centrifuged for 5 minutes at 1000 g at 2-8ºC within 1 hour of collection and plasma was harvested into two, approximately equal aliquots.
The plasma samples were stored at ≤ -60ºC until packed on dry ice and delivered to the analytical laboratory for analysis.
An LC-MS/MS method was used to analyse the concentrations of test article in the plasma samples.

It is noted that the storage stability of the analytes in plasma matrix was not addressed as part of the method validation.

Instrument Conditions

Instrument:	Agilent 1200 HPLC
Software:	Agilent Mass Hunter
(MPA) Mobile Phase A:	0.1% formic Acid in Deionised Water
(MPB) Mobile Phase B:	Acetonitrile
Diluent:	Acetonitrile: Deionised Water (50:50, v/v)
Flush Port Solvent:	Acetonitrile: Deionised Water (50:50, v/v)
Biological Matrix:	Mouse plasma
Stock Solution:	100 µg/mL test article/test article metabolite in diluent
Working Stock Solution:	20000 µg/mL test article/test article metabolite in diluent
QC Stock Solution:	100 µg/mL test article/test article metabolite in diluent
QC Working Stock:	20000 µg/mL test article/test article metabolite in diluent
Internal Standard:	98.1 ng/mL internal standard in acetonitrile
Column:	Agilent Zorbax Eclipse Plus C18 rapid resolution HD, 50 mm x 2.1 mm, 1.8 µm particles
Column Temperature:	25 °C
Autosampler Temperature:	5 °C
Injection volume:	10 µL
Flow rate:	0.6 mL/min
Injections/Sample:	1
Run Time:	2 minutes
Linear regression:	y = ax + b
Retention time:	Pirimicarb: approximately 0.319 minutes R31805: approximately 0.221 minutes
Elution mode:	Isocratic
(MPA:MPA) %:	50:50

Analytical Conditions for MS

Instrument:	Agilent QQQ6430 Mass Spec
Ion Source:	Electrospray Ionisation (ESI)
Polarity:	Positive
Scan Type:	MRM
Capillary Voltage:	4000 V
Gas Temperature:	300 °C
Nebulizer:	20 psi
Gas flow:	7 L/min
Precursor Ion:	239.2 m/z for Pirimicarb (168 m/z for R31805) ((284 m/z for IS)
Product Ion:	72.2 m/z for pirimicarb (71 m/z for R31805) (252 m/z for IS)
Fragmentor Voltage:	150 V for pirimicarb (125 V for R31805) (120V for IS)
Collision Energy:	20 V for pirimicarb (30 for R31805) (10 V for IS)
Cell Accelerator Voltage:	7V
Dwell:	200 ms

Summary of Validation Data

Sample matrix	Fortification level (ng/mL)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Plasma	Pirimicarb			
	10	79 – 81 (80)	2.8 (3)	5 - 100 ng/mL
	50	105 – 111 (110)	4.8 (3)	n = 7
	90	104 – 129 (114)	0.9 (3)	R= 0.998
	Overall	79 – 129 (101)	17.3 (9)	
	R31805			
	10	107 – 113 (111)	2.8 (3)	5 - 100 ng/mL
	50	93 – 102 (97)	4.8 (3)	n = 7
	90	94 – 99 (97)	0.9 (3)	R= 0.997
	Overall	93 – 113 (102)	7.5 (9)	

Specificity: For both pirimicarb and R31805, representative chromatograms were shown for the plasma matrix blank (mouse plasma with K_2EDTA as anticoagulant), the lowest and highest concentration calibration solutions in matrix, the highest and lowest fortifications levels in matrix and the test samples at 1, 4 and 24 hour intervals. No significant interference was observed at the retention times of interest.
Linearity: For both pirimicarb and R31805, linearity was demonstrated using 7 calibration solutions in the range of 5 - 100 ng/mL. The correlation coefficients were >0.99 for both analytes. Representative calibrations have been presented for both analytes. Matrix matches standards were used to generate the calibration plots. The analytical calibration extends over a range appropriate to the lowest and highest nominal concentration of the analyte in relevant analytical solutions.
Accuracy: For both pirimicarb and R31805, three fortification levels were used to determine the accuracy of the method 10, 50, 90 ng/mL. The mean recoveries were mostly in the range of 70-110%, except for pirimicarb at the 90 ng/mL fortification level (mean recovery: 114%) and R31805 at the 10 ng/mL (mean recovery: 111%).
Precision: Three recovery determinations were made at each fortification level from which the relative standard deviation (RSD) was determined. The reported RSD was ≤20% at each level. It is noted that the data set at each fortification level is low (n=3).
LOQ: For both pirimicarb and R31805 the LOQ of the method is 10 ng/mL

Conclusion: The LC-MS/MS method used to support study AE77RC.DRF000M.BTL is not strictly validated in accordance with SANCO/3029/99 rev. 4. The number of recoveries at each fortification is low (3) and for each analyte, the mean recovery ate one fortification level is >110% and, therefore, outside the range of acceptability. However, it is noted that the mean recoveries were not significantly over 110% and the corresponding precision is small. On this basis, the method can be considered fit for regulatory purposes.

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

No specific methods for the support of operator exposure studies were developed.

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

The following risk assessment methods for food of plant origin were submitted for the original approval of pirimicarb (Vol.3, Annex B, Section B5, October 2003):

Crop	Analyte	Method	Reference
Apple Tomato Potato Bean	Pirimicarb R34836	GCMS	Method: PPRAM 15/2 Study: RJ1056B GM Cullen, 1993
Apple Tomato Lettuce Sugar beet Cabbage Wheat grain Wheat straw	Pirimicarb R34836 R238177	GC BPX5 column with thermionic nitrogen specific detection	Method: RAM 265/01 Study: RJ1806B KJ Harradine, 1995
Potato Apple Cereal grain Cereal forage Cereal Straw	Pirimicarb R34836 R238177	GC BPX5 column with thermionic nitrogen specific detection	Method: RAM 265/02 Study: CEMR-622 N B Coombe, 1996
Oil Seed Rape Seed Lettuce Maize grain Beans	Pirimicarb R34836 R238177	LC-MS/MS	Method: RAM 265/03 Study: CLE38/229-D2140 Wright, D R, 1998
Tobacco Plum Orange fruit Orange pulp	Pirimicarb R34836 R238177	LC-MS/MS	Method: RAM 265/04 Study: CLE38/256-2140 A. Croucher, 2000
Tomato Oil Seed Rape Grain	Pirimicarb R34836 R238177	LC-MS/MS	Method: RAM 265/04 Study: 20265 AM Doran and GM McGuire

In Vol.3, Annex B, Section B5 of the DAR (October 2003), analytical method RAM 265/01 and its independent laboratory validation, RAM 265/02, were evaluated and validated for the determination of pirimicarb, R34836 and R238177 with an LOQ of 0.01 mg/kg in dry commodities (wheat grain) and an LOQ of 0.05 mg/kg in straw. Evaluation/validation of the remaining submitted studies was not presented the DAR.

Where new studies have been submitted that rely on the methods supplied for the Annex I approval of pirimicarb, the original study reports have been reopened and the methods validated in accordance with SANCO/3029/99 rev. 4.

Additional analytical methods have been developed for the determination of residues of pirimicarb on crops for the purpose of renewal:

Crop	Analyte	Method	Reference
Maize whole cob Maize grain Maize forage Maize residual plant at harvest	Pirimicarb R34836 R238177	LC-MS/MS	Method: RAM 265/04 Study: CLE 38/299-03R Croucher A, 2002
Wheat grain Sugar beet roots Sugar beet leaves	Pirimicarb R34836	LC-MS/MS	Method: GRM039.04A Report: TK0252946 Tsui, 2015

Report:	**Cullen, GM. (1993), Pirimicarb: Determination of Pirimicarb and its Carbamate Metabolite in Crops – Analytical Method Validation, Report RJ 1056B**

Previous evaluation	In DAR (2003) for original approval

Validation data was provided for GCMS method PPRAM 15/2 for the determination of pirimicarb and its metabolite R34836 (desmethyl pirimicarb) in four crop matrices: apples, tomatoes, potatoes and broad beans.

Method Summary

Samples were extracted with methanol and filtered. The methanol was removed by rotary evaporation and the sample residue taken up in n-hexane and allowed to stand overnight in 0.1M hydrochloric acid, during which time any desmethyl formamido pirimicarb was converted to desmethyl pirimicarb. Sample extracts were then cleaned by liquid-liquid partition.

Final determination of pirimicarb and its desmethyl metabolite was by packed column (3% OV17) gas-liquid chromatography using a thermionic nitrogen specific detector; by comparison of peak area for the recovery samples against that obtained for a standard solution containing known concentrations of both pirimicarb and desmethyl pirimicarb.

Instrument Parameters

System:	Varian 3400 Series with thermionic nitrogen specific detector and Varian 8000 autosampler
Column:	3% OV17 on Chromosorb WHP 100-120 mesh 1.8 metre x 2 mm i.d.
Temperature:	250°C
Detector:	300 °C
Carrier Gas:	Helium 30 mL/min
Detector gases:	Hydrone 4.5 mL/min Air 185 min/min
Injection Volume:	4 µL
Data Handling System:	V.G. Multichrom

Summary of Validation Data (pirimicarb)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Pirimicarb				
Apple	0.02*	74 – 77 (76)	2.8 (2)	
	0.1	62 – 82 (72)	19.6 (2)	
	0.5	67 – 71 (69)	4.1 (2)	-
	5	82 – 74 (78)	7.2 (2)	
	Overall	62 – 82 (74)	9.1 (8)	
Tomato	0.02*	83 – 84 (84)	0.8 (2)	
	0.1	77 – 84 (81)	6.1 (2)	
	0.5	57 – 91 (76)	3.7 (4)	-
	5	68 – 84 (76)	14.9 (2)	
	Overall	62-82 (78)	16.5 (10)	
Broad Beans	0.02	111 – 164 (134)	15.8 (4)	
	0.1*	87 – 111 (97)	10.0 (4)	
	0.5	84 – 85 (84)	0.6 (3)	-
	5	80 – 86 (83)	3.1 (3)	
	Overall	80 – 111 (89)	10.3 (10)	
Potatoes	0.02	65 – 74 (68)	6.2 (4)	
	0.1	61 – 75 (69)	9.5 (4)	
	0.5*	67 – 71 (69)	4.1 (2)	-
	5	73 (73)	0 (2)	
	Overall	61 – 75 (69)	6.5 (12)	

*Due to the presence of an interfering peak in the chromatogram, the accuracy of measurement of the pirimicarb component was unreliable and atypically high recovery values obtained. As a result, the 0.02 fortification has been excluded from the overall range and precision and the 0.1 mg.kg fortification is considered the LOQ.

Summary of Validation Data (R34836)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
R34836				
Apple	0.02*	74 – 91 (83)	14.6 (2)	
	0.1	67 – 91(79)	21.5 (2)	
	0.5	74 – 78 (76)	3.7 (2)	-
	5	72 – 90% (81)	15.7 (2)	
	Overall	67 – 91 (80)	12.1 (8)	
Tomato	0.02*	83 – 87 (85)	3.3 (2)	
	0.1	84 – 85 (85)	0.8 (2)	
	0.5	68 – 96 (80)	1.8 (4)	-
	5	79 – 88 (84)	7.6 (2)	
	Overall	66 – 96 (83)	11.3 (10)	
Broad Beans	0.02	59 – 87 (76)	16.7 (4)	
	0.1*	63 – 84 (70)	14.0 (4)	
	0.5	79 – 87 (83)	4.8 (3)	-
	5	78 – 84 (82)	3.9 (3)	
	Overall	83 – 87 (77)	11.6 (10)	
Potatoes	0.02	54 – 63 (58)	7.1 (4)	
	0.1	53 – 75 (65)	19.2 (4)	
	0.5*	68 – 70 (69)	2.0 (2)	-
	5	69 – 75 (73)	5.9 (2)	
	Overall	53 – 76 (64)	13.8 (12)	

Specificity: For each matrix, representative chromatograms were provided for the matrix blanks, analytical standards and the fortified matrix (highest and lowest fortification level). Significant interferences arising from the crop matrix was observed at the retention times of interest in broad beans at the lowest fortification level.
Linearity: Linearity data was not provided as part of this study.
Accuracy: For pirimicarb and desmethyl pirimicarb, 4 fortification levels were used to determine the accuracy: 0.02, 0.1, 0.5 and 5 mg/kg. The mean recoveries were mostly in the range 70-110%. For pirimicarb in apple, the mean recovery at the 0.5 mg/kg fortification is 69% and in potato the mean recovery is 68% at the lowest fortification (0.02 mg/kg). For desmethyl pirimicarb in potato, the recoveries at the three lowest fortification levels (0.02, 0.05 and 0.1 mg/kg) are <70%.
Precision: For each plant matrix, between two and four recovery determinations were made at each fortification level from which the relative standard deviation (RSD) was determined. The reported RSD was ≤20% at each level, except for the 0.1 mg/kg of desmethyl pirimicarb in apple (%RSD: 21.5). However, it is noted that the data set at each fortification level is low (n=2).
LOQ: For pirimicarb the LOQ is 0.02 mg/kg in apple and tomato. In broad beans the LOQ is 0.1 mg/kg due to interference from the crop matrix at 0.02 mg/kg, and in potatoes the LOQ is 0.5 mg/kg due to the mean recovery being <70% at the lowest fortification level. For desmethyl pirimicarb the LOQ is 0.02 mg/kg in all matrices except potato (LOQ: 0.5 mg/kg) due to the mean recovery being <70% at the three lowest fortification levels.

Conclusion: GCMS method PPRAM 15/2 method is not validated in accordance with SANCO/3029/99 rev. 4 given that linearity data has not been presented and <5 recovery determinations have been made at each fortification level.

Report:	**Harradine, KJ. (1995), Pirimicarb: Validation of a method for the determination of pirimicarb and its carbamate metabolites in brassica, cereal grain and straw, pome fruit, fruiting vegetables (edible peel), leafy Vegetables, Root and Tuber Vegetables. Report RJ 1806B**

Previous evaluation	In DAR (2003) for original approval

Gas chromatography method RAM 265/01 (GC BPX5 column with thermionic nitrogen specific detection) was validated for the determination of pirimicarb and its metabolites desmethyl pirimicarb (R34836) and R238177 in seven crop matrices: apples, tomatoes, lettuce, sugar beet, cabbage, wheat grain and straw.

Method Summary

20 g portions of crop samples (10 g for straw) were extracted by maceration with methanol. After filtration, the methanol was removed by rotary evaporation and the sample residuum re-suspended with hexane and 0.1 M hydrochloric acid solution.

The samples were then left to stand at room temperature overnight during which time any residues of desmethyl formamido (R34885) pirimicarb that were present in the extracts would be converted to and determined as desmethyl pirimicarb (R34836). The hexane layer was then discarded and the residual aqueous layer was further cleaned up by partitioning with ethyl acetate. The ethyl acetate was then discarded and the aqueous phase made basic by addition of sodium hydroxide solution. Any pirimicarb and its carbamate metabolite residues present were then partitioned into dichloromethane which was subsequently evaporated to dryness and the residue re-suspended in acetone prior to analysis by gas chromatography with thermionic nitrogen specific detection.

Instrument Parameters:

Column:	BPX 5 25 m x 0.32 mm, 0.5 µm film
Carrier Gas:	Helium at 4.0 mL/min
Temperature program:	70 °C hold for 1 minute and rise at 20°C per minute to 250°C then immediately rise at 50°C/min to 300 °C and hold for 2 minutes.
Injector Program:	40 °C hold for 0.1 minutes and rise at 150 °C/min to 250 °C and hold for 12 minutes.
Detector temperature:	300°C
Hydrogen Flow:	4.5 mL/min
Air Flow:	170 mL/min
Make up Flow:	26 mL/min
Bead Current:	3.25A
Injection Volume:	2 µL
Solvent Plug Size:	1 µL
Injection Speed:	2µL per second

Summary of Validation Data (Pirimicarb)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Pirimicarb				
Lettuce	0.01* 0.02 0.05 0.1 0.5 Overall	70 – 81 (76) 80 – 86 (82) 81 – 86 (84) 81 – 91 (85) 74 – 100 (87) 70 – 100 (83)	6.9 (4) 3.2 (3) 2.5 (4) 4.6 (4) 10.9 (4) 8.0 (19)	0.01 – 1.0 µg/mL N=6 R=0.99999
Tomato	0.01 0.02 0.05 0.1 0.5 Overall	70 – 94 (80) 74 – 89 (80) 59 – 97 (85) 81 – 95 (88) 80 – 84 (81) 59 – 97 (83)	11.7 (4) 7.5 (4) 18.0 (4) 5.7 (4) 2.0 (4) 11.0 (20)	0.01 – 1.0 µg/mL N=6 R=0.99999
Cabbage	0.05 0.1 0.5 Overall	73 – 81 (77) 62 – 97 (78) 74 – 94 (84) 62 – 97 (80)	4.3 (3) 14.5 (4) 9.1 (4) 13.0 (11)	0.01 – 1.0 µg/mL N=6 R=0.99999
Wheat Grain	0.01 0.02 0.05 0.1 0.5 Overall	80 – 95 (86) 75 – 90 (84) 79 – 86 (84) 78 – 79 (80) 80 – 87 (83) 75 – 95 (83)	7.2 (4) 6. 9 (4) 3.2 (4) 1.9 (4) 3.4 (4) 6.0 (20)	0.01 – 1.0 µg/mL N=6 R=0.99999
Wheat Straw	0.05 0.10 0.20 0.50 1.0 Overall	87 – 106 (96) 69 – 94 (91) 88 – 98 (92) 88 – 97 (92) 88 – 96 (93) 69 – 106 (91)	8.3 (4) 11.1 (4) 4.2 (4) 4.2 (3) 4.2 (4) 8.0 (19)	0.01 – 1.0 µg/mL N=6 R=0.99999
Apple	0.01 0.02 0.05 0.1 0.5 Overall	76 – 90 (81) 68 – 84 (78) 67 – 82 (75) 75 – 87 (81) 80 – 85 (83) 67 – 90 (79)	6.9 (4) 7.8 (4) 7.8 (4) 5. 7 (4) 2.8 (4) 7.0 (20)	0.01 – 1.0 µg/mL N=6 R=0.99999
Sugar Beet	0.01 0.02 0.05 0.1 0.5 Overall	76 – 99 (91) 66 – 83 (75) 77 – 83 (79) 74 – 79 (76) 59 – 79 (69) 59 – 99 (78)	9.9 (4) 8.1 (4) 3.1 (4) 2.8 (4) 11.0 (4) 12.0 (20)	0.01 – 1.0 µg/mL N=6 R=0.99999

*Limit of Quantification

Summary of Validation Data (R34836)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
R34836				
Lettuce	0.01* 0.02 0.05 0.1 0.5 Overall	81 – 119 (98) 82 – 102 (90) 84 – 92 (87) 82 – 94 (88) 77 – 103 (91) 77 – 119 (91)	15.9 (4) 9.4 (3) 3.6 (4) 4.8 (4) 10.4 (4) 11.0 (19)	0.01 – 1.0 µg/mL N=6 R=0.99992
Tomato	0.01 0.02 0.05 0.1 0.5 Overall	84 – 94 (88) 86 – 92 (89) 65 – 91 (82) 90 – 99 (95) 92 – 106 (98) 65 – 106 (90)	4.5 (4) 3.1 (4) 12.4 (4) 3.7 (4) 5.3 (4) 9.0 (20)	0.01 – 1.0 µg/mL N=6 R=0.99999
Cabbage	0.05 0.1 0.5 Overall	98 – 100 (99) 71 – 114 (91) 87 – 104 (94) 71 – 114 (94)	0.8 (3) 17.8 (4) 6.7 (4) 12.0 (11)	0.01 – 1.0 µg/mL N=6 R=0.99999
Wheat Grain	0.01 0.02 0.05 0.1 0.5 Overall	70 – 80 (75) 73 – 89 (77) 82 – 90 (85) 77 – 86 (82) 88 – 96 (90) 70 – 96 (82)	5.8 (4) 8.8 (4) 4.0 (4) 4.0 (4) 4.8 (4) 9.0 (20)	0.01 – 1.0 µg/mL N=6 R=0.99999
Wheat Straw	0.05 0.10 0.20 0.50 1.0 Overall	82 – 113 (96) 70 – 101 (91) 91 – 119 (100) 97 – 107 (100) 96 – 108 (103) 70 – 119 (98)	14.8 (4) 13.1 (4) 11.0 (4) 4.0 (3) 4.2 (4) 12.0 (19)	0.01 – 1.0 µg/mL N=6 R=0.99999
Apple	0.01 0.02 0.05 0.1 0.5 Overall	74 – 87 (79) 73 – 78 (76) 71 – 82 (77) 77 – 85 (81) 85 – 88 (86) 71 – 88 (80)	6.3 (4) 3.0 (4) 6.0 (4) 5.6 (4) 1.5 (4) 6.0 (20)	0.01 – 1.0 µg/mL N=6 R=0.99999
Sugar Beet	0.01 0.02 0.05 0.1 0.5 Overall	88 – 97 (92) 78 – 88 (82) 74 – 90 (85) 82 – 89 (86) 8 – 92 (86) 74 – 97 (86)	3.99 (4) 5.02 (4) 7.53 (4) 3.02 (4) 4.31 (4) 6 (20)	0.01 – 1.0 µg/mL N=6 R=0.99999

*Limit of Quantification

Summary of Validation Data (R238177)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
R238177				
Lettuce	0.01* 0.02 0.05 0.1 0.5 Overall	71 – 92 (81) 85 – 88 (86) 81 – 87 (84) 81 – 93 (87) 76 – 101 (89) 71 – 101 (85)	9.4 (4) 1.4 (3) 3.0 (4) 5.7 (4) 10.5 (4) 8.0 (19)	0.01 – 1.0 µg/mL N=6 R=0.99995
Tomato	0.01 0.02 0.05 0.1 0.5 Overall	70 – 99 (79) 82 – 88 (86) 83 – 102 (94) 92 – 100 (98) 90 – 101 (95) 70 – 102 (90)	13.9 (4) 2.6 (4) 8.0 (4) 4 (4) 4.5 (4) 11.1 (20)	0.01 – 1.0 µg/mL N=6 R=0.99995
Cabbage	0.05 0.1 0.5 Overall	84 – 88 (86) 65 – 114 (87) 84 – 101 (92) 65 – 114 (89)	2.4 (3) 23.5 (4) 7.7 (4) 13.8 (11)	0.01 – 1.0 µg/mL N=6 R=0.99995
Wheat Grain	0.01 0.02 0.05 0.1 0.5 Overall	89 – 104 (98) 85 – 97 (90) 80 – 90 (86) 75 – 80 (78) 83 – 91 (86) 75 – 104 (88)	6.1 (4) 4.6 (4) 4.7 (4) 3.0 (4) 3.8 (4) 8.7 (20)	0.01 – 1.0 µg/mL N=6 R=0.99995
Wheat Straw	0.05 0.10 0.20 0.50 1.0 Overall	97 – 104 (101) 67 – 100 (90) 94 – 108 (99) 93 – 101 (96) 91 – 100 (96) 67 – 108 (97)	3.0 (4) 13.4 (4) 6.0 (4) 4.3 (3) 3.7 (4) 8.7 (19)	0.01 – 1.0 µg/mL N=6 R=0.99995
Apple	0.01 0.02 0.05 0.1 0.5 Overall	71 – 85 (76) 69 – 75 (72) 70 – 80 (76) 74 – 82 (79) 80 – 85 (83) 69 – 85 (77)	7.8 (4) 3.0 (4) 5.4 (4) 4.8 (4) 2.8 (4) 7.1 (20)	0.01 – 1.0 µg/mL N=6 R=0.99995
Sugar Beet	0.01 0.02 0.05 0.1 0.5 Overall	78 – 100 (90) 85 – 88 (87) 74 – 88 (84) 83 – 90 (87) 82 – 93 (87) 74 – 99 (86)	11.0 (4) 1.3 (4) 7.8 (4) 3.2 (4) 5.0 (4) 6.2 (20)	0.01 – 1.0 µg/mL N=6 R=0.99995

*Limit of Quantification

Specificity: Representative chromatograms were provided for each blank matrix, a mixed standard solution of the 3 analytes (highest concentration calibration solution) and the fortified matrix (mixed analyte fortification; highest and lowest fortification level).
Linearity: For each individual analyte, linearity was demonstrated in a range from 0.01 – 1.0 µg/mL with 6 non-matrix matched standards. The concentrations of analyte in the LC-MS/MS solutions were 0.02 µg/mL at the 0.01 mg/kg fortification level, and 1 µg/mL at the 0.05 mg/kg fortification level. Therefore, the analytical calibration does not extend over a range appropriate to the highest nominal concentrations of analyte in the analytical solutions +20%. The matrix effects have not been addressed.
Accuracy: four fortification levels were used to determine the accuracy of the method in terms of each analyte: 0.01 mg/kg (LOQ), 0.02, 0.05, 0.1 and 0.5 mg/kg (50 x LOQ). For cabbage samples, only three fortification levels were used to determine the accuracy (0.05, 0.1 and 0.5 mg/kg). The mean recoveries were in range 70-110%, except at the 0.5 mg/kg fortification of pirimicarb in sugar beet where the mean recovery was slightly outside this range (69%).
Precision: Four recovery determinations were made at each fortification level from which the relative standard deviation (RSD) was determined, except for lettuce and straw where samples were lost at the 0.02 mg/kg and 0.5 mg/kg fortification levels respectively. The reported RSD was ≤20% at each level. No outliers were identified.
LOQ: The LOQ for each analyte in each matrix is 0.01 mg/kg (the lowest fortification level with an acceptable mean recovery and acceptable RSD). For cabbage and wheat straw samples, the LOQ is set as 0.05 mg/kg.

Conclusion: The method is not strictly validated in accordance with SANCO/3029/99 rev. 4 as only four recovery determinations were made at each fortification levels and, therefore, the data set is limited (the guidelines require five determinations). In addition, the analytical calibration does not extend over a range appropriate to the highest nominal concentrations of analyte in the analytical solutions +20%. Non matrix-matched standards were used for the calibration and the matrix effects were not addressed.
It is noted that this method was considered as a monitoring method for the Annex I approval of pirimicarb. The method is not fully validated in accordance with SANCO 825/00 rev. 8.1 since, in addition to the above deficiencies, the method does not employ a confirmatory technique. The method has not been validated for all four matrix groups specified by SANCO 825/00 rev. 8.1.

Report:	**Coombe, N.B. (1996), Independent Validation of Standard Operating Procedure RAM 265/02, Report Number: CEMR-622**

Previous evaluation	In DAR (2003) for original approval

Independent laboratory validation was provided for the determination of pirimicarb and its metabolites R34836 (desmethyl pirimicarb) and R238177 in five crop matrices: potato, apple, cereal grain, cereal forage, cereal straw. The samples were then subjected to the full analytical procedure and analysed by gas chromatography (GC BPX5 column with thermionic nitrogen specific detection).

Method Summary

Residues of pirimicarb and its metabolites are extracted by maceration with methanol. After filtration, the methanol is removed by rotary evaporation and the samples are re-suspended and partitioned with hexane and 0.1M HCl, The samples are left to stand overnight. The hexane layer is discarded and the acidic aqueous layer further cleaned by partitioning with ethyl acetate. The ethyl acetate layer is discarded and the aqueous layer made basic by addition of sodium hydroxide solution. Pirimicarb and its carbamate metabolites are then extracted into dichloromethane. The dichloromethane is evaporated to dryness and the residuum redissolved in acetone. Final quantitation of residues is by GC with thermionic nitrogen specific detector.

<u>Instrument Parameters</u>

Column:	BPX 5 25 m x 0.32 mm, 0.5 µm film
Carrier Gas:	Helium at 4.0 mL/min
Temperature program:	70 °C hold for 1 minute and rise at 20°C per minute to 250°C then immediately rise at 50°C/min to 300 °C and hold for 2 minutes.
Injector Program:	40 °C hold for 0.1 minutes and rise at 150 °C/min to 250 °C and hold for 12 minutes.
Detector temperature:	300°C
Hydrogen Flow:	4.5 mL/min
Air Flow:	170 mL/min
Make up Flow:	26 mL/min
Bead Current:	3.25A
Injection Volume:	2 µL
Solvent Plug Size:	1 µL
Injection Speed:	2µL per second

Under these conditions the retention times of pirimicarb and its metabolite are between 8 and 9 minutes.

Summary of Validation Data (Pirimicarb)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Pirimicarb				
Potato	0.01* 0.05 Overall	87 – 88 (88) 94 – 96 (95) 87 – 96 (91)	0.8 (2) 1.5 (2) 4.8 (4)	40 – 200 ng/mL N=5 R=0.9995
Apple	0.01* 0. 05 Overall	80 – 97 (89) 89 – 93 (91) 80 – 97 (90)	13.6 (2) 3.1 (2) 8.1 (4)	40 – 200 ng/mL N=5 R=0.9995
Cereal Grain	0.01* 0.05 Overall	81 – 87 (84) 97 – 99 (98) 81 – 99 (91)	5.1 (2) 1.4 (2) 9.3 (4)	40 – 200 ng/mL N=5 R=0.9995
Cereal Forage	0.01* 0.05 Overall	101 – 103 (102) 88 – 91 (90) 88 – 103 (96)	1.4 (2) 2.4 (2) 7.7 (4)	40 – 200 ng/mL N=5 R=0.9995
Cereal Straw	0.01* 0.05 Overall	110 – 113 (112) 94 – 96 (95) 94 – 113 (103)	1.9 (2) 1.5 (2) 9.3 (4)	40 – 200 ng/mL N=5 R=0.9995

*Limit of Quantification

Summary of Validation Data (R34836)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
R34836				
Potato	0.01* 0.05 Overall	106 – 110 (108) 99 – 103 (101) 99 – 110 (104.5)	2.6 (2) 2.8 (2) 4.5 (4)	40 – 200 ng/mL N=5 R=0.9996
Apple	0.01* 0. 05 Overall	90 – 92 (91) 96 – 100 (98) 90 – 100 (95)	1.6 (2) 2.9 (2) 4.7 (4)	40 – 200 ng/mL N=5 R=0.9996
Cereal Grain	0.01* 0.05 Overall	92 – 103 (98) 109 – 115 (112) 92 – 115 (105)	5.1 (2) 1.4 (2) 9.3 (4)	40 – 200 ng/mL N=5 R=0.9996
Cereal Forage	0.01* 0.05 Overall	91 – 95 (93) 89 – 93 (91) 89 – 95 (92)	1.4 (2) 2.4 (2) 7.7 (4)	40 – 200 ng/mL N=5 R=0.9996
Cereal Straw	0.01* 0.05 Overall	80 – 96 (83) 91 – -93 (95) 80 – 93 (88)	5.1 (2) 1.5 (2) 6.6 (4)	40 – 200 ng/mL N=5 R=0.9996

*Limit of Quantification

Summary of Validation Data (R238177)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
R238177				
Potato	0.01* 0.05 Overall	74 – 78 (76) 98 – 107 (103) 74-107 (89)	3.7 (2) 6.2 (2) 17.7 (4)	40 – 200 ng/mL N=5 R=0.9949
Apple	0.01* 0. 05 Overall	75 – 93 (84) 96 – 99 (98) 75 – 99 (91)	15.1 (2) 2.2 (2) 11.9 (4)	40 – 200 ng/mL N=5 R=0.9949
Cereal Grain	0.01* 0.05 Overall	84 – 92 (88) 94 (94) 84 – 92 (91)	6.4 (2) 0 (2) 5.2 (4)	40 – 200 ng/mL N=6 R=0.9949
Cereal Forage	0.01* 0.05 Overall	84 – 89 (93) 86 – 88 (87) 84 – 89 (87)	4.1 (2) 1.6 (2) 2.6 (4)	40 – 200 ng/mL N=5 R=0.9949
Cereal Straw	0.01* 0.05 Overall	86 – 91 (89) 88 – 92 (90) 86 – 92 (89)	4.0 (2) 3.1 (2) 3.1 (4)	40 – 200 ng/mL N=5 R=0.9949

*Limit of Quantification

Specificity: Representative chromatograms were provided for each blank matrix, a mixed standard solution of the 3 analytes (highest concentration calibration solution) and the fortified matrix (mixed analyte fortification; highest and lowest fortification level). It is noted that the representative chromatograms are not clearly labelled.
Linearity: For each individual analyte, linearity was demonstrated in a range from 40-200 ng/mL with 6 non-matrix matched standards. The concentrations of analyte in the LC-MS/MS solutions were 40 ng/mL at the 0.01 mg/kg fortification level, and 200 ng/mL at the 0.05 mg/kg fortification level. Therefore, the analytical calibration does not extend over a range appropriate to the lowest and highest nominal concentrations of analyte in the analytical solutions ±20%.
Accuracy: For pirimicarb, desmethyl pirimicarb, and R238177, 2 fortification levels were used to determine the accuracy: 0.01 mg/kg (LOQ) and 0.05 mg/kg (5 x LOQ). The mean recoveries were in range 70-110%, except at the 0.05 mg/kg fortification of desmethyl pirimicarb in cereal grain where the mean recovery was slightly outside this range (112%). It is noted that the recoveries have been corrected with respect to the unfortified control samples.
Precision: Two recovery determinations were made at each fortification level from which the relative standard deviation (RSD) was determined. The reported RSD was ≤20% at each level. No outliers were identified.
LOQ: The LOQ for each analyte in each matrix is 0.01 mg/kg (the lowest fortification level with an acceptable mean recovery and acceptable RSD).

Conclusion: The method is not fully validated in accordance with SANCO/3029/99 rev. 4. In terms of the accuracy of the method, only two recovery determinations were made at each fortification level and, therefore, the data set is limited. Linearity has not been demonstrated in a range appropriate to the lowest and highest nominal concentrations of analyte in the analytical solutions ±20%.
It is noted for the Annex I approval of pirimicarb, these data were considered as the independent laboratory validation of method RAM 265/01. The method is not fully validated in accordance with SANCO 825/00 rev. 8.1 since, in addition to the above deficiencies, the method does not employ a confirmatory technique. The method has not been validated for all four matrix groups specified by SANCO 825/00 rev. 8.1.

Report:	**Wright, DR (1998), Transfer of Standard Operating Procedure RAM 265/03 to Covance Laboratories Ltd in four crop matrices Report Number: CLE 38/229/D2140**

Previous evaluation	None. Submitted for the purposes of renewal

LC-MS/MS method RAM 265/03 was validated for the determination of pirimicarb and its metabolites R34836 (desmethyl pirimicarb) and R238177 in four crop matrices: oil seed rape (OSR) seed, lettuce maize grain and beans with edible pods.

Method Summary

Residues of pirimicarb and its metabolites were extracted by maceration with methanol. After filtration, the methanol was removed by rotary evaporation and the samples were re-suspended and partitioned with hexane and 0.1M HCl, The samples are left to stand overnight. The hexane layer was discarded and the acidic aqueous layer further cleaned by partitioning with ethyl acetate. The ethyl acetate layer was discarded and the aqueous layer made basic by addition of sodium hydroxide solution. Pirimicarb and its carbamate metabolites were then extracted into dichloromethane. Final determination was by high performance liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS).

Instrument Parameters

Pump:	Jasco PU-980
Autosampler:	Gilson model 231
Dilutor:	Gilson 401
Analytical Column:	Luna Phenyl-Hexyl 5 µm, 50 x 2 mm (id)
Flow rate:	0.2 mL/min
Mobile phase:	50/50 v/v acetonitrile/water with 20 mM ammonium acetate
Program:	Isocratic
Injection Volume:	20 µL
Reservoir Solvent:	50/50 v/v acetonitrile/water with 20 mM ammonium acetate.
Analysis time:	3 mins
Mode of Operation:	APcI positive (MS/MS)
Collision gas:	Argon
Collision gas pressure:	$3.0x10^{-4}$ mBAr
Probe temperature:	450 °C
Source temperature:	150 °C
HV lens:	0.3V

Compound	**Parent Ion**	**Daughter Ion**	**Dwell (secs)**	**Coll Energy (eV)**	**Cone (V)**
R34836	225.00	71.90	0.2	40	25
Pirimicarb	239.00	71.90	0.2	20	30
R238177	255.00	71.90	0.2	50	30

Inter channel delay:	0.02s
Capillary voltage:	3.25 KV
Bath gas flow (N_2):	300 L/Hr
Nebulizer gas flow (N_2):	On
APcI Sheath gas (N_2):	150 L/Hr

Summary of Validation Data (Pirimicarb)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Pirimicarb				
Oilseed Rape Seed	0.01* 0.1 0.5 Overall	85 – 89 (87) 83 – 85 (84) 85 – 90% (88) 83 – 90 (86)	2.3 (2) 1.2 (2) 2.9 (2) 2.9 (6)	0.005-0.5 µg/mL N=7 R=0.999163
Lettuce	0.01* 0.10 0.50 Overall	74 – 90 (82) 88 – 101 (100) 89 – 105 (97) 88 – 105 (91)	9.8 (2) 6.9 (2) 8.3 (2) 11.0 (6)	0.005-0.5 µg/mL N=7 R=0.999163
Maize Grain	0.01* 0.10 0.50 Overall	85 – 101 (93) 99 – 101 (100) 98 – 98 (98) 85 – 101 (97)	8.6 (2) 1.0 (2) 0 (2) 5.7 (6)	0.005-0.5 µg/mL N=7 R=0.999163
Beans with Edible Pods (Runner Beans)	0.01* 0.10 0.50 Overall	80 – 100 (90) 78 – 82 (80) 85 – 101 (93) 78 – 101 (88)	11.1 (2) 2.5 (2) 8.6 (2) 10.6 (6)	0.005-0.5 µg/mL N=7 R=0.999163

*Limit of Quantification

Summary of Validation Data (R34836)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
R34836				
Oilseed Rape Seed	0.01* 0.1 0.5 Overall	86 – 92 (87) 90 – 93 (84) 89 – 91 (88) 86 – 93 (90)	3.4 (2) 1.6 (2) 1.7 (2) 2.6 (6)	0.005-0.5 µg/mL N=7 R=0.997645
Lettuce	0.01* 0.10 0.50 Overall	87 – 95 (82) 86 – 101 (100) 93 – 107 (97) 86 – 107 (95)	4.4 (2) 8.0 (2) 7.0 (2) 7.8 (6)	0.005-0.5 µg/mL N=7 R=0.997645
Maize Grain	0.01* 0.10 0.50 Overall	79 – 95 (87) 90 – 100 (95) 101 – 107 (104) 79 – 107 (95)	9.2 (2) 5.3 (2) 2.9 (2) 9.4 (6)	0.005-0.5 µg/mL N=7 R=0.997645
Beans with Edible Pods (Runner Beans)	0.01* 0.10 0.50 Overall	79 – 98 (89) 82 – 85 (83.5) 84 – 109 (97) 82 – 109 (88)	10.7 (2) 1.8 (2) 13.0 (2) 11.8 (6)	0.005-0.5 µg/mL N=7 R=0.997645

*Limit of Quantification

Summary of Validation Data (R238177)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
R238177				
Oilseed Rape Seed	0.01* 0.1 0.5 Overall	82 – 97 (90) 83 – 94 (89) 85 – 92 (89) 82 – 97 (89)	11.9 (2) 8.8 (2) 5.6 (2) 7.1 (6)	0.005-0.5 µg/mL N=7 R=0.999690
Lettuce	0.01* 0.10 0.50 Overall	85 – 90 (88) 88 – 97 (93) 93 – 108 (101) 85 – 108 (94)	4.0 (2) 6.9 (2) 10.6 (2) 8.8 (6)	0.005-0.5 µg/mL N=7 R=0.999690
Maize Grain	0.01* 0.10 0.50 Overall	72 – 92 (82) 90 – 96 (93) 104 – 105 (105) 72 – 105 (93)	17.2 (2) 4.6 (2) 0.7 (2) 12.9 (6)	0.005-0.5 µg/mL N=7 R=0.999690
Beans with Edible Pods (Runner Beans)	0.01* 0.10 0.50 Overall	86 – 106 (96) 82 – 88 (85) 83 – 106 (95) 82 – 106 (95)	14.7 (2) 5.0 (2) 17.2 (2) 12.2 (6)	0.005-0.5 µg/mL N=7 R=0.999690

*Limit of Quantification

Specificity: For each matrix/analyte combination, representative chromatograms were provided for the matrix blank, the lowest calibration standard and the fortified matrix (lowest and highest fortification levels). Significant interference was not observed at the retention times of interest.
Linearity: For each analyte, linearity was demonstrated from 6 non-matrix matched calibration solutions in a range from 0.005-0.5 µg/mL. The concentrations of analyte in the LC-MS/MS solutions were 0.01 ng/mL at the 0.01 mg/kg fortification level, 0.1 ng/mL at the 0.1 mg/kg fortification level, and 0.5 ng/mL at the 0.5 mg/kg fortification level. Therefore, the analytical calibration does not extend over a range appropriate to the highest nominal concentrations of analyte in the analytical solutions ±20%. However, as a linear relationship has been established, this is not of concern.
Accuracy: For each analyte, 3 fortification levels were used to determine the accuracy: 0.01 mg/kg (LOQ), 0.1 mg/kg (10 x LOQ) and 5.0 mg/kg (>10 x LOQ). The mean recoveries were in the range of acceptability (70-110%).
Precision: Two recovery determinations were made at each fortification level from which the relative standard deviation (RSD) was determined. The reported RSD was ≤20% at each level. No outliers were identified.
LOQ: The LOQ, as defined as the lowest fortification level with an acceptable mean recovery and acceptable RSD, is 0.01 mg/kg for each analyte in each matrix.

Conclusion: The method is not strictly validated in accordance with SANCO/3029/99 rev. 4 as only two recovery determinations were made at each fortification level and, therefore, the data set is limited.

Report:	**Croucher, A. (2002), Pirimicarb: Validation of analytical method CLE 38/299-03R for the determination of pirimicarb and metabolites residues in Maize, Report Number: CLE 38/299-03R**

Previous evaluation	None. Submitted for the purposes of renewal

LC-MS/MS method RAM 265/04 was validated for the determination of pirimicarb and metabolites R34836 (desmethyl pirimicarb) and R238177 in maize (whole cobs, grain, fodder and residual plant at harvest).

Method Summary

Samples were extracted by homogenisation with methanol. After filtration, the methanol was removed by rotary evaporation and the samples re-suspended in hexane and 0.1M hydrochloric acid. The samples were left to stand overnight during which time any residues of R34885 were converted to R034836. The hexane layer was discarded and the acidic aqueous fraction further cleaned-up by partitioning with ethyl acetate. The ethyl acetate was discarded and the aqueous solution made basic by the addition of sodium hydroxide solution.

Pirimicarb and its carbamate metabolites were partitioned into dichloromethane, evaporated to dryness and re-suspended in acetonitrile: water for final determination by liquid chromatography coupled to a triple quadrupole mass spectrometer (LC-MS/MS).

Instrument parameters

Instrument:	Quattro 1	
Column:	Phenomenex Luna 5µ phenyl-hexyl, 50 x 2 mm (id)	
Mobile Phase:	Acetonitrile/water with 20 mM ammonium acetate (1:1, v/v)	
Flow rate:	0.2 mL/min	
Programme:	Isocratic	
Retention Times:	Desmethyl pirimicarb (1.1 minutes)	
	Pirimicarb (1.6 minutes)	
	R238177 (1.2 minutes)	
Injection volume:	20 µL	
Ionisation mode:	APcI positive (MS/MS)	
Ion Monitored:	R34836:	225.0 to 71.9
	Pirimicarb:	239 to 71.9
	R238177:	255 to 71.9
Cone voltage:	R34836:	25 V
	Pirimicarb:	30 V
	R238177:	30 V
Collision Energy:	R34836:	40 eV
	Pirimicarb:	20 eV
	R238177:	50 eV
Dwell Time:	0.2 seconds	
Inter channel delay:	0.02 seconds	
CID gas:	argon	
CID gas cell pressure:	3.0×10^{-4} mbar	
Bath gas:	300 L/hr (nitrogen)	
ESI nebuliser:	15 L/hr (nitrogen)	
Capillary voltage:	3.25 kV	
APcI Sheath gas:	150 L/Hr	
Probe Temperature:	450 °C	
Source Temperature:	150 °C	
HV lens:	0.03V	

Summary of Validation Data (Pirimicarb)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Pirimicarb				
Maize whole cobs	0.01* 0.1 Overall	83 – 87 (85) 85 – 90 (88) 83 – 90 (86)	1.8 (5) 2.7 (5) 3.0 (10)	0.005-0.5 µg/mL N=6 R=0.9997
Maize grains	0.01* 0.1 Overall	77 – 94 (88) 57 – 91 (80) 57 – 94 (84)	7.5 (5) 18.2 (5) 13.7 (10)	0.005-0.5 µg/mL N=6 R=0.9988
Residual plant (at harvest)	0.01* 0.1 Overall	89 – 107 (94) 87 – 94 (92) 87 – 107 (93)	7.8 (5) 3.8 (5) 6.0 (10)	0.005-0.5 µg/mL N=6 R=0.9999
Maize fodder	0.01* 0.1 Overall	84 – 96 (90) 83 – 91 (89) 83 – 96 (89)	4.9 (5) 3.8 (5) 4.2 (10)	0.005-0.5 µg/mL N=6 R=0.9992

*Limit of quantification

Summary of Validation Data (R34836)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
R34836				
Maize whole cobs	0.01* 0.1 Overall	84 – 97 (90) 90 – 93 (91) 84 – 97 (91)	6.2 (5) 1.3 (5) 4.3 (10)	0.005-0.5 µg/mL N=6 R=1.0000
Maize grains	0.01* 0.1 Overall	87 – 100 (94) 65 – 94 (84) 65 – 100 (89)	5.0 (5) 15.5 (5) 12.1 (10)	0.005-0.5 µg/mL N=6 R=0.9983
Residual plant (at harvest)	0.01* 0.1 Overall	88 – 102 (95) 87 – 93 (91) 87 – 102 (93)	5.2 (5) 2.5 (5) 4.6 (10)	0.005-0.5 µg/mL N=6 R=0.9973
Maize fodder	0.01* 0.1 Overall	89 – 100 (95) 90 – 95 (93) 89 – 100 (94)	4.5 (5) 2.1 (5) 3.6 (10)	0.005-0.5 µg/mL N=6 R=0.9994

Summary of Validation Data (R238177)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
R238177				
Maize whole cobs	0.01* 0.1 Overall	84 – 89 (90) 82 – 93 (88) 82 – 93 (89)	3.8 (5) 5.4 (5) 4.5 (10)	0.005-0.5 µg/mL N=6 R=.9992
Maize grains	0.01* 0.1 Overall	86 – 96 (91) 68 – 92 (84) 68 – 96 (87)	5.0 (5) 15.5 (5) 12.1 (10)	0.005-0.5 µg/mL N=6 R=0.9979
Residual plant (at harvest)	0.01* 0.1 Overall	86 – 92 (89) 87 – 90 (88) 86 – 92 (88)	3.4 (5) 1.6 (5) 2.6 (10)	0.005-0.5 µg/mL N=6 R=0.9981
Maize fodder	0.01* 0.1 Overall	85 – 96 (90) 84 – 90 (88) 84 – 96 (89)	4.3 (5) 3.1 (5) 3.9 (10)	0.005-0.5 µg/mL N=6 R=0.9995

Specificity: For each matrix/analyte combination, representative chromatograms were provided of the blank matrix, the highest and lowest concentration calibration standards and the matrix fortified matrix (LOQ and 10xLOQ level). No significant interferences arising from the crop matrices have been observed at the retention times of interest.
Linearity: For each analyte, the linearity of the method was demonstrated with a calibration plot for solutions in the range of 0.005-0.5 µg/mL. The concentrations of analyte in the LC-MS/MS solutions were 0.01 µg/mL at the 0.01 mg/kg fortification level, and 0.1 µg/mL at the 0.1 mg/kg fortification level. Therefore, the analytical calibration extends over a range appropriate to the lowest and highest nominal concentrations of analyte in the analytical solutions ±20%.
No significant matrix effects (>30% of LOQ level) were reported and so matrix matched linearity standards (i.e. solvent standards in control matrix extracts) were not required for quantification.
Accuracy: For each analyte, two fortification levels were used to determine the accuracy: 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 x LOQ). The mean recoveries were in the acceptable range 70-110%.
Precision: Five recovery determinations were made at each fortification level from which the relative standard deviation (RSD) was determined. The reported RSD was ≤20% at each level. No outliers were identified.
LOQ: The LOQ, as defined as the lowest fortification level with an acceptable mean recovery and acceptable RSD, is 0.01 mg/kg.

Conclusion: LC-MS/MS method RAM 265/04 is satisfactorily validated in accordance with SANCO/3029/99 rev. 4 for the determination of pirimicarb, desmethyl pirimicarb and R238177 in maize matrices with an LOQ of 0.01 mg/kg.

Report:	**Tsui, G. (2015), Pirimicarb - Analytical Method GRM039.04A for the Determination of Pirimicarb and its Metabolite Desmethyl Pirimicarb in Crops, Report Number: TK0252946**

Previous evaluation	None. Submitted for the purposes of renewal

LC-MS/MS method GRM039.04A was successfully validated for the determination of pirimicarb and its metabolite desmethyl pirimicarb (R34836) in four crop matrices: wheat grain, wheat straw, sugar beet roots and sugar beet leaves with tops.

Method Summary

20 g sub samples of crop (10 g for straw) were extracted by homogenisation with methanol. Extracts were centrifuged and aliquots (1 mL = 0.2 g or 0.05 g for straw) were evaporated, hydrolysed with 0.1 M HCl and diluted with 0.1 M ammonium acetate solution, followed by filtration. Final determination was by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).

Storage

The stability of the final extracts was assessed in all matrices after storing the sample sets in a refrigerator (1 – 8 °C) for at least 7 days, before re-analysing against freshly prepared calibration standards. Consistent fortification results (within 70 - 110% range and RSD <20% after storage), quantified using the primary transition, demonstrate stability of pirimicarb in the final extracts for at least 8, 14, 15 and 15 days in wheat straw, wheat grain, sugar beet leaves with tops and sugar beet roots respectively, and demonstrate stability of desmethyl pirimicarb for at least 8, 14, 23 and 41 days in wheat straw, wheat grain, sugar beet leaves with tops and sugar beet roots respectively.

Pirimicarb Recovery Data After 8-15 Days Storage at 1-8°C (Primary Transition *m/z* 239 → 72)

Matrix	**Storage Interval (days)**	**Fortification Level**	**Recoveries % range (mean)**	**%RSD (n)**
Wheat Grain	0	0.01	100 – 104 (102)	1.7 (6)
	14	0.01	96 – 103 (99)	2.6 (6)
Wheat Straw	0	0.01	105 – 113 (110)	3.0 (6)
	8	0.01	104 – 107 (107)	1.7 (6)
Sugar Beet Roots	0	0.01	107 – 113 (109)	2.1 (6)
	15	0.01	107 – 111 (108)	3.2 (6)
Sugar Beet Leaves with Tops	0	0.01	105 – 112 (110)	2.8 (6)
	15	0.01	104 – 113 (107)	3.5 (6)

Desmethyl Pirimicarb Recovery Data After 8-41 Days Storage at 1-8°C (Primary Transition *m/z* 225 → 72)

Matrix	**Storage Interval (days)**	**Fortification Level**	**Recoveries % range (mean)**	**%RSD (n)**
Wheat Grain	0	0.01	89 – 91 (90)	0.7 (6)
	14	0.01	85 – 91 (88)	2.4 (6)
Wheat Straw	0	0.01	102 – 112 (108)	3.8 (6)
	8	0.01	106 – 110 (109)	2.8 (6)
Sugar Beet Roots	0	0.01	104 – 110 (108)	2.1 (6)
	41	0.01	101 – 113 (108)	4.5 (6)
Sugar Beet Leaves with Tops	0	0.01	107 – 111 (110)	1.2 (6)
	23	0.01	106 – 113 (109)	2.5 (6)

Instrument parameters

Chromatograph:	Agilent 1100 HPLC with an AB Sciex API5000 mass spectrometer
Detector:	AB Sciex API5000 triple quadrupole mass spectrometer with Analyst™ software version 1.6.2.
Pump and Degasser:	Agilent 1100 series binary pump fitted with degasser
Autosampler:	Presearch CTC PAL HTS
Gas Supply:	Peak Scientific Genius 3031 gas station
Column Oven:	Agilent 1100 series
Column size:	Phenomenex Kinetex C18, 50 mm length, 4.6 mm i.d., 2.6 µm particle
Column temperature:	20°C ±1°C
Injection volume:	10 µL
Stop Time:	5 minutes
Injection protocol:	Analyse calibration standard after 3 to 4 sample injections
Mobile Phase:	Solvent 1: 10 mM ammonium acetate (aq) Solvent 2: Acetonitrile

Time (mins)	% solvent 1	% solvent 2	Flow rate (mL/min)
0	80	20	1.0
0.5	80	20	1.0
2.5	20	80	1.0
3.5	20	80	1.0
3.6	80	20	1.0
5	80	20	1.0

Under these conditions the retention times of pirimicarb and desmethyl pirimicarb are 2.4 – 2.5 minutes and 1.9 – 2.0 minutes, respectively.

Mass Spectrometer Conditions

Mass Spectrometer :	API5000
Interface :	TurboIonSpray
Polarity :	Positive
Curtain gas (CUR) :	30 (arbitrary units)
Temperature (TEM) :	500 °C
Ion spray voltage :	5500 V
Collision gas setting (CAD) :	5 V
Gas 1 (GS1) :	50 (arbitrary units)
Gas 2 (GS2) :	50 (arbitrary units)
Interface heater (ihe) :	On

MRM Conditions	**Pirimicarb primary transition**	**Pirimicarb confirmatory transition**	**R34836 primary transition**	**R34836 confirmatory transition**
Q1 m/z	239	239	225	225
Q3 m/z	72	182	72	168
Dwell time	100 ms	100 ms	100 ms	100 ms
Resolution Q1	Unit	Unit	Unit	Unit
Resolution Q3	Unit	Unit	Unit	Unit
Declustering potential (DP)	86 V	86 V	66 V	66 V
Entrance potential (EP)	10 V	10 V	10 V	10 V
Collision Energy (CP)	29 V	23 V	33 V	21 V
Collision cell exit potential (CXP)	12 V	12 V	28 V	10 V

Summary of Validation Data (Pirimicarb)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Wheat Grain		Pirimicarb (m/z 239 to72) Quantification		
	0.01* 0.5 Overall	100 – 104 (102) 91 – 97 (94) 91 – 104 (98)	1.7 (6) 2.5 (6) 4.7 (12)	0.105 – 10.5 ng/mL (0.000525-0.0525 mg/kg) N=7 R=0.9966
		Pirimicarb (m/z 239 to 182) Confirmation		
	0.01* 0.5 Overall	88 – 93 (90) 85 – 94 (90) 85 – 94 (90)	2.0 (6) 3.4 (6) 2.6 (12)	0.105 – 10.5 ng/mL (0.000525-0.0525 mg/kg) N=7 R=0.9999
Wheat Straw		Pirimicarb (m/z 239 to72) Quantification		
	0.01* 2.0 Overall	105 – 113 (110) 100 – 106 (103) 100 – 113 (106)	3.0 (6) 2.2 (6) 4.2 (12)	0.105 – 10.5 ng/mL (0.002-0.210 mg/kg) N=7 R=0.9954
		Pirimicarb (m/z 239 to 182) Confirmation		
	0.01* 2.0 Overall	89 – 111 (102) 96 – 105 (100) 89 – 111 (101)	7.3 (6) 2.9 (6) 5.5 (12)	0.105 – 10.5 ng/mL (0.002-0.210 mg/kg) N=7 R=0.9991
Sugar Beet Roots		Pirimicarb (m/z 239 to72) Quantification		
	0.01* 0.5 Overall	107 – 113 (109) 102 – 111 (108) 102 – 113 (109)	2.1 (6) 2.9 (6) 2.6 (12)	0.105 – 10.5 ng/mL (0.000525-0.0525 mg/kg) N=7 R=0.9971
		Pirimicarb (m/z 239 to 182) Confirmation		
	0.01* 0.5 Overall	103 – 111 (107) 99 – 109 (105) 99 – 111 (106)	2.7 (6) 3.7 (6) 3.2 (12)	0.105 – 10.5 ng/mL (0.000525-0.0525 mg/kg) N=7 R=0.9998
Sugar Beet Leaves with Tops		Pirimicarb (m/z 239 to72) Quantification		
	0.01* 5.0 Overall	105 – 112 (110) 94 – 101 (97) 94 – 112 (103)	2.8 (6) 3.0 (6) 7.1 (12)	0.105 – 10.5 ng/mL (0.000525-0.0525 mg/kg) N=7 R=0.9955
		Pirimicarb (m/z 239 to 182) Confirmation		
	0.01* 5.0 Overall	106 – 112 (108) 91 – 101 (94) 91 – 112 (100)	2.2 (6) 4.4 (6) 8.0 (12)	0.105 – 10.5 ng/mL (0.000525-0.0525 mg/kg) N=7 R=0.9995

*Limit of quantification

Summary of Validation Data (R34836)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Wheat Grain		R34836 (m/z 225 to 72) Quantification		
	0.01* 0.5 Overall	89 – 91 (90) 86 – 94 (90) 86 – 94 (90)	0.7 (6) 3.2 (6) 2.2 (12)	0.0974-9.74 ng/mL (0.000487-0.0487 mg/kg) N=7 R=0.9997
		R34836 (m/z 225 to168) Confirmation		
	0.01* 0.5 Overall	80 – 88 (84) 85 – 94 (88) 80 – 94% (86)	3.6 (6) 3.8 (6) 4.6 (12)	0.0974-9.74 ng/mL (0.000487-0.0487 mg/kg) N=7 R=0.9989
Wheat Straw		R34836 (m/z 225 to 72) Quantification		
	0.01* 2.0 Overall	102 – 112 (108) 105 – 109 (107) 102 – 112 (107)	3.8 (6) 1.6 (6) 2.8 (12)	0.0974-9.74 ng/mL (0.00195 – 0.195 mg/kg) N=7 R=0.9981
		R34836 (m/z 225 to168) Confirmation		
	0.01* 2.0 Overall	104 – 111 (107) 104 – 111 (108) 104 – 111 (108)	2.4 (6) 2.6 (6) 2.5 (12)	0.0974-9.74 ng/mL (0.00195 – 0.195 mg/kg) N=7 R=0.9988
Sugar Beet Roots		R34836 (m/z 225 to 72) Quantification		
	0.01* 0.5 Overall	104 – 110 (108) 98 – 108 (103) 98 – 110 (106)	2.1 (6) 3.3 (6) 3.5 (12)	0.0974-9.74 ng/mL (0.000487-0.0487 mg/kg) N=7 R=0.9968
		R34836 (m/z 225 to168) Confirmation		
	0.01* 0.5 Overall	101 – 106 (103) 90 – 100 (97) 90 – 106 (100)	1.7 (6) 3.9 (6) 4.4 (12)	0.0974-9.74 ng/mL (0.000487-0.0487 mg/kg) N=7 R=0.9996
Sugar Beet Leaves with Tops		R34836 (m/z 225 to 72) Quantification		
	0.01* 5.0 Overall	107 – 111 (110) 91 – 99 (94) 91 – 111 (102)	1.2 (6) 3.5 (6) 8.6 (12)	0.0974-9.74 ng/mL (0.000487-0.0487 mg/kg) N=7 R=0.9971
		R34836 (m/z 225 to168) Confirmation		
	0.01* 5.0 Overall	107 – 111 (108) 89 – 97 (92) 89 – 111 (100)	1.2 (6) 3.5 (6) 9.0 (12)	0.0974-9.74 ng/mL (0.000487-0.0487 mg/kg) N=7 R=0.9997

*Limit of quantification

Specificity: For each matrix and each analyte, representative chromatograms of the matrix blanks, the lowest concentration matrix matched calibration standard and the fortified matrix (highest and lowest fortification levels) were provided. In each instance a chromatogram was provided for both the primary and confirmatory mass transitions.
Linearity: For pirimicarb, linearity was demonstrated in a range from 0.105-10.5 ng/mL and for desmethyl pirimicarb, linearity was demonstrated in a range from 0.0974-9.74 ng/mL. The concentrations of analyte in the LC-MS/MS solutions were 2 ng/mL at the 0.01 mg/kg fortification level, and 100 ng/mL at the 0.5 mg/kg fortification level. For straw samples, the concentrations of analyte in the LC-MS/MS solutions were 0.5 ng/mL at the 0.01 mg/kg fortification level, and 100 ng/mL at the 25 mg/kg fortification level. Therefore, the analytical calibration extends over a range appropriate to the lowest and highest nominal concentration of the analyte in relevant analytical solutions + at least 20%.
Significant matrix effects (suppression or enhancement, $\geq \pm 10\%$) were observed in the crop matrices tested during method validation. Therefore, matrix-matched linearity standards (i.e. solvent standards in control matrix extracts) were used for quantification.
Accuracy: For both pirimicarb and desmethyl pirimicarb, 2 fortification levels were used to determine the accuracy: 0.01 mg/kg (LOQ) and a second level at >10 x LOQ. The mean recoveries were in range 70-110%.
Precision: Six recovery determinations were made at each fortification level from which the relative standard deviation (RSD) was determined. The reported RSD was ≤20% at each level. No outliers were identified.
LOQ: The LOQ, as defined as the lowest fortification level with an acceptable mean recovery and acceptable RSD, is 0.01 mg/kg.

Conclusion: The method is satisfactorily validated in accordance with SANCO/3029/99 rev. 4 for the determination of pirimicarb and R34836 in wheat grain, wheat straw, sugar beet roots and sugar beet leaves with tops with an LOQ of 0.01 mg/kg.

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

Report:	**Peither, A. (2014), Pirimicarb - Effect on Survival, Growth and Reproduction of Daphnia magna in a Semi-Static Test over Three Weeks in a Higher Tier Test Design with Sediment, Report Number: D84807**

Previous evaluation	None. Submitted for the purposes of renewal

Preparation of Test Samples

The test and control samples were thawed at room temperature for 3 hours and shaken manually to obtain homogeneous sample solutions. The samples were completely diluted with acetonitrile by a factor of 1.25. If necessary, they were further diluted into the calibration range with test water/acetonitrile (v/v; 80/20) before they were analysed. Following the dilutions, the final nominal concentrations of test item were in the range of 1.60-3.2 µg/L.

Instrument parameters

Autosampler: CTC PAL
Pump: High pressure gradient with 2 Shimadzu LC-10AD and a Shimadzu SCL System Controller
Detector: MDS Sciex API 4000; triple stage quadrupole mass spectrometer
Column: Inertisil ODS-3 (GL Sciences); 2.1 mm x 50mm; 3 µm
Eluent A: 95 vol. water + 5 vol. methanol + 0.1 vol. formic acid;5 mM ammonium formate
Eluent B: 5 vol. water + 95 vol. methanol + 0.1 vol. formic acid;5 mM ammonium formate
Gradient:

Minutes	% Eluent A	% Eluent B
0	50	50
2.0	30	70
3.0	0	100
3.5	0	100
3.6	50	50
5.0	50	50

Flow rate: 300 µL/minute
Injection volume: 5 µL
Ionization Mode: ESI
Heater Gas Temp. 400 °C
Spray Voltage: 5500 V
Scan Mode: Multiple reaction monitoring (MRM)
MS/MS Conditions:

Active Ingredient	Precursor Ion	Product Ion
Pirimicarb	239.1	182.1

Retention time: Pirimicarb: 1.6 minutes

Summary of Validation Data

Matrix	**Analyte**	**LOQ (µg/L)**	**Recovery fortification level (µg/L)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**
Water	Pirimicarb	1.97	1.97 34.7 Overall	101-108 (104%) 102-110 (106%) 101-110 (105%)	2.92 (6) 3.00 (6) 2.94 (12)	0.0997-10.8 µg/L (corresponding to ~6-340 w/v in test samples) N=7 R=0.9993

Specificity: Comparison of the chromatogram of a biological control sample and the test solution showed no interference at the retention time of pirimicarb.
Linearity: Linearity was demonstrated in the range 0.0997 to 10.8 µg/L. The test item solutions were diluted into the calibration range with test water/acetonitrile before they were analysed.
Accuracy: Two fortification levels were used to determine the accuracy: 1.97 µg/L (LOQ) and 34.7 µg/L (>10 x LOQ). The mean recoveries were in the range of 80-100%.
Precision: The precision was reported as repeatability of recovery at each fortification level (six determinations were made at each fortification level). The RSD at each fortification level and the overall RSD was ≤20%.

Conclusion: The method is fully validated for the determination of pirimicarb in solutions of test water in accordance with SANCO/3029/99 rev. 4.

Report:	**Wallace S. J., Smyth D. V., Shillabeer N., (2002), YF7904B (Pirimicarb formulation): Toxicity to the sediment reworker *Chironomus riparius* of a 500 g kg-1 WG formulation, Report Number: BL7239/B**

Previous evaluation	None. Submitted for the purposes of renewal

Sample Preparation
Aqueous samples of pirimicarb were extracted into toluene and analysed by gas chromatography using a thermionic detector. The samples were quantified against standards of analytical ingredient, in toluene, prepared from an acetone stock.

Instrument Parameters
Column: 25 m x 0.53 mm id silica
Column stationary phase: CP-Sil-19-CB
Column temperature: 200°C
Injection port temperature: 250°C
Injection volume: 1.0 µL
Carrier gas: helium @ 15 mL/min
Detector: thermionic specific
Detector gases: nitrogen @ 30 mL/min
Air @ 180 mL/min
Hydrogen @5.0 mL/min
Detector temperature: 300°C
Detector range: 12
Retention time: ~4.6 minutes

Summary of Validation Data

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Reconstituted water	Pirimicarb	0.25	0.25	92 (92%)	0 (3)	-
			0.5	96-100% (97%)	2.4 (3)	
			Overall	92-96% (95%)	3.5 (6)	

Specificity: Chromatograms were presented for a 0.5 mg/L solution of pirimicarb standard and the nominal 1.05 mg/L formulation solution (corresponding to 0.5 mg/L active substance). A chromatogram was not provided for the matrix blank.
Linearity: The linearity of the method was not addressed.
Accuracy: Two fortification levels were used to determine the accuracy: 0.25 mg/L (LOQ) and 0.5µg/L (2 x LOQ). The mean recoveries were in range 80-100%.
Precision: Three recovery determinations were made at each fortification level. The %RSD at each fortification level was <20%.
LOQ: The method is validated with an LOQ of 0.25 mg/L (lowest concentration tested at which an acceptable mean recovery (70-110%) with an acceptable RSD (≤20%) has been determined).

Conclusion

The method is not fully validated in accordance with SANCO/3029/99 rev. 4 for the determination of pirimicarb in solutions of reconstituted water for the following reasons:

- The specificity of the method has not been addressed since a chromatogram was not provided for the matrix blank; however, given the method is a straightforward extraction into toluene from water, followed by GC analysis, specificity is unlikely to be a concern. The submitted chromatograms also show a sharp peak without any significant perturbation of the baseline.
- The linearity of the method has not been addressed; however, chromatograms demonstrate a sharp signal at 0.5 mg/L, indicative of the detector being sensitive at least to a level around and slightly below the NOEC. So, whilst linearity is not addressed in accordance with SANCO/3029/99 rev. 4, there is sufficient confidence that the response of the detector is linear at an appropriate concentration for the NOEC.
- Only three recovery determinations were made at each fortification level. SANCO/3029/99 rev. 4 stipulates that 5 recovery determinations should be made at each fortification level. It is not clear if the fortifications were made to blank water samples or the test solutions.

However it is considered that there is sufficient data to support an LOQ of the method is 0.25 mg/L. It is noted that there is no data to support the LOQ of 0.0069 mg/L reported in the study, or the reported concentration of pirimicarb in the test solutions after 28 days (0.0016 mg/L).

Overall, it is clear that the method is not validated in accordance with SANCO guidance; however, the method is a simple extraction into toluene from water, followed by GC analysis. The biggest challenge with this method is the high recovery at Day 0 (130% of nominal) resulting in Ecotoxicology taking forward a NOEC of 0.65 mg/L. Due to the high recovery, it is not possible to conclude whether the method overestimated the amount of analyte, or whether the amount of pirimicarb added to the system was too high as a result of laboratory error.

This results in uncertainty with the initial mean measured NOEC (0.65 mg/l) proposed from this study. This uncertainty should be considered further by the ecotoxicology specialist. The NOEC cannot be confidently established on the basis of this study however potentially the nominal NOEC (0.50 mg/l) could be used as a conservative approach. Furthermore if this species is not considered to be driving the ecotoxicology risk assessment then this uncertainty may be considered to be acceptable. Please refer to the RAR Volume 3, CA (AS), Section B.9 for the further consideration of this analytical uncertainty from an ecotoxicology perspective.

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

B.5.2.1. Methods for the determination of all components included in the monitoring residue definition in or on food and feed of plant and animal origin

a) Plant matrices

Residue Definition

The EFSA conclusion on the peer review of pirimicarb (2005) indicates that the residue definition for monitoring in food of plant origin is sum of pirimicarb, R34836 (desmethyl pirimicarb) and R34885 (desmethyl formamido pirimicarb), expressed as pirimicarb. However, the EFSA reasoned opinion on pirimicarb (2014) proposed to change the residue definition for enforcement by excluding R34836 and R34885; the parent compound was deemed to be a sufficient marker of the residue. At renewal, the residue definition for monitoring in food of plant origin is pirimicarb.

Monitoring methods

Analytical methods for the determination of residues of pirimicarb and desmethyl pirimicarb in crops were described in the EU DAR (Vol.3, Annex B, Section B5, October 2003). The validation data for these methods is covered in section B.5.1.2.5. It is noted that these methods are not fully validated in accordance with SANCO/825/00 rev. 8.1, though this guidance document was not in force at the time of original approval (2003).

Matrices	Analyte	Method	LOQ	Reference
Wheat (grain, straw)	Pirimicarb R34836	GC-NPD	0.01 mg/kg (grain) 0.05 mg/kg (straw)	Method: RAM 265/01 Study: RJ1806B KJ Harradine, 1995
ILV - Wheat (grain, straw)	Pirimicarb R34836	GC-NPD	0.01 mg/kg (grain) 0.05 mg/kg (straw)	Method: RAM 265/02 Study: CEMR-622 N B Coombe, 1996

No new monitoring methods have been proposed. However, the Applicant for the renewal of pirimicarb states that there is a QuEChERS method in combination with HPLC-MS/MS available for the analysis of pirimicarb, with an LOQ of 0.01 mg/kg in high water content, high oil content, acidic and dry commodities. The Applicant submitted validation data for this QuEChERS method. There is insufficient information to conclude that the method is validated with respect to all the required criteria in SANCO/825/00 rev. 8.1 and, therefore, there is a data gap for a monitoring method for food of plant origin.

b) Animal Matrices

Residue Definition

The EFSA conclusion on the peer review of pirimicarb (2005) indicates that the residue definition for monitoring in food of animal origin is pirimicarb. The residue definition was based on the available livestock metabolism studies, which showed no parent or other carbamate compounds in edible tissues even following dosing at levels in excess of the expected practical level of exposure of livestock. It was further concluded that the monitoring of animal products is unnecessary in terms of consumer safety and, therefore, an analytical method for food of animal origin was not required due to the fact that no MRL was proposed.
The EFSA reasoned opinion on pirimicarb (2014) indicates that default MRLs of 0.05* mg/kg are in place for all animal commodities, and that a modification to this value to 0.01* mg/kg is recommended for poultry products. It is noted that the residue definition for enforcement is pirimicarb in poultry and the sum of pirimicarb and R34836 (desmethyl pirimicarb), expressed as pirimicarb for other products of animal origin (ruminant and swine).
At renewal, the residue definition for monitoring in food of animal origin is the sum of 2-amino-5,6-dimethyl-4-hydroxypyrimidine (NOA422676 (R31680)) and 5,6-dimethyl-2-methylamino-4-hydroxypyrimidine (R34865), expressed as pirimicarb.

Monitoring methods

Analytical methods for the determination of residues of pirimicarb, R34836 and R34885 (desmethyl formamido pirimicarb) in animal matrices were evaluated during the first Annex 1 review of pirimicarb and summaries of the method and validation data are provided in the EU DAR (Vol.3, Annex B, Section B5, October 2003). It is noted that these methods are not fully validated in accordance with SANCO/825/00 rev. 8.1, though this guidance document was not in force at the time of original approval (2003).

Matrices	Analyte	Method	Reference
Milk Eggs Tissues	Pirimicarb R34836 R34885	GLC-NPD	Method: Residue Analytical Method 38 Report: PPRAM 38 Edwards, M.J. and Dick, J., 1978
Milk Muscle Kidney Liver Egg	Pirimicarb R34836	GC/MSD	Method: DFG-S19 Report: ZEN-9503 Tillkes, V.M., 1995

A new monitoring method utilising LC-MS/MS techniques has been proposed for the determination of pirimicarb in animal matrices (method DFG S 19). Analytical validation and independent laboratory validation data for the DFG S 19 analytical method have been provided. However, it is noted that the ILV has been performed for only two animal matrices (milk and egg). In accordance with SANCO/825/00 rev 8.1, it may be sufficient to perform an ILV with two animal matrices, provided that the primary method is identical for all matrices (milk, eggs, meat, fat and liver/kidney). While the primary method for milk, eggs, meat and liver/kidney is identical, the method for fat is different. On this basis, an ILV for fat matrices would be required to support DFG S 19.

Crop	Analyte	Method	Reference
Milk Eggs Liver Kidney Meat Fat	Pirimicarb	LC-MS/MS	Method: DFG S 19 Report: SYN-0501 Lakaschus, S. (2005)
ILV - Milk ILV - Eggs	Pirimicarb	LC-MS/MS	Method: DFG S 19 Report: IF-05/00362966 Reichert, N. (2005)

It is noted that at renewal the residue definition for monitoring in food of animal origin is the sum of 2-amino-5,6-dimethyl-4-hydroxypyrimidine (NOA422676 (R31680)) and 5,6-dimethyl-2-methylamino-4-hydroxy pyrimidine (R34865), expressed as pirimicarb. On this basis, a monitoring method that complies with the new residue definition is required.

Method DFG S 19: Determination of pirimicarb in animal matrices

Report:	**Lakaschus, S., (2005), Validation of Multi-Residue Method DFG S 19 (L 00.00-34) For The Determination of Residues of Pirimicarb in Animal Tissues with LC-MS/MS Detection, Report Number: SYN-0501V**

Previous evaluation	None. Submitted for the purposes of renewal

Validation data was provided for the determination of pirimicarb in six animal matrices: milk, eggs, liver, kidney, meat and fat. The animal species of the samples was not indicated. The samples were fortified with various levels of pirimicarb and were then subjected to the full analytical procedure and analysed by LC-MS/MS.

Method Summary

Meat, liver and kidneys were homogenised in a food cutter with dry ice. For the preparation of the egg specimens the shell was removed and the material was homogenised using a cutter. For the fat and milk specimens no further pre-treatment was performed. All specimens were maintained frozen until the extraction procedure.

Meat, milk, liver, kidney and eggs (Module E8)

The specimen material (5 g were used for each matrix) was ground with a 1:1 mixture of sea sand and sodium sulfate using a pestle until homogeneous consistency was reached. The mixture was transferred onto a chromatographic column containing sodium sulfate and eluted with 250 mL of 2:1 n-hexane/acetone. The eluate was collected and evaporated to dryness at 40°C bath temperature using rotary evaporation. The residue was dissolved in 15 mL of 1:1 ethyl acetate/cyclohexane. Samples were cleaned up using gel permeation chromatography; the collected GPC eluate was evaporated to less than 5 mL and then made up to 5 mL with ethyl acetate. An aliquot of this cleaned up sample (2.5 mL) was then evaporated and dissolved in 0.5 mL methanol before dilution with 0.1 % acetic acid to 2.5 mL (milk and meat) or with methanol: 0.1 % acetic acid (20:80 v/v) to 10 mL (eggs, liver and kidney).

Fat (Module E6)

To the fat (5 g) was added 10 mL of 1:1 ethyl acetate/cyclohexane and the mixture was kept at 60°C for 10 minutes in a drying oven. The solution was drained through glass wool into a 50 mL volumetric flask. The extraction step was repeated once with 10 mL of 1:1 ethyl acetate/cyclohexane. The ethyl acetate/cyclohexane phases were combined and made up to 50 mL. Samples were cleaned up using gel permeation chromatography; the collected GPC eluate was evaporated to less than 5 mL and then made up to 5 mL with ethyl acetate. An aliquot of this cleaned up sample (2.5 mL) was then evaporated and dissolved in 0.5 mL methanol before dilution with 0.1 % acetic acid to 2.5 mL.

Instrument Parameters

System	Hewlett-Packard Series 1100 HPLC (Agilent Technologies)
Column	Phenomenex LUNA C18(2), 2.0 x 150 mm
Column Temperature	30°C
Injection Volume	30 µL
Mobile Phase Conditions	A: methanol + 0.05% acetic acid B: water + 0.05% acetic acid

Time	**%A**	**%B**	**Flow (mL/min)**
-4.0 -> 0	10	90	0.6
0.0 -> 0.01	10	90	0.4
0.01 -> 1.0	10	90	0.4
1.0 -> 1.01	60	40	0.4
1.01 -> 6.0	90	10	0.4
6.0 -> 8.0	90	10	0.4

Valco Valve:	0.0 – 4.0 to Waste 1.0 4.0 – 7.9 to MS
Retention Times (approx.)	Pirimicarb ~6.7 min
Total Run Time	12 min

Mass Spectrometer Conditions

MS System:	**PE-Sciex API 4000 tandem mass spectrometer**			
Analyte Monitored	Ion Monitored	Declustering Potential	Collision Energy	Dwell Time (Seconds)
Pirimicarb	239→182	+56 V	+27 V	0.5
Pirimicarb	239→72	+56 V	+39 V	0.5
Ion Mode:		Positive Multiple Reaction Monitoring (MRM)		
Capillary Voltage:		+5000V		
Ion spray Turbo Heater:		375°C		
Gas Flow 1:		40		
Gas Flow 2:		60		
Curtain Gas Flow:		35		

Summary of Validation Data

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Milk		Pirimicarb (m/z 239 to182) Quantification		
	0.01* 0.10 Overall	91 - 118 (107) 90 - 107 (101) 90 - 118 (104)	10 (5) 6.5 (5) 8.7 (10)	0.200-50.0 ng/mL n =6 R= 0.9998
		Pirimicarb (m/z 239 to 72) Confirmation		
	0.01* 0.10 Overall	93 - 119 (110) 86 - 100 (95) 90 – 101 (102)	9.3 (5) 5.6 (5) 11 (10)	0.200-50.0 ng/mL n =6 R= 0.9958
Eggs		Pirimicarb (m/z 239 to182) Quantification		
	0.01* 0.10 Overall	91 - 97 (94) 93 - 95 (94) 91 - 97 (94)	3.0 (5) 1.2 (5) 2.1 (10)	0.200-50.0 ng/mL n =6 R= 0.9994
		Pirimicarb (m/z 239 to 72) Confirmation		
	0.01* 0.10 Overall	95 – 100 (98) 94 – 96 (95) 94 – 100 (96)	1.8 (5) 0.8 (5) 1.9 (10)	0.200-50.0 ng/mL n =6 R= 0.995
Liver		Pirimicarb (m/z 239 to182) Quantification		
	0.01* 0.10 Overall	76 - 88 (81) 74 - 79 (77) 74 - 88 (79)	5.7 (5) 2.5 (5) 4.8 (10)	0.200-50.0 ng/mL n =6 R= 0.9994
		Pirimicarb (m/z 239 to 72) Confirmation		
	0.01* 0.10 Overall	80 – 91 (84) 76 – 80 (79) 76 – 91 (81)	5.4 (5) 1.9 (5) 5.2 (10)	0.200-50.0 ng/mL n =6 R= 0.9947
Meat		Pirimicarb (m/z 239 to182) Quantification		
	0.01* 0.10 Overall	96 - 100 (97) 94 - 100 (98) 94 - 100 (96)	1.8 (5) 2.6 (5) 2.0 (10)	0.200-50.0 ng/mL n =6 R= 0.9997
		Pirimicarb (m/z 239 to 72) Confirmation		
	0.01* 0.10 Overall	98 - 101 (100) 90 - 94 (92) 90 – 101 (96)	1.1 (5) 1.6 (5) 4.2 (10)	0.200-50.0 ng/mL n =6 R= 0.9966
Kidney		Pirimicarb (m/z 239 to182) Quantification		
	0.01* 0.10 Overall	74 - 99 (86) 81 - 86 (82) 74 - 99 (84)	11 (5) 3.3 (5) 7.7 (10)	0.200-50.0 ng/mL n =6 R= 0.9996
		Pirimicarb (m/z 239 to 72) Confirmation		
	0.01* 0.10 Overall	76 - 102 (89) 82 - 90 (86) 76 – 102 (87)	11 (5) 3.4 (5) 7.9 (10)	0.200-50.0 ng/mL n =6 R= 0.9959

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Fat		Pirimicarb (m/z 239 to182) Quantification		
	0.01* 0.10 Overall	96 - 106 (101) 95 - 107 (101) 95 - 107 (101)	4.7 (5) 4.5 (5) 4.3 (10)	0.200-50.0 ng/mL n =6 R= 0.9998
		Pirimicarb (m/z 239 to 72) Confirmation		
	0.01* 0.10 Overall	98 - 110 (104) 96 - 108 (103) 96 – 110 (104)	5.3 (5) 4.4 (5) 4.6 (10)	0.200-50.0 ng/mL n =6 R= 0.9966

*Limit of quantification

Specificity: For each animal matrix, representative chromatograms were provided for the lowest to highest concentration calibration standards, the matrix blank and the fortified samples (LOQ and 10xLOQ). No significant peak interferences were observed at the retention time of pirimicarb. Chromatograms were provided for the 2 mass transitions which were monitored for quantification and confirmation purposes.
Linearity: Linearity was demonstrated using 6 calibration solutions in the range of 0.200-50.0 ng/mL. The correlation coefficients were > 0.99 in all cases. Representative calibration lines are presented in the report for both primary and confirmatory ion transitions. The linear range covers 30% of the LOQ to above 20% of the highest level for each matrix. Matrix matches standards were not used however it was demonstrated that the matrix effects were not significant (<20%).
Recovery and Repeatability: The accuracy of the method was assessed by fortifying 5 control samples of each matrix with 0.01 mg/kg (LOQ) and 0.1 mg/kg (10xLOQ) pirimicarb. All mean recoveries at each fortification level were within the acceptable range of 70 to 120%. Two control blanks were analysed for each matrix. All RSDs at each fortification level were within the acceptable range of <20%.
LOQ: The LOQ of the method is 0.01 mg/kg. This complies with the guideline LOQ set out in SANCO/825/00 revision 8.1 for food of animal origin.
Confirmation of Identity: The mass transition 239->182 was used for quantification and the transition 239->72 was used for confirmation.
Storage Stability: Extracts were stored for no longer than 9 days after extraction before their last injection for analysis. All recoveries are in the acceptable range of 70 – 120% and, therefore, demonstrate that gamma cyhalothrin is stable for at least 9 days in the final extracts after the first injection.
Extraction Efficiency: Residues of pirimicarb parent compound are not expected ≥LOQ in food of animal origin and are, therefore, in accordance with SANCO/825/00 rev. 8.1. There is no need to address the efficiency of the extraction procedures.

Conclusion: Method DFG S 19 is validated in accordance with SANCO/825/00 revision 8.1 with an LOQ of 0.01 mg/kg in animal products.

ILV of Method DFG S 19: Determination of pirimicarb in animal matrices

Report:	**Reichart, N. (2005), Independent Laboratory Validation of the DFG Method S19 for the Determination of Residues of Pirimicarb in Matrices of Animal Origin, Report Number: IF-05/00362966**

Previous evaluation	None. Submitted for the purposes of renewal

Independent laboratory validation was provided for the determination of pirimicarb in two animal matrices: milk and eggs. The samples were fortified with various levels of pirimicarb. The samples were then subjected to the full analytical procedure and analysed by high performance liquid chromatography with mass spectrometric detection (Method DFG S 19; LC/MS/MS).

Method Summary

Milk and eggs (Module E8)

As described by Lakaschus, S., (2005, Report Number: SYN-0501V).

Instrument Parameters

The LCMS/MS conditions were adapted for the ILV:

LC-MS/MS Unit: Applied Biosystems, API 3000
Column: Phenomenex Synergi Fusion-RP 80A (150mm x 3.0mm 4.0 µm)
Solvent System: Eluent 1: Methanol + 0.05% acetic acid
Eluent 2: De-ionised water + 0.05% acetic acid
Flow: 400 mL/min
Gradient:

Time [min]	**Eluent C [%]**	**Eluent D [%]**
0.00	20	80
0.50	20	80
4.00	90	10
12.00	90	10
12.10	20	80
15.00	20	80

Oven Temperature: 30°C
Injection Volume: 20
Thermostat temperature: 10
Split (detector/waste) 1:1
Switching intervals: 0 to 6 mins (to waste)
6 to 10 mins (to LC-MS)
10 to 16 mins (to waste)

Mass Spectrometer Conditions

Retention Window (min)	10	10
Ion Spray Voltage	5500	5500
Temperature	400	400
Scan Type	MRM	MRM
Q1 Mass	239	239
Q3 Mass	182	72
Declustering Potential	26	26
Focusing Potential	140	140
Collision Energy	23	39
Collision cell exit Pot.	12	4
Entrance Potential	10	10
Ion energy 1 [V] (IE1)	1	1
Ion energy 3 [V] (IE3)	0.4	0.4
Dwell [msec]	500	500
Deflector	-220	-220
Channel electron multiplier [V]	2200	2200

Summary of Validation Data

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Milk		Pirimicarb (m/z 239 to182) Quantification		
	0.01* 0.10 Overall	71 - 104 (92) 88- 105 (98) 71 - 105 (95)	14 (5) 6 (5) 10 (10)	0.1998 – 19.98 ng/mL n = 8 R= 0.99987
		Pirimicarb (m/z 239 to 72) Confirmation		
	0.01* 0.10 Overall	70 - 103 (92) 87 - 103 (97) 70 - 103 (95)	14 (5) 6 (5) 10 (10)	0.1998 – 19.98 ng/mL n = 8 R= 0.99987
Eggs		Pirimicarb (m/z 239 to182) Quantification		
	0.01* 0.10 Overall	61 - 81 (70) 57 - 82 (73) 57 - 82 (72)	13 (5) 14 (5) 13 (10)	0.1998 – 19.98 ng/mL n = 8 R= 0.99987
		Pirimicarb (m/z 239 to 72) Confirmation		
	0.01* 0.10 Overall	61 – 80 (70) 56 - 82 (72) 56 - 82 (71)	13 (5) 15 (5) 13 (10)	0.1998 – 19.98 ng/mL n = 8 R= 0.99987

* Limit of quantification

Specificity: For each matrix, representative chromatograms were demonstrated for the matrix blanks, the lowest and highest concentration calibration solutions and the fortified samples (LOQ and 10xLOQ). No significant peak interferences were observed at the retention time of pirimicarb. Chromatograms were provided for the quantification mass transition.
Linearity: Linearity was demonstrated using 8 calibration solutions in the range of 0.1998-19.98 ng/mL. The correlation coefficients were > 0.99 in all cases. A representative calibration line is presented in the report for the 20 µL injections. The linear range covers 30% of the LOQ to above 20% of the highest level for each matrix. Matrix matches standards were not used however it was demonstrated that the matrix effects were not significant (<20%).
Recovery and Repeatability: The accuracy of the method was assessed by fortifying 5 control samples of each matrix with 0.01 mg/kg (LOQ) and 0.1 mg/kg (10xLOQ) pirimicarb. All mean recoveries at each fortification level were within the acceptable range of 70 to 120%. Two control blanks were analysed for each matrix. All RSDs at each fortification level were within the acceptable range of <20%.
LOQ: The LOQ of the method is 0.01 mg/kg. This complies with the guideline LOQ set out in SANCO/825/00 revision 8.1 for food of animal origin.
Confirmation of Identity: The mass transition 239->182 was used for quantification and the transition 239->72 was used for confirmation.
Storage Stability: Extracts were stored for no longer than 3 days after extraction before their last injection for analysis. The mean recoveries at each fortification level were in the range of acceptability, although it is noted that for egg some of the individual results lie outside this range.
Extraction Efficiency: Residues of pirimicarb parent compound are not expected ≥LOQ in food of animal origin and, therefore, in accordance with SANCO/825/00 rev. 8.1. There is no need to address the efficiency of the extraction procedures.
Conclusion: Method DFG S 19 is validated in accordance with SANCO/825/00 revision 8.1 with an LOQ of 0.01 mg/kg in animal products. However, it is noted that the ILV has been performed for only two animal matrices (milk and egg). In accordance with SANCO/825/00 rev 8.1., it may be sufficient to perform an ILV with two of the animal matrices, provided that the primary method is identical for all matrices (milk, eggs, meat, fat and liver/kidney). While the primary method for milk, eggs, meat and liver/kidney is identical, the method for fat is different. On this basis, an ILV for fat matrices would be required to support DFG S 19.

B.5.2.2. Methods for the determination of all components included for monitoring purposes in the residue definitions for soil and water

a) Soil

Residue Definition

The EFSA conclusion on the peer review of pirimicarb (2005) indicates that the residue definition for monitoring in soil is parent pirimicarb, and potentially R34836 (desmethyl pirimicarb), R34885 (desmethyl formamido pirimicarb) and R35140 (pending on assessment on effects on soil non-target microorganisms).

The Applicant for the renewal proposes a revised residue definition for monitoring in soil of pirimicarb (parent only). This is considered acceptable based on the assessments conducted by fate and behaviour and ecotoxicology specialists for the renewal of pirimicarb.

Monitoring methods

The following methods were evaluated during the first Annex 1 review of pirimicarb and summaries of the method and validation data are provided in the DAR (Vol.3, Annex B, Section B5, October 2003).

Matrices	**Analyte**	**Method**	**Reference**
Soil (Sandy Loam)	Pirimicarb R34836	HPLC-fluorescence GC-NPD HPLC-MS-MS	Method: RAM 274/02 Study: RJ1983B Kwiatkowsi A. & Robinson, N.J., 1996
ILV - Soil (Sandy Loam and Clay Loam)	Pirimicarb R34836	GC-NPD	Method: RAM 274/02 Study: CEMR-0660 Bolton, A. 1997

It was concluded in the DAR that method RAM 274/02 is validated for pirimicarb and desmethyl pirimicarb with an LOQ of 0.01 mg/kg in soil. It is noted that this method is not fully validated in accordance with SANCO/825/00 rev. 8.1, though this guidance document was not in force at the time of original approval (2003). No new monitoring methods for pirimicarb in soil were submitted in support of the renewal of pirimicarb.

According to guidance document SANCO/825/00 rev. 8.1, the limit of quantification for monitoring methods in soil should be 0.05 mg/kg. However, if the LC_{50} for the most sensitive non-target organism is <0.05 mg/kg (=75 g/ha) then the LOQ must comply with this value. None of the soil organism endpoints are <0.05 mg/kg and, therefore, the LOQ of 0.01 mg/kg is considered sufficiently low.

Method RAM 274/02: Determination of pirimicarb in soil

Report:	**Kwiatkowski A.S., Robinson N.J. (1996), Pirimicarb and Desmethyl Pirimicarb: Analytical Method and Validation of a Method for the Determination of Residues in Soil, Report Number: RJ1983B**

Previous evaluation	In DAR (2003) for original approval

Validation data were provided for analytical method RAM 274/02, used for monitoring pirimicarb and desmethyl pirimicarb in soil. Two different soil matrices were used to generate the validation data: Speyer 2.2 and Hyde Farm (sandy loam). The samples were analysed by HPLC-Fluorescence/GC-NPD/HPLC-MS-MS.

Method

Soil samples (20 g) were weighed out in extraction vessels and extracted in 1:1 v/v acetone: 1M aqueous ammonium chloride (2 x 100 mL). An aliquot of the extract (2 g; 20 mL) was transferred to a separating funnel (washing with 10 mL water), acidified with 1 M hydrochloric acid (1 mL) and cleaned up by liquid-liquid partition with diethyl ether (10 mL). The organic layer was discarded and the aqueous extract was partitioned into dichloromethane (2 x 10 mL) under basic conditions (2 cm^3 NaOH solution). The dichloromethane was evaporated to dryness and redissolved in a known volume of suitable solvent prior to analysis by HPLC-fluorescence (2 mL), HPLC-MS-MS (2 mL) or GC-NPD (1 mL).

Soil samples

Speyer 2.2 soil was sampled from East Jubilee Field at Jealott's Hill Research Station, Bracknell, Berkshire, RG42 6ET, UK. Hyde Farm soil (Sandy Loam) was sampled from Hyde Farm, Pinkey's Green, Maidenhead, Berkshire, UK. The samples were not described in terms of pH and organic matter/carbon content, as per SANCO/825/00 rev. 8.1.

Instrument Parameters (HPLC-Fluorescence)

Instrument:	Hewlett Packard 1050 series gradient pump/autosampler
Detector:	Perkin-Elmer LC-240
Column:	KR100-5C18 (25 cm x 3.2 mm internal diameter)
Guard Column:	KR100-5C18-10C5
Mobile phase:	Solvent A - 0.05 M phosphate buffer pH = 7 Solvent B - Acetonitrile

Gradient:

Time (mins)	**0**	**2**	**20**
Solvent A (%)	75	75	50
Solvent B (%)	25	25	50

Flow rate:	0.8 mL/min or 1.0 mL/min	
Injection volume:	25 µL	
Detection:	Excitation wavelength:	240 nm
	Emission wavelength:	382 nm
Retention Time:	Pirimicarb:	~5 minutes
	Desmethyl pirimicarb:	~12 minutes

Instrument Parameters (GC-NPD)

Instrument:	Varian 3400 series capillary gas chromatograph fitted with a Varian 8100 series, a nitrogen/phosphorous detector, and a septum equipped programmable injector (SPI).
Column:	BPX 5 25 m x 0.32 mm i.d. (0.5 µm film thickness)
GC Temperature:	70 °C, hold for 1 minute and rise at 20°C per programme minute to 250°C then immediately rise at 50°C per minute to 300°C and hold for 2 minutes.
Injector Mode:	SPI with buffer large volume injection liner packed with a small amount of silanised glass wool to trap non-volatile components
Injection Port:	40 °C, hold for 0.1 minute and rise at 150°C per minute to 250°C and hold for 12 minutes.

Injection Volume:	2 µL	
Detector temperature:	300 °C	
Carrier Gas:	Helium at 4 mL/min	
Hydrogen flow:	4 mL/min	
Air Flow:	170 mL/min	
Make up Flow:	26 mL/min	
Bead Current:	3.25A	
Solvent plug size:	1 µL	
Injection speed:	2 µL	
Retention Time:	Pirimicarb:	~8 minutes
	Desmethyl pirimicarb:	~9 minutes

Instrument Parameters (HPLC-MS-MS)

Instrument:	Perkin Elmer Binary LC 250 pump fitted with a Perkin Elmer Advanced LC Sample Processor ISS200 and a PE-SCIEX API 111 triple quadrupole mass spectrometer in the positive ion mode	
Column:	Kromasil 100-5C18 (5 cm x 4.6 mm internal diameter)	
Mobile Phase:	30:70% acetonitrile:water + 20 mM ammonium acetate	
Flow Rate:	1 mL/min	
Injection Volume:	50 µL (The volume may be varied dependent on the instrument response at the time of analysis)	
Ionisation Mode:	Heated nebulizer (APCI) positive ion	
Temperature of Heated Nebulizer:	480 °C	
Auxiliary Gas:	UHP Nitrogen (1.8 L/min)	
Nebulizer Gas:	UHP Nitrogen (60 psi)	
Collision gas:	Ar/N_2 (10%)	
Retention Time:	Pirimicarb:	~2.5 minutes
	Desmethyl pirimicarb:	~1 minutes

Protonated molecular ions generated in the ion source (pirimicarb, m/z 255 and Desmethyl pirimicarb, m/z 239) are selected and subjected to further fragmentation by collisional activation. The largest ion (pirimicarb, m/z 198 and Desmethyl pirimicarb, m/z 182) in the resulting daughter spectra are then monitored and used for quantitative analysis.

Summary of Validation Data (HPLC-Fluorescence)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Pirimicarb				
Speyer 2.2	0.01*	94 – 103 (98.5)	4.7 (4)	0.01 – 0.5 µg/mL n = 5 R= 0.99981
	0.02	101 – 102 (101.5)	0.7 (2)	
	0.05	94 – 99 (96.5)	3.7 (2)	
	0.10	95 – 97 (96)	1.5 (2)	
	0.50	89 – 97 (93)	6.1 (2)	
	Overall	89 – 103 (97)	3.3 (12)	
Hyde Farm	0.01*	86 – 94 (89)	4.1 (4)	
	0.02	92 – 93 (92.5)	0.8 (2)	
	0.05	90 – 91 (90.5)	0.8 (2)	
	0.10	82 – 91 (86.5)	7.4 (2)	
	0.50	76 – 87 (81.5)	9.5 (2)	
	Overall	76 – 94 (88)	4.5 (12)	

* Limit of quantification

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Desmethyl pirimicarb				
Speyer 2.2	0.01*	80 – 89 (84.5)	4.4 (4)	0.01 – 0.5 µg/mL n = 5 R= 0.9998
	0.02	89 – 92 (90.5)	2.3 (2)	
	0.05	91 – 93 (92)	1.5 (2)	
	0.10	90 – 93 (91.5)	2.3 (2)	
	0.50	85 – 94 (89.5)	7.1 (2)	
	Overall	80 – 94 (90)	3.5 (12)	
Hyde Farm	0.01*	71 – 79 (75)	5.4 (4)	
	0.02	75 – 75 (75)	0.0 (2)	
	0.05	73 – 74 (73.5)	1.0 (2)	
	0.10	73 – 76 (74.5)	2.9 (2)	
	0.50	64 – 74 (69.0)	10.3 (2)	
	Overall	64 – 79 (73.4)	3.9 (12)	

* Limit of quantification

Summary of Validation Data (GC-NPD)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Pirimicarb				
Speyer 2.2	0.01*	77 – 83 (80.5)	3.3 (4)	0.01 – 10 µg/mL n = 7 R= 0.99985
	0.02	83 – 100 (91.5)	13.1 (2)	
	0.05	84 – 91 (87.5)	5.7 (2)	
	0.10	85 – 88 (86.5)	2.5 (2)	
	0.50	81 – 85 (83)	3.4 (2)	
	Overall	77 – 100 (85.8)	5.6 (12)	
Hyde Farm	0.01*	77 – 94 (89)	8.9 (4)	
	0.02	88 – 112 (100)	17.0 (2)	
	0.05	89 – 91 (90)	1.6 (2)	
	0.10	86 – 94 (90)	6.3 (2)	
	0.50	99 – 99 (99)	0.0 (2)	
	Overall	86 – 112 (94)	6.7 (12)	

* Limit of quantification

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Desmethyl pirimicarb				
Speyer 2.2	0.01*	73 – 85 (78)	4.4 (4)	0.01 – 10 µg/mL n = 8 R= 0.999789
	0.02	83 – 89 (86)	4.9 (2)	
	0.05	85 – 87 (86)	1.6 (2)	
	0.10	84 – 88 (86)	3.3 (2)	
	0.50	82 – 89 (86)	5.8 (2)	
	Overall	73 – 89 (84)	4.0 (12)	
Hyde Farm	0.01*	70 – 122 (91)	24.1 (4)	
	0.02	87 – 126 (106.5)	25.9 (2)	
	0.05	89 – 102 (95.5)	9.6 (2)	
	0.10	92 – 98 (95)	4.5 (2)	
	0.50	105 – 114 (109.5)	5.8 (2)	
	Overall	98 – 122 (100)	14 (12)	

* Limit of quantification

Summary of Validation Data (HPLC-MS-MS)

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Pirimicarb				
Speyer 2.2	0.01*	80 – 86 (84)	3.4 (4)	0.01 – 0.5 µg/mL n = 5 R= 0.999974
	0.02	87 – 88 (87.5)	0.8 (2)	
	0.05	93 – 93 (93)	0.0 (2)	
	0.10	90 – 92 (91)	1.6 (2)	
	0.50	92 – 98 (95)	4.5 (2)	
	Overall	80 – 98 (90.1)	2.1 (12)	
Hyde Farm	0.01*	98 – 118 (105)	8.3 (4)	
	0.02	106 – 111 (108.5)	3.3 (2)	
	0.05	111 – 111 (111.0)	0.0 (2)	
	0.10	104 – 113 (108.5)	5.9 (2)	
	0.50	101 – 110 (105.5)	6.0 (2)	
	Overall	98 – 118 (108)	4.7 (12)	

* Limit of quantification

Sample matrix	Fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Desmethyl pirimicarb				
Speyer 2.2	0.01*	83 – 87 (85)	2.0 (4)	0.01 – 0.5 µg/mL n = 5 R= 0.999945
	0.02	83 – 87 (85)	3.3 (2)	
	0.05	88 – 88 (88)	0.0 (2)	
	0.10	85 – 87 (86)	1.6 (2)	
	0.50	85 – 93 (87)	6.4 (2)	
	Overall	83 – 93 (87)	2.7 (12)	
Hyde Farm	0.01*	95 – 99 (96)	2.1 (4)	
	0.02	105 – 106 (105.5)	0.7 (2)	
	0.05	103 – 103 (103)	0.0 (2)	
	0.10	98 – 101 (99.5)	2.1 (2)	
	0.50	97 – 99 (98.0)	1.4 (2)	
	Overall	98 – 106 (100.4)	1.3 (12)	

*Limit of quantification

Specificity: For each analytical technique, representative chromatograms were provided for the analytical standards (highest calibration level), matrix blanks (unknown soil type) and the fortified samples (0.05 mg/kg level). No significant interference was observed between the matrix blank and the analytes of interest, however it is noted that a chromatograms were not provided for the lowest calibration standard or the lowest fortification level for comparison.
Calibration: Linear calibration functions were established ranging from 0.01–0.5 µg/mL for pirimicarb/desmethyl pirimicarb (5 levels) by injecting standard solutions. The calibration response for both analytes was shown to be linear with a correlation coefficient (r) of >0.99. Representative calibration lines are presented in the report for each type of analytical method. Matrix matched standards were not used and the matrix effects were not addressed.
Recovery and Repeatability: The accuracy of the method was assessed by fortifying control samples of each type of soil with 0.01 (LOQ) to 0.5 mg/kg (50xLOQ) pirimicarb/desmethyl pirimicarb. Four samples were fortified at the LOQ, and two samples at each of the subsequent levels.
For pirimicarb, all mean recoveries at each fortification level were within the acceptable range of 70 to 120%. All RSDs at each fortification level were within the acceptable range of <20%. No outliers were identified.
For desmethyl pirimicarb, not all the mean recoveries were within the acceptable range of 70 to 120% and not all RSDs at each fortification level were <20%. However, as desmethyl pirimicarb does not form part of the residue definition for monitoring, these deficiencies are not considered to be of concern.
LOQ: The limit of quantification of the method is 0.01 mg/kg. According to SANCO/825/00, rev. 8.1, the limit of quantification of a method must meet 0.05 mg/kg for residues in soil.
Confirmation: Confirmation was achieved by independent analytical techniques (GC and HPLC) and alternative detectors (HPLC-fluorescence and HPLC-MS-MS).
Storage Stability: Standard solutions were stored at ≤9°C and replaced with fresh standards after a period of 4 months. The storage and handling of extracts was not reported.

Conclusion: Method RAM 274/02 is not strictly validated in accordance with SANCO/825/00 rev. 8.1. In terms of the specificity of the method, clearly labelled chromatograms of standards at the lowest calibration level, matrix blanks (each matrix) and samples fortified at the lowest fortification level for each analyte/matrix combination were not provided. Where mass spectrometry has been used, mass/product ion spectra have not been provided.

For the calibration, matrix matched standards were not used and the matrix effects have not been addressed. It is not clear that the linear range covers from 30% of the LOQ to 20% above the highest level.

In terms of the precision and repeatability, the LOQ of the method was determined with only 4 recovery samples rather than the 5 stipulated in the guidelines. At each of the higher levels only 2 recoveries were reported which is not considered sufficient to derive a %RSD. The storage stability of extracts was not addressed.

It is noted that independent laboratory validation was also submitted in support of this method (Bolton, A. 1997). Additional validation data was provided for the GC-NPD analytical technique. Independent laboratory validation is not a requirement for monitoring methods in soil. The ILV data is not validated in accordance with SANCO/825/00 rev. 8.1 and is not considered to add additional information to the method. On this basis, the ILV data has not been presented.

Due to the deficiencies with Method RAM 274/02, it considered that a data requirement should be set for the determination of residues of pirimicarb in soil with an LOQ of 0.01 mg/kg validated in line with SANCO/825/00 rev.8.1.

b) Water

Residues Definitions

Ground Water: The EFSA conclusion on the peer review of pirimicarb (2005) indicates that the residue definition for enforcement in ground water is pirimicarb and R35140 (pending on assessment on potential ground water contamination).

The Applicant for the renewal proposes a revised residue definition for monitoring in ground water of pirimicarb. This is considered acceptable based on the assessments conducted by fate and behaviour and ecotoxicology for the renewal of pirimicarb.

Surface Water: The EFSA conclusion on the peer review of pirimicarb (2005) indicates that the residue definition for enforcement in surface water is pirimicarb.

The Applicant for the renewal proposes a revised residue definition for monitoring in surface water of pirimicarb, R34836 (desmethyl pirimicarb), R34885 (desmethylformamido pirimicarb), and R35140 based on the results of toxicological and ecotoxicological testing.

Based on the available data, a residue definition of pirimicarb, R34885 and R34836 has been proposed by ecotoxicology specialists. A gap has been set for further data to address the ecotoxicological relevance of metabolite R35140.

Monitoring methods

The following methods were evaluated during the first Annex 1 review of pirimicarb and summaries of the method and validation data are provided in the DAR (Vol.3, Annex B, Section B5, October 2003).

Matrices	**Analyte**	**Method**	**Reference**
River water Sea water Ground water Drinking Water	Pirimicarb R34836 R34885 R238177	GC-MSD	Method: RAM 360/01 Study: TMJ4568B Robinson, N.J., 2001
Tap Water	Pirimicarb R34836	HPLC-fluorescence	Method: RAM 277/01 Study: RJ2027B Harradine, K.J., 1996

It was concluded in the DAR that GC-MSD method RAM 360/01 is validated for pirimicarb, R34836, R34885 and R238177 with an LOQ of 0.1 µg/L in water matrices. The Applicant for the renewal of pirimicarb is relying on this monitoring method and, therefore, it has been re-evaluated for the purposes of renewal.

It is noted that method RAM 277/01 was validated for pirimicarb and R34836 with an LOQ of 0.1 µg/L for the original ("Annex 1") review of pirimicarb. Since this method is not considered to add any additional information, it has not been re-evaluated for the renewal.

The Applicant for renewal has developed a new monitoring method for metabolite R35140 in water, as a result of the proposed change to the residue definition for surface water. This method has been evaluated against SANCO/825/00 revision 8.1 for the purpose of renewal.

Matrices	**Analyte**	**Method**	**Reference**
Surface Water Ground Water Drinking	R35140	LC/MS/MS	Method: GRM039.02A Report: GRM039-02A Langridge, G. (2011)

Method RAM 360/01: Determination of pirimicarb, R34836, R3488, R238177 in water (surface, ground and drinking water)

Report:	**Robinson, N.J. (2001), Pirimicarb and its Carbamate Metabolites: Validation of a Residue Analytical Method for the Determination of Residues in Water, Report Number: TMJ4568B**

Previous evaluation	In DAR (2003) for original approval

Analytical method RAM 360/01 has been developed and validated for the determination of pirimicarb and its carbamate metabolites R34836, R34885 and R238177 in water using GC-MSD.

Method Summary

(i) Hydrolysis (Conversion of metabolite R34885 to R34836)

Transfer 50 mL aliquots of the water sample to be analysed into round bottomed flasks (100 mL). Acidify samples with concentrated HCl (0.5 mL). Stopper the flasks and allow the sample to stand at room temperature overnight period (18-24 hours). After this time, the samples are neutralised by the addition of sodium hydrogen carbonate (approximately 0.25 g) until a pH of 7-8 is reached.

(ii) Solid Phase Extraction Clean-Up Procedure

One Waters Oasis HLB SPE cartridge (60mg/3mL) was wet with methanol (allow to percolate; do not allow to dry) for each sample to be analysed followed by water (allow to percolate; do not allow to dry). The water samples from step (i) were loaded onto the SPE cartridges (allow to percolate; do not allow to dry). Pirimicarb, R34836 and R238177 were retained on the SPE cartridge.

The empty sample tubes were washed with water (2x1 mL) and the risings were added to the cartridge.

Suitable collection vials (were placed under each port, as required. The cartridges were eluted with dichloromethane (1 mL), under gravity or drawn through under low vacuum at a rate of approximately 2 mL/min to the level of the top frit collecting the column eluate. A high vacuum was applied for approximately 5 seconds to collect the excess solvent from the SPE cartridges.

The samples were evaporated to dryness in a heating block at 30 °C under a stream of dry air and re-dissolve the sample in acetonitrile (0.5 mL) with ultrasonification.

(iii) Derivatisation of R238177

Metabolite R238177 is derivatized with bis(trimethylsilyl)trifluoroacetamide (BSTFA) + I% trimethylchlorosilane (TMCS) to form the corresponding trimethylsilyl derivative since the derivative has better properties for chromatography. Neither pirimicarb nor R34836 react under these conditions.

For quantification purposes, the mixed analytical standards of pirimicarb, R034836 and R238177 were treated with bis(trimethylsilyl)trifluoroacetamide (BSTFA) + I% trimethylchlorosilane (TMCS).

Samples

The method was tested in 4 different water matrices as shown in the following table:

Water Type	Source	pH	Silt Content (% w/w)	Dissolved Organic Carbon (DOC) (mg/L)	Total Hardness $CaCO_2$ (mg/L)
River Water	River Thames, Maidenhead, Berkshire, UK	8.02	0.004	16.1	342
Sea Water	Beach N^{o}. 24, Hayling Island, Hampshire, UK	8.01	N/A	4.5	5987
Ground Water	Sheeplands Farm, Twyford Road, Wargrave, Berkshire, UK	7.95	< 0.10	6.5	591.8
Drinking Water	Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK	7.25	N/A	< 1.0	332

Instrument Parameters

A Hewlett Packard HP 6890 gas chromatograph fitted with HP5973 series mass selective detector has been found to be suitable for this analysis.

Primary Method

Column:	BPX**5** 30 m x 0.25 mm id, 0.25 µm df
Injection port:	Split/splitless injector operating in splitless mode with a carbofit gooseneck splitless liner
Carrier gas and head pressure:	Helium at 1.0 ml/min constant flow with a pressure pulse injection of 30 psi for 1 minute
Splitless time:	1.0 minute
Injection volume:	3 µL
Injector temperature:	250 °C
Transfer line temperature:	280 °C
Temperature programme:	100 °C hold for 1 minute then programme at 15 °C to 300 °C and hold for 1 minute

MSD Conditions

Ionisation mode:	EI
System calibration:	Autotune

Acquisition Parameters

Compound Name	**Low Mass Resolution**	**SIM MODE**	
Pirimicarb	Yes	Target Ion Qualifier Retention Time	166 m/z 238 m/z 10.2 min
R34836	Yes	Target Ion Qualifier Retention Time	152 m/z 224 m/z 10.4 min
R238177	Yes	Target Ion Qualifier Retention Time	254 m/z 326 m/z 11.7 min

Confirmatory method

A different stationary phase was used for the confirmatory method (BPX35 column vs BPX5). A BPX5 column consists of 5% phenyl polysilphenylene siloxane while the BPX35 column consists of 35% phenyl polysilphenylene siloxane.

Column:	BPX**35** 30 m x 0.25 mm id, 0.25 µm df	
Injection port:	Split/splitless injector operating in splitless mode with a carbofit gooseneck splitless liner	
Carrier gas and head pressure:	Helium at 1.0 ml/min constant flow with a pressure pulse injection of 30 psi for 1 minute	
Splitless time:	1.0 minute	
Injection volume:	3 µL	
Injector temperature:	250 °C	
Transfer line temperature:	280 °C	
Temperature programme:	100 °C hold for 1 minute then programme at 15 °C to 300 °C and hold for 1 minute	
Retention Times:	Pirimicarb:	8.6 min
	R034836:	9.0 min
	R238177:	9.8 min

Summary of Validation Data (Pirimicarb)

Sample matrix	Fortification level (µg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
River Water		Pirimicarb Quantification		
	0.1* 1.0 Overall	83 – 88 (86) 86 – 90 (88) 83 – 90 (87)	2.4 (5) 1.7 (5) 2.3 (10)	0.005 - 1 µg/mL n =6 R= 0.9996
		Pirimicarb Confirmation		
	0.1* 1.0 Overall	88 – 92 (90) 84 – 91 (88) 84 – 92 (89)	1.7 (5) 3.5 (5) 2.8 (10)	0.005 - 1 µg/mL n =6 R= 0.9996
Sea Water		Pirimicarb Quantification		
	0.1* 1.0 Overall	82 – 86 (84) 83 – 91 (89) 82 – 91 (86)	2.1 (5) 3.6 (5) 3.9 (10)	0.005 - 1 µg/mL n =6 R= 0.9996
		Pirimicarb Confirmation		
	0.1* 1.0 Overall	85 – 88 (87) 83 – 86 (85) 83 – 88 (86)	1.3 (5) 1.6 (5) 1.9 (10)	0.005 - 1 µg/mL n =6 R= 0.9996
Ground Water		Pirimicarb Quantification		
	0.1* 1.0 Overall	79 – 91 (85) 87 – 95 (93) 79 – 95 (89)	5.8 (5) 4.4 (5) 6.7 (10)	0.005 - 1 µg/mL n =6 R= 0.9996
		Pirimicarb Confirmation		
	0.1* 1.0 Overall	81 – 87 (84) 88 – 93 (91) 81 – 93 (87)	2.6 (5) 2.5 (5) 4.9 (10)	0.005 - 1 µg/mL n =6 R= 0.9996
Drinking Water		Pirimicarb Quantification		
	0.1* 1.0 Overall	83 – 93 (88) 88 – 99 (93) 83 – 99 (90)	4.3(5) 5.9 (5) 5.5 (10)	0.005 - 1 µg/mL n =6 R= 0.9996
		Pirimicarb Confirmation		
	0.1* 1.0 Overall	91 – 96 (93) 92 – 98 (95) 91 – 98 (94)	1.9 (5) 3.2 (5) 2.7 (10)	0.005 - 1 µg/mL n =6 R= 0.9996

*Limit of quantification

Summary of Validation Data (R34836)

Sample matrix	Fortification level (µg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
River Water		R34836 Quantification		
	0.1* 1.0 Overall	80 – 95 (85) 84 – 89 (86) 80 – 95 (86)	6.7 (5) 2.8 (5) 4.9 (10)	0.005 - 1 µg/mL n =6 R= 0.9991
		R34836 Confirmation		
	0.1* 1.0 Overall	90 – 97 (92) 84 – 89 (87) 84 – 97 (90)	3.2 (5) 2.4 (5) 4.1 (10)	0.005 - 1 µg/mL n =6 R= 0.9991
Sea Water		R34836 Quantification		
	0.1* 1.0 Overall	78 – 83 (80) 80 – 90 (85) 78 – 90 (83)	2.6 (5) 4.4 (5) 4.5 (10)	0.005 - 1 µg/mL n =6 R= 0.9991
		R34836 Confirmation		
	0.1* 1.0 Overall	73 – 81 (77) 78 – 83 (80) 73 – 83 (79)	4.1 (5) 2.2 (5) 3.4 (10)	0.005 - 1 µg/mL n =6 R= 0.9991
Ground Water		R34836 Quantification		
	0.1* 1.0 Overall	84 – 88 (87) 89 – 95 (92) 84 – 95 (89)	2.3 (5) 2.4 (5) 4.0 (10)	0.005 - 1 µg/mL n =6 R= 0.9991
		R34836 Confirmation		
	0.1* 1.0 Overall	88 – 97 (93) 89 – 93 (90) 88 – 97 (92)	4.1 (5) 2.2 (5) 3.4 (10)	0.005 - 1 µg/mL n =6 R= 0.9991
Drinking Water		R34836 Quantification		
	0.1* 1.0 Overall	79 – 89 (84) 86 – 97 (90) 79 – 97 (87)	4.4 (5) 5.5 (5) 6.2 (10)	0.005 - 1 µg/mL n =6 R= 0.9991
		R34836 Confirmation		
	0.1* 1.0 Overall	79 – 89 (84) 86 – 97 (90) 79 – 97 (87)	4.4 (5) 5.5 (5) 6.2 (10)	0.005 - 1 µg/mL n =6 R= 0.9991

*Limit of quantification

Summary of Validation Data (R283177)

Sample matrix	Fortification level (µg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
River Water		R283177 Quantification		
	0.1* 1.0 Overall	81 – 84 (82) 88 – 89 (88) 81 – 89 (85)	1.4 (5) 0.5 (5) 4.1 (10)	0.005 - 1 µg/mL n =6 R= 0.9994
		R283177 Confirmation		
	0.1* 1.0 Overall	79 – 86 (81) 85 – 91 (88) 79 – 91 (85)	3.4 (5) 2.7 (5) 4.8 (10)	0.005 - 1 µg/mL n =6 R= 0.9994
Sea Water		R283177 Quantification		
	0.1* 1.0 Overall	84 – 88 (86) 88 – 94 (92) 84 – 94 (89)	1.7 (5) 2.5 (5) 4.1 (10)	0.005 - 1 µg/mL n =6 R= 0.9994
		R283177 Confirmation		
	0.1* 1.0 Overall	78 – 83 (80) 85 – 89 (87) 78 – 89 (83)	2.3 (5) 1.9 (5) 4.7 (10)	0.005 - 1 µg/mL n =6 R= 0.9994
Ground Water		R283177 Quantification		
	0.1* 1.0 Overall	85 – 93 (89) 92 – 97 (95) 85 – 97 (92)	3.4 (5) 2.3 (5) 4.5 (10)	0.005 - 1 µg/mL n =6 R= 0.9994
		R283177 Confirmation		
	0.1* 1.0 Overall	79 – 82 (81) 90 – 94 (92) 79 – 94 (86)	1.6 (5) 2.2 (5) 7.0 (10)	0.005 - 1 µg/mL n =6 R= 0.9994
Drinking Water		R283177 Quantification		
	0.1* 1.0 Overall	84 – 92 (86) 88 – 95 (91) 84 – 95 (89)	3.7 (5) 3.4 (5) 4.4 (10)	0.005 - 1 µg/mL n =6 R= 0.9994
		R283177 Confirmation		
	0.1* 1.0 Overall	78 – 82 (80) 87 – 94 (90) 78 – 94 (85)	2.2 (5) 3.5 (5) 6.6 (10)	0.005 - 1 µg/mL n =6 R= 0.9994

*Limit of quantification

Specificity: For the primary and confirmatory method in the ground water matrix, representative chromatograms were provided for the 0.1 µg/mL calibration standard, the matrix blanks, the fortified matrix (highest and lowest fortification levels) for each of the analytes. In each case a chromatogram was shown for the qualifier and target mass ions. Chromatograms were not provided for any of the other water matrices that were analysed. GC-MSD mass spectra were shown for each analyte.
Matrix Effects: An assessment of the GC-MSD matrix effects was made. No significant matrix effects (>20%) were observed for any of the analytes in any of the water matrices.

Water Type	Matrix Effect		
	Pirimicarb	**R34836**	**R238177**
River Water	6% enhancement	12% enhancement	2% enhancement
Sea Water	4% enhancement	8% enhancement	5% enhancement
Groundwater	6% enhancement	5% enhancement	5% enhancement
Drinking Water	2% enhancement	No enhancement	4% enhancement

Linearity: Linearity was demonstrated for each analyte using 6 calibration solutions in the range of 0.005 - 1 µg/mL. As significant matrix effects were not observed, matrix matched standards were not used. The concentration range covers from <30 % of the LOQ to >20 % above the highest concentration of solution injected for the determination of the recovery.
Recovery and Repeatability: The accuracy of the method was assessed by fortifying 5 control samples of each matrix with 0.1 µg/L (LOQ) and 1.0 µg/L (10xLOQ) of each analyte. All mean recoveries at each fortification level were within the acceptable range of 70 to 120%. Control blanks were analysed for each matrix. All RSDs at each fortification level and for each analyte were within the acceptable range of <20%.
LOQ: The LOQ of the method is 0.1 µg/L. According to guidance document SANCO/825/00 rev. 8.1, the limit of quantification for monitoring methods in surface water must comply with the lowest effect concentrations in aquatic species. The lowest effect endpoint for pirimicarb is 0.9 µg/l for the long term risk to Daphnia (NOEC endpoint). For drinking water or groundwater the limit of quantification must meet 0.1 µg/L. Therefore, the LOQ is considered to be sufficiently low.
Confirmation of Identity: A different stationary phase was used for the confirmatory method (BPX35 column vs BPX5). A BPX5 column consists of 5% phenyl polysilphenylene siloxane while the BPX35 column consists of 35% phenyl polysilphenylene siloxane. The stationary phases are considered sufficiently different to be considered independent analytical methods.
Storage Stability: Standard solutions were stored at ≤7°C and replaced with fresh standards after a period of 4 months. It is also stated that external standard recoveries were used to validate any work stoppages (overnight/weekend). The samples are stored in sealed vessels at ≤7°C.
Derivatisation: Metabolite R283177 was derivatized to form the corresponding trimethylsilyl derivative since the derivative has better properties for chromatography. The reference standards of metabolite R283177 were derivatized using the same procedure in order to produce the calibration plot. The efficiency and precision of the derivatisation step was not demonstrated with analyte in sample matrix against pure derivative. It is noted that R283177 does not form part of the residue definitions for monitoring in surface and groundwater.

Conclusion: Method RAM 360/01 is not strictly validated in accordance with SANCO/825/00 revision 8.1 since the specificity of the method has not been adequately addressed for all matrices. However the other validation data is considered to be acceptable. On this basis the method is considered fit for regulatory purposes for the determination of residues of pirimicarb, R34836 and R283177 in water matrices with an LOQ of 0.1 µg/L.

Method GRM039.02A: Determination of metabolite R35140 in water (surface, ground and drinking water)

Report:	**Langridge, G. (2011), Validation of Residue Method GRM039.02A for the determination of the Pirimicarb metabolite R35140 in Surface Water, Groundwater and Drinking Water Report Number: GRM039-02A**

Previous evaluation	None. Submitted for the purposes of renewal

Analytical method GRM039.02A has been developed for the determination of residues of R35140 (2-amino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate) in surface water, groundwater and drinking water at the LOQ of 0.05 µg/L. The samples were analysed by high performance liquid chromatography with mass spectrometric detection (LC/MS/MS).

Method Summary

A water sample (5 mL) was transferred to a suitable tube. One Waters Oasis HLB SPE cartridge (60mg/3mL) was wet with methanol (allow to percolate; do not allow to dry) for each sample to be analysed followed by water (allow to percolate; do not allow to dry). The water samples were loaded onto the SPE cartridges (allow to percolate; do not allow to dry). R35140 is retained on the SPE cartridge.
The empty sample tubes were washed with methanol/HPLC water (10/90, v/v) (1 mL) and the risings were added to the cartridge. The cartridges were dried under vacuum for 15 minutes.
Suitable collection vials (e.g. suitable graduated glass vials) were placed under each port, as required. The cartridges were eluted with methanol (1 mL), under gravity or drawn through under low vacuum at a rate of approximately 1 mL/min to the level of the top frit collecting the column eluate. A high vacuum was applied for approximately 5 seconds to collect the excess solvent from the SPE cartridges. R35140 was eluted in this step.
The final volume was adjusted to 2 mL with the addition of 1 mL HPLC water. An aliquot was transferred to a suitable autosampler vial ready for final determination by LC-MS/MS. The final sample concentration was 2.5 mL/mL (or 0.0025 L/mL).

Samples

The analytical method was validated with surface water, groundwater and drinking water.

Matrix	**Source**	**pH**	**Silt Content (mg/L)**	**Dissolved Organic Carbon (DOC) (mg/L)**	**Total Hardness as $CaCO_3$ (mg/L)**
Surface Water	River Loddon Charvil	7.8	<100	5.83	206.2
Ground Water	Well at Fawley Lodge, Henley on Thames	7.6	<100	0.64	292.3
Drinking Water	CEMAS	7.7	<100	2.48	261.2

Instrument Parameters

Chromatography Conditions

Pump:	Agilent 1200 series binary pump SL model number G1312B
Degasser:	Agilent 1200 series model number G1379B
Column Oven:	Agilent 1200 series model number G1316B
Detector:	Applied Biosystems API5500 triplequadrupole mass spectrometer with AnalystTM software version 1.5.2
Autosampler:	Agilent 1200 series model number G1367C
Gas Supply:	Peak Scientific N300DR gas station
Column:	Phenomenex Luna C18 (50 x 2.0 mm, 2.5µm)
Column Oven Temperature:	25°C
Injection volume:	10 µL
Stop Time:	5.0 minutes
Injection protocol:	Analyse calibration standard after 3 to 4 sample injections
Mobile phase:	1: 0.01% Formic Acid in HPLC water 2: Methanol

Mobile Phase Composition:

Time (min)	% Solvent 1	% Solvent 2	Flow, mL/min
0.0	90	10	0.5
3.5	50	50	0.5
3.8	5	95	0.5
4.0	90	10	0.5
5.0	90	10	0.5

Retention time: 1.1 minutes

Mass Spectrometer Conditions

Interface:	TurboIonSpray
Polarity:	Positive
Curtain gas (CUR):	Nitrogen set at 40 (arbitrary units)
Temperature (TEM):	550 °C
Ion spray voltage:	5000 V
Collision gas setting (CAD):	Nitrogen set at 7 (arbitrary units)
Gas 1 (GS1):	Air set at 30 (arbitrary units)
Gas 2 (GS2):	Air set at 35 (arbitrary units)
Interface heater (ihe):	On
Scan type:	MRM

MRM Conditions

	R35140 primary transition	**R35140 confirmatory transition I**
Q1 m/z:	211.1	211.1
Q3 m/z:	71.9	154.1
Dwell time:	100 ms	100 ms
Resolution Q1:	Unit	Unit
Resolution Q3 :	Unit	Unit
Declustering potential (DP):	83 V	83 V
Entrance potential (EP):	10 V	10 V
Collision energy (CE):	30 V	19 V
Collision cell exit potential (CXP):	8 V	18 V

Summary of Validation Data

Sample matrix	Fortification level (µg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Surface Water		R35140 (m/z 211.1 to 71.9) Quantification		
	0.05* 0.50 Overall	89 – 98 (93) 97 – 100 (98) 89 – 100 (96)	3.4 (5) 1.0 (5) 3.8 (10)	0.03 - 5 µg/L n =6 R= 0.9999
		R35140 (m/z 211.1 to 154.1) Confirmation		
	0.05* 0.50 Overall	84 – 95 (89) 94 – 96 (95) 84 – 96 (92)	3.6 (5) 1.1 (5) 5.4 (10)	0.03 - 5 µg/L n =6 R= 0.9999
Ground Water		R35140 (m/z 211.1 to 71.9) Quantification		
	0.05* 0.50 Overall	93 – 95 (94) 97 – 103 (101) 93 – 103 (97)	0.9 (5) 2.5 (5) 3.7 (10)	0.03 - 5 µg/L n =6 R= 0.999
		R35140 (m/z 211.1 to 154.1) Confirmation		
	0.05* 0.50 Overall	89 – 93 (91) 96 - 102 (99) 89 – 102 (95)	1.9 (5) 2.4 (5) 4.8 (10)	0.03 - 5 µg/L n =6 R= 0.9999
Drinking Water		R35140 (m/z 211.1 to 71.9) Quantification		
	0.05* 0.50 Overall	97 – 103 (100) 101 – 107 (105) 97 – 107 (102)	2.6 (5) 2.1 (5) 3.5 (10)	0.03 - 5 µg/L n =6 R= 0.9999
		R35140 (m/z 211.1 to 154.1) Confirmation		
	0.05* 0.50 Overall	93 – 99 (97) 101 – 107 (105) 93 – 107 (101)	2.6 (5) 2.0 (5) 4.6 (10)	0.03 - 5 µg/L n =6 R= 0.9999

* Limit of quantification

Specificity: Representative chromatograms were demonstrated for the matrix blanks, the 0.1 µg/L calibration solution and the fortified samples (LOQ and 10xLOQ). No significant peak interferences were observed at the retention time of pirimicarb. Chromatograms were provided for the 2 mass transitions which were monitored for quantification and confirmation purposes.
Matrix Effects: The effect of different water matrices on the LC-MS/MS response was assessed by preparing standards in the presence of each matrix and comparing the peak areas of R35140 against non-matrix standards at an equivalent concentration. No significant enhancement or suppression of detector response was observed in the presence of any of the water matrices.
Linearity: Linearity was demonstrated using 6 calibration solutions in the range of 0.03-5.0 µg/L. The correlation coefficients were > 0.99 for all matrices. Non-matrix calibration standards were used for quantification. The concentration range covers from <30 % of the LOQ to >20 % above the highest level
Recovery and Repeatability: The accuracy of the method was assessed by fortifying 5 control samples of each matrix with 0.05 µg/L (LOQ) and 0.5 µg/L (10xLOQ) pirimicarb. All mean recoveries at each fortification level were within the acceptable range of 70 to 120%. Control blanks were analysed for each matrix. All RSDs at each fortification level were within the acceptable range of <20%.
LOQ: The LOQ of the method is 0.05 µg/L. According to guidance document SANCO/825/00 rev. 8.1, the limit of quantification for monitoring methods in surface water must comply with the lowest effect concentrations in aquatic species. The lowest effect endpoint for pirimicarb is 0.9 µg/l for the long term risk to Daphnia (NOEC

endpoint). For drinking water or groundwater the limit of quantification must meet 0.1 µg/L. Therefore, the LOQ is considered to be sufficiently low.
Confirmation of Identity: The mass transition 211.1->71.9 was used for quantification and the transition 211.1->154.1 was used for confirmation.
Storage Stability: The storage stability of extracts in surface was demonstrated for a period of 7 days against a freshly prepared calibration standard. Recoveries were in the range of 85-91% (mean: 88%) with an RSD of 2.9 (5 samples).

Matrix	**Analyte**	**Recovery fortification level (µg/L)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**
Surface Water	R35140	0.05	85 - 91 (88)	2.9 (5)

Conclusion: GRM039.02A is validated in accordance with SANCO/825/00 revision 8.1 for the detection of metabolite R35140 with an LOQ of 0.05 µg/L in water matrices.

B.5.2.3. Methods for the analysis in air of the active substance and relevant breakdown products formed during or after application

Residue Definition

The EFSA conclusion on the peer review of pirimicarb (2005) indicates that the residue definition for monitoring in air is pirimicarb.

Monitoring methods

The following method was evaluated during the first ("Annex 1") review of pirimicarb and a summary of the method and validation data are provided in the DAR (Vol.3, Annex B, Section B5, October 2003).

Matrix	**Analyte**	**Method**	**Reference**
Air	Pirimicarb	GC-MSD	Method: RAM 359 Study: TMJ4568B Crook, S., 2001

It was concluded in the DAR that GC-MSD method RAM 359 is validated for pirimicarb with an LOQ of 6 µg/m^3 in water. No new monitoring methods for pirimicarb in air were submitted in support of the renewal of pirimicarb. The Applicant for the renewal of pirimicarb is relying on this monitoring method and, therefore, it has been re-evaluated for the purposes of renewal.

Method RAM 359: Determination of pirimicarb in air

Report:	**Crook, S. (2001), Pirimicarb Validation of a Confirmatory Residue Analytical Method for the Determination of Residues in Air Report Number: TMJ4572B**

Previous evaluation	In DAR (2003) for original approval.

A monitoring method using GC-MSD was developed and validated for the determination of pirimicarb in air.

Method Summary

Air was drawn through sampling tubes containing two layers of Tenax adsorbent at a rate of 2 L/min for a period of six hours, using pre-calibrated motorised pumps. After this time period the Tenax adsorbent was removed from the tubes and residues of pirimicarb were desorbed by ultrasonication in toluene (5 mL). Aliquots were transferred to autosampler vials for final determination by gas-liquid chromatography with mass selective detection (GC-MSD) using two fragment ions (m/z > 100).

Air Samples

The method was validated in 2 different conditions of temperature and humidity:
(i) 20°C at 45% relative humidity
(ii) 35 °C at > 90 % relative humidity

Conditions (ii) are acceptable for method validation in air as per SANCO/825/00 revision 8.1.

Sorbent Characteristics

Tenax sampling tubes contain polymer based sorbents and, therefore, proof of adsorbance is not considered necessary.

Instrument Validation

The analysis was performed using a Hewlett Packard HP6890 gas liquid chromatograph fitted with a HP5973 series mass selective detector.

Column:	SGE BPX-5 (5% phenylmethylpolysiloxane) 30 m x 0.25 mm x 0.25 µm film thickness
Injection port:	Split/splitless injector operating in splitless mode with a 4 mm i.d. double gooseneck splitless liner packed with a small amount of glass wool
Carrier gas + head pressure:	Helium at 1.0 ml min^{-1} constant flow
Injection mode:	Pulsed splitless (pulse pressure 30.0 psi for 1.00 minute)
Splitless time:	1.0 minute
Injection volume:	3 µl
Injection temperature:	250 °C
Transfer line temperature:	280 °C
Temperature programme:	100 °C hold for 1 minute then programme at 15 °C per minute to 300 °Cand hold for 1 minute.
MSD Conditions	
Electron energy:	70 eV
System Calibration:	Autotune
Acquisition Parameters:	

Compound Name	**Low Mass Resolution**	**SIM**	**MODE**
Pirimicarb	Yes	Target Ion Qualifier 1 Retention Time	166 m/z 238 m/z 10.2 min

Summary of Validation Data

Sample matrix	Fortification level (µg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Pirimicarb				
20°C at 45% relative humidity	Control	-	- (2)	0.005 – 1.0 µg/mL (equivalent to 0.015 – 3.0 ng on column) n = 5 R= 0.9999
	4.0*	80 – 91% (84%)	6.3 (5)	
	40.0	83 – 92 % (87%)	3.9 (5)	
	Overall	80 – 92% (86%)	4.9 (10)	
35°C at 80% relative humidity	Control	-	- (2)	
	4.0*	85 – 113% (98%)	11.0 (5)	
	40.0	67 – 98% (73%)	4.9 (5)	
	Overall	67 – 113% (86%)	18.1 (10)	

*The limit of quantification (LOQ) is 6.0 µg/m^3, equivalent to 4.0 µg adsorbed on the Tenax.

Selectivity: Representative chromatograms were demonstrated for the control blanks, the 1.0 µg/mL calibration solution (highest concentration calibration standard) and the fortified samples (LOQ and 10xLOQ). No significant peak interferences were observed at the retention time of pirimicarb. Chromatograms were provided for 2 mass ions which were monitored. A chromatogram was provided of a mass spectrum.
Linearity: Linearity was demonstrated using 5 calibration solutions in the range of 0.005 – 1.0 µg/mL (equivalent to 0.015 – 3.0 ng on column). The correlation coefficients were >0.99. Non-matrix calibration standards were used for quantification; significant matrix effects are not anticipated. It is not clear if the concentration range covers from <30 % of the LOQ to >20 % above the highest level.
Recovery and Repeatability: The accuracy of the method was assessed by fortifying 5 control samples of each matrix with 4.0 µg (LOQ) and 40 µg (10xLOQ) pirimicarb. All mean recoveries at each fortification level were within the acceptable range of 70 to 120%. Control blanks were analysed for each matrix. All RSDs at each fortification level were within the acceptable range of <20%.
LOQ: The LOQ of the method is 6.0 µg/m^3, equivalent to 4.0 µg adsorbed on the Tenax. In accordance with the guidance document SANCO/825/00 rev. 8.1, (reference: pages 21-22), if a limit of quantification was established according to Council Directive 98/24/EC, the supported LOQ from the analytical monitoring method for determining residues in air should comply with this value. No limit has been established for pirimicarb according to Council Directive 98/24/EC. Therefore, the LOQ should comply with the concentration, c, calculated according to the equation given in SANCO/825/00 rev. 8.1.
Using the $AOEL_{systemic}$ of 0.035 mg/kg bw/day (as there is no AOEL specifically for inhalation and there are not believed to be any issues with local effects), the concentration c is calculated as follows:

$$c = AOEL_{systemic} \cdot \frac{safety\ factor \cdot body\ weight}{air\ intake}$$

With safety factor: 0.1; body weight: 60[kg]; air intake: 20 [m^3/day]

$c = AOEL_{systemic} \cdot 300$ [µg/m^3]
$c = 0.035 \times 300$

$c = 10.5$ µg/m^3

This calculated concentration, c, is higher than the supported LOQ from the analytical monitoring method for determining residues in air of 6 µg/m^3 and the LOQ is, therefore, considered to be acceptable.
Confirmation of Identity: While two mass ions were monitored (166 m/z, 238 m/z), the method validation was not presented in terms of each ion. It is acceptable to conclude that further confirmatory methods are not required for the method to determine residues in air given that sufficient confirmatory data is provided for methods of analysis in water.
Storage Stability: According to the method procedure, if the analysis is not to be completed in the same day then the sampling tube is to be stored overnight at <7°C. If storage for longer than overnight is required, it is recommended that the tubes are stored deep-frozen at ≤-18°C. The storage stability of the analyte loaded onto the sorbent has not been reported.

Standard solutions were stored at ≤7°C; it is stated that the pirimicarb analytical standard is assumed to be stable for four months when stored ≤7°C.
It is also stated that the analytical procedure can be stopped at various points for overnight/weekend breaks and that external standard recoveries will validate any work stoppages. The samples are stored in sealed vessels at ≤7°C.
Breakthrough: The retention capacity of the sorbent material has not been proven.
Extractability: Data on the extractability of the analyte from the sorbent was not submitted.

Conclusion: Method RAM 359 is not strictly validated in accordance with SANCO/825/00 rev. 8.1. In terms of the specificity of the method, a clearly labelled chromatogram of the lowest calibration level has not been provided. It is not clear that the linear range covers from 30% of the LOQ to 20% above the highest level.

The retention capacity of the sorbent material has not been addressed. The extractability and storage stability of pirimicarb on the sorbent has not been addressed, although it is acknowledged that these two considerations are described as 'desirable' rather than necessary by SANCO/825/00 rev. 8.1.

Although it is recognised that there are deficiencies with Method RAM 359, the accuracy and precision data for pirimicarb is considered acceptable. On this basis the method is considered fit for regulatory purposes for the determination of residues of pirimicarb in air with an LOQ of 6 µg/m^3.

B.5.2.4. Methods for the analysis in body fluids and tissues for active substances and relevant metabolites

Body Fluids and Tissues

Residue Definition

The EFSA conclusion on the peer review of pirimicarb (2005) does not state a residue definition for monitoring in body fluids and tissues. However, pirimicarb has a harmonised classification of Acute toxicity 3. Furthermore, the EFSA conclusion on the peer review of pirimicarb (2005) states that metabolites R34836 and R34885 have a *'similar or higher level of toxicity'* to the parent compound.

The Applicant for renewal argues that it is known that carbamate containing compounds are not detected in animals fed with parent and, therefore, a method which measures parent only is considered adequate.

Based on the renewal submission, the residue definition for monitoring in body fluids and tissues has been proposed as pirimicarb and the major metabolite R34865.

Monitoring methods

The following method was evaluated during the first Annex 1 review of pirimicarb and a summary of the method and validation data are provided in the DAR (Vol.3, Annex B, Section B5, October 2003).

Matrices	**Analyte**	**Method**	**Reference**
Human plasma Dog Plasma	Pirimicarb	LC-UV LC-MS	Method: CTL/R/1441 Study: RJ1056B Hall, M.G., 2000

It was concluded in the DAR that LC-UV LC-MS method CTL/R/1441 is validated for pirimicarb in human and dog plasma with LOQs of 4 ng/mL and 11 ng/mL respectively. No new monitoring methods for pirimicarb in body fluids and tissues were submitted in support of the renewal of pirimicarb. The Applicant for the renewal of pirimicarb is relying on method CTL/R/1441 and, therefore, it has been re-evaluated for the purposes of renewal.

According to SANCO/825/00 rev. 8.1, a method must also be validated for body tissues (either meat, liver or kidney). However, the guidance also states that *'If a primary method for food of animal origin (Section 4) with sufficient sensitivity covers the respective tissue no additional method or validation study for body tissue is required'*. The guidance document states that the LOQ shall meet 0.1 mg/kg for body tissues.

As per section B.5.2.1.1., method DFG S19 multi-residue method has been fully validated in accordance with SANCO/825/00 revision 8.1 for the determination of pirimicarb in meat, liver and kidney with an LOQ of 0.01 mg/kg. Therefore, this method is considered suitable for the determination of pirimicarb in body tissues.

Based on the renewal submission, the residue definition for monitoring in body fluids and tissues has been proposed as pirimicarb and the major metabolite R34865. It is noted that there are currently no monitoring methods available in body fluids and tissues for this metabolite.

Method CTL/R/1441: Determination of pirimicarb in body fluids

Report:	**Hall M G (2000), Pirimicarb: Determination in human and animal plasma by LC-UV and LC-MS, Report Number CTL/R/1441**

Previous evaluation	In DAR (2003) for original approval.

A monitoring method using LC-UV and LC-MS analytical techniques was developed and validated for the determination of pirimicarb in body fluids.

Method Summary

Samples of blood (typically 5 mL) were taken into collection tubes containing lithium heparin anticoagulant and mixed well. The samples were centrifuged and the plasma was transferred to a clean tube, plasma samples were stored frozen at approximately -20 °C until analysed.

Aliquots (0.5 mL) of each sample were made alkaline with 1 mL 0.1 M sodium hydroxide and mixed well. To each tube was added 5 mL of methyl-t-butyl ether. The samples were vortex mixed for 10 minutes and then centrifuged at 2500 rpm for 5 minutes. A 4 mL aliquot of each extract was transferred to a clean tube. The extracts were evaporated to dryness at less than 30°C using a stream of nitrogen.

The residues were then re-dissolved in 200 µL of either acetonitrile/50 mM ammonium acetate buffer (50:50 v/v) for analysis by LC-UV or acetonitrile/1 mM ammonium acetate buffer (50:50 v/v) for analysis by LC-MS. The extracts were transferred to autosampler vials for analysis.

Instrument Validation

Primary Method (LC-UV)

Analytical column: 250 x 4.6 mm i.d. packed with 5 µm particle size Spherisorb ODS-2
Pre-column: Hichrom RPB cartridge guard column
Mobile phase: Acetonitrile/50 mM Ammonium acetate buffer (40:60v/v)
Flow rate: 1 mL/min
Injection volume: 50 µL
Detection: The analytes were quantified using UV detection at 245 nm
Typical retention times using the above conditions and UV detection were 8.7 minutes.

Confirmatory method (LC-MS)

Analytical column: 250 x 4.6 mm i.d. packed with 5 µm particle size Spherisorb ODS-2
Pre-column: Hichrom RPB cartridge guard column
Mobile phase: Solvent A: Acetonitrile
Solvent B: 1 mM ammonium acetate

Gradient:

Time (minutes)	%A	%B
0.0	50	50
8.0	30	70
9.0	30	70
9.01	50	50
11.0	50	50

Flow rate: 1 mL/min
Injection volume: 5 µL
Detection: Quantitation was performed using the ion chromatograms recorded for m/z = 182.0

m/z	Identity
239.2	[M+H]+
182.0	[M-$(CH_3)_2$NC]+
72.0	[$(CH_3)_2$NCO]+

Typical retention times using the above conditions and UV detection were 5.4 minutes.

Summary of Validation Data (Primary method: LC-UV)

Sample matrix	Fortification level (ng/mL)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Pirimicarb				
Human plasma	50*	99.5 – 123 (112)	8.7% (5)	20 - 5000 ng/mL n = 11 R= 1.000
	100	88 – 91 (89)	1.7% (5)	
	300	84 – 90 (87)	2.4% (5)	
	900	79 – 86 (83)	3.6% (5)	
	2000	84 – 89 (86)	2.7% (5)	
	Overall	79 - 123 (91)	12.5% (25)	
Dog plasma	50*	85 – 104 (90)	7.5% (5)	
	100	84 – 92 (92.5)	3.6% (5)	
	300	89 – 92 (90.5)	1.4% (5)	
	900	92 – 94 (86.5)	0.9% (5)	
	2000	94 – 10 (81.5)	3.0% (5)	
	Overall	84 – 104 (88)	4.7% (25)	

*The limit of quantification (LOQ) is 50 ng/mL

Summary of Validation Data (Confirmatory method: LC-MS)

Sample matrix	Fortification level (ng/mL)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Pirimicarb				
Human plasma	20*	75.5 – 113 (92)	15.8% (5)	10 – 500 ng/mL n = 11 R= 1.000
	500	70 – 91 (84)	10.4% (5)	
	Overall	70 – 113 (90)	11.9% (10)	
Dog plasma	20*	81 – 94 (84.5)	6.1% (5)	
	500	77 – 100 (86.5)	9.8% (5)	
	Overall	77 – 100 (84.9)	8.4% (10)	

*The limit of quantification (LOQ) is 20 ng/mL

Selectivity: For the primary method (UV detection) representative chromatograms were shown for both the blank and fortified human plasma matrix (LOQ and 40xLOQ level). Chromatograms were not shown for the lowest calibrated level or the blank/fortified dog plasma matrix.
For the confirmatory method (MS detection), representative chromatograms were shown for a matrix blank of unknown origin. Chromatograms were in turn shown for the fortified unknown matrix (50 ng/mL and 500 ng/mL). Chromatograms were presented for each of the 3 mass ions of interest for each fortification level (72, 182 and 239.2 m/z). A positive ion mass spectrum for pirimicarb was also provided. A chromatogram was not provided for the lowest calibrated level or the lowest fortification level.
It is noted that none of the submitted chromatograms were clearly labelled.
Linearity: The linearity of the method was addressed with a calibration plot for the primary method (UV detection). Matrix matches standards were used to generate the calibration plot; it is not clear from which matrix the calibration plot data has been generated. The correlation coefficient is reported to be 1.00 for both human and dog plasma. It is not clear exactly how many calibration levels were tested, but the number is >5. For the primary method the calibration range covers from <30 % of the LOQ to >20 % above the highest level.
Calibration plots were not shown for the confirmatory method (MS detection). It is not clear if the concentration range covers from <30 % of the LOQ to >20 % above the highest level.
Recovery and Repeatability: For the primary method, the accuracy of the method was assessed by fortifying 5 control samples of each matrix with 50 µg to 2000 ng/mL pirimicarb (5 fortification levels). All mean recoveries at each fortification level were within the acceptable range of 70 to 120%. Control blanks were analysed for each matrix. All RSDs at each fortification level were within the acceptable range of <20%.

For the confirmatory method, the accuracy of the method was assessed by fortifying 5 control samples of each matrix with 20 µg and 500 ng/mL pirimicarb. All mean recoveries at each fortification level were within the acceptable range of 70 to 120%. Control blanks were analysed for each matrix. All RSDs at each fortification level were within the acceptable range of <20%.
LOQ: The LOQ of the primary method is 50 ng/mL while the LOQ of the confirmatory method is 20 ng/mL. According to SANCO/825/00 rev. 8.1, the limit of quantification must meet 0.05 mg/L for body fluids. Therefore, the LOQ is considered to be sufficiently low.
Confirmation: Confirmation was achieved by an independent confirmatory technique (alternative detector). It is noted that the study report does not provide sufficient validation data in terms of the calibration and selectivity of the confirmatory method.
Storage stability: The storage and handling of standard solutions/extracts was not reported. The storage stability of standard solutions and extracts was not addressed.

Conclusion: Method CTL/R/1441 is not strictly validated in accordance with SANCO/825/00 rev. 8.1. There are deficiencies in terms of selectivity and linearity of the method in terms of both the primary and confirmatory techniques. The storage stability of standard solutions extracts was not addressed.

Although it is recognised that there are deficiencies with Method CTL/R/1441, the accuracy and precision data is considered acceptable. On this basis, the method is considered fit for regulatory purpose for the determination of residues of pirimicarb in body fluid with an LOQ of 0.05 mg/L.

B.5.3. REFERENCES RELIED ON

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 4.1.1/01	Kirchner, J.	2011	Pirimicarb - Determination of PP62 in technical material Syngenta Syngenta Crop Protection, LLC, Greensboro, NC, USA, SA-52/1 Not GLP not published Syngenta File No PP62_10070	N	N	n/a	SYN	N
KCA 4.1.1/02	Kirchner, J.	2011	Pirimicarb technical (PP62) - Validation of analytical method SA-52/1 Syngenta Syngenta Crop Protection, LLC, Greensboro, NC, USA, TK0059421 GLP not published Syngenta File No PP62_10072	N	Y	New data; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 4.1.1/03	Kirchner, J.	2011	Pirimicarb - Determination of by-products in PP62 Syngenta Syngenta Crop Protection, LLC,	N	N	n/a	SYN	N

			Greensboro, NC, USA, SB-52/1 Not GLP not published Syngenta File No PP62_10069 **This is CONFIDENTIAL INFORMATION***					
KCA 4.1.1/04	Kirchner, J.	2011	Pirimicarb technical (PP62) - Validation of analytical method SB-52/1 Syngenta Syngenta Crop Protection, LLC, Greensboro, NC, USA, TK0041747 GLP not published Syngenta File No PP62_10073 **This is CONFIDENTIAL INFORMATION***	N	Y	New data; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 4.1.1/05	Grunenwald, S.	2011	Mass spectral identification of pirimicarb technical and six related impurities Syngenta Syngenta Crop Protection, Inc., Greensboro, USA, 10474133 Not GLP not published Syngenta File No PP62_10109 **This is CONFIDENTIAL INFORMATI**	N	N	n/a	SYN	N

			ON*					
KCA 4.1.2/01	Bruce, S.	2017	Pirimicarb - Oral (Gavage) Proof of Exposure Study in Mice, Report Number: AE77RC.DRF 000M.BTL	N	Y	New data; eligible for data protection according to SANCO/1257 6/2012	SYN	N
KCA 4.1.2/02	Cullen, GM.	1993	Pirimicarb: Determination of Pirimicarb and its Carbamate Metabolite in Crops – Analytical Method Validation, Report RJ 1056B	N	-	-	SYN	Y - DAR
KCA 4.1.2/03	Harradine, KJ.	1995	Pirimicarb: Validation of a method for the determination of pirimicarb and its carbamate metabolites in brassica, cereal grain and straw, pome fruit, fruiting vegetables (edible peel), leafy Vegetables, Root and Tuber Vegetables. Report RJ 1806B	N	-	-	SYN	Y - DAR
KCA 4.1.2/04	Coombe, N.B.	1996	Independent Validation of Standard Operating Procedure RAM 265/02, Report Number: CEMR-622	N	-	-	SYN	Y - DAR
KCA 4.1.2/05	Wright, DR	1998	Transfer of Standard Operating Procedure RAM 265/03 to Covance	N	-	-	SYN	Y - DAR

			Laboratories Ltd in four crop matrices Report Number: CLE 38/229/D2140					
KCA 4.1.2/06	Croucher A.	2002	Pirimicarb: Validation of an Analytical Method for the Determination of Pirimicarb and Metabolite Residues in Tobacco, Stone Fruits and Citrus Fruit Syngenta Crop Protection AG, Basel, Switzerland Covance Laboratories, Harrogate, United Kingdom, 38/256-D2140 GLP, not published Syngenta File No PP62/1101	N	Y	New data; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 4.1.2/07	Tsui G.	2015	Pirimicarb - Validation of Analytical Method GRM039.04A for the Determination of Pirimicarb and its Metabolite Desmethyl Pirimicarb in Crops Syngenta Battelle UK Ltd, Chelmsford, Essex, UK, TK0252946 GLP not published Syngenta File No PP62_10268	N	Y	New data; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 4.1.2/08	Peither, A.	2014	Pirimicarb - Effect on	N	Y	New data; eligible for	SYN	N

			Survival, Growth and Reproduction of Daphnia magna in a Semi-Static Test over Three Weeks in a Higher Tier Test Design with Sediment, Report Number: D84807			data protection according to SANCO/12576/ 2012		
KCA 4.1.2/09	Wallace S. J., Smyth D. V., Shillabeer N.	2002	Wallace S. J., Smyth D. V., Shillabeer N., (2002), YF7904B (Pirimicarb formulation): Toxicity to the sediment reworker Chironomus riparius of a 500 g kg-1 WG formulation, Report Number: BL7239/B	N	Y	New data; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 4.2/01	Lakaschus S.	2005	Validation of Multi-Residue Method DFG S19 (L00.00-34) For the Determination of Residues of Pirimicarb in Animal Tissues With LC-MS/MS Detection Syngenta Crop Protection AG, Basel, Switzerland Dr. Specht & Partner Chem. Laboratorien GmbH, Hamburg, Germany, SYN-0501V GLP	N	Y	New data; eligible for data protection according to SANCO/12576/ 2012	SYN	N

			not published Syngenta File No PP62/1468					
KCA 4.2/02	Reichert N.	2005	Independent Laboratory Validation of the DFG Method S19 for the Determination of Residues of Pirimicarb in Matrices of Animal Origin Syngenta Crop Protection AG, Basel, Switzerland Institut Fresenius, Taunusstein, Germany, IF-05/00362966 GLP not published Syngenta File No PP62/1469	N	Y	New data; eligible for data protection according to SANCO/12576/ 2012	SYN	N
KCA 4.2/03	Kwiatkowski AS, Robinson NJ	1996	Pirimicarb and Desmethyl Pirimicarb: Analytical Method and Validation of a Method for the Determination of Residues in Soil. Zeneca Agrochemicals, Jealott's Hill, United Kingdom Zeneca Agrochemicals, Jealott's Hill, United Kingdom, RJ1983B GLP, not published Syngenta File No PP62/0557	N	-	-	SYN	Y - DAR
KCA	Robinson,	2001	Pirimicarb and	N	-	-	SYN	Y - DAR

4.2/04	N.J.		its Carbamate Metabolites: Validation of a Residue Analytical Method for the Determination of Residues in Water, Report Number: TMJ4568B					
KCA 4.2/05	Langridge G.	2011	R35140 - Validation of Residue Method GRM039.02A for the Determination of the Pirimicarb metabolite R35140 in Surface Water, Groundwater and Drinking Water Syngenta CEMAS, North Ascot, United Kingdom, CEMR-5184-REG GLP not published Syngenta File No R035140_10000	N	Y	New data; eligible for data protection according to SANCO/12576/ 2012	SYN	N

IIA, 4.2/06	Crooks S J	2001	Pirimicarb: Validation of a Residue Analytical Method for the Determination of Residues in Air Samples Syngenta Crop Protection AG, Basel, Switzerland Zeneca Agrochemicals, Jealott's Hill, United Kingdom, TMJ4572B Not GLP, not published Syngenta File No PP62/0941	N	-	-	SYN	DAR
IIA, 4.2/07	Hall MG	2000	Pirimicarb: Determination in Human and Animal Plasma by LC-UV and LC-MS. Central Toxicology Laboratory Zeneca Report No. CTL/R/1441 GLP, Unpublished	N	-	-	SYN	Y - DAR