European Commission

Renewal Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

Mecoprop-P

Volume 3 – B.5 (AS)

Rapporteur Member State : United Kingdom
Co-Rapporteur Member State : Ireland

Version History

When	What
31/03/2016	Initial Renewal Assessment Report (RAR)

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B.5. METHODS OF ANALYSIS

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

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

a) Active substance

Report:	CA 1.11/01, Mahmood, T. (2014a)
Title	Analysis of seven batches of Mecoprop-P TGAI Report No. 14/0861
Guidelines:	Regulation (EC) No 1107/2009 repealing Council Directives 71/117/EEC and 91/414/EEC PMRA Regulatory Directive 98-04 OPPTS 830 Series
GLP:	Yes
Deviations	None
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012.

The mecoprop-P content in the technical grade active substance is determined by a reverse phase chiral HPLC external standard method. The following conditions were noted:

HPLC conditions
Column: Nucleodex alpha PM. 20 cm x 4 mm i.d. 5µm film thickness.
Column temperature: 30^{0}C
Mobile phase: 65 % methanol / 35 % buffer solution
Buffer solution: 50 mM NaH_2PO_4 adjusted to pH 3 with phosphoric acid
Flow rate: 0.8 mL/min
Detector wavelength: 280 nm
Injector volume: 10 µL
Run time: 20 minutes

The method for determining optical ratio is included in the confidential section of the dossier; see Volume 4 of mecoprop-P RAR.

Method validation is reported in Table 5.1-1.

Table Error! No text of specified style in document.-1Summary of method validation

	Linearity	Precision, %RSD (n)	Fortification levels and recovery, %	Interference
Mecoprop-P in TGAI	*ca.* 0.5 – 2.5 mg/mL [equiv. to *ca.* 25 – 125 % nominal content] n = 5 r = 0.9999	0.32 (5) (at 91.82 % w/w) Horwitz %RSD$_r$ = 1.36	Not required	Chromatograms of blank, test sample and analytical standards (racemic sample) showed no interference at retention times of interest: mecoprop-P = *ca.* 6.5 min. mecoprop (S-) = *ca.* 8.5 min.

Conclusion

The method is validated in accordance with the EU guidance SANCO/3030/99/rev. 4.

b) Significant and relevant impurities

See Volume 4 of mecoprop-P RAR.

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

All studies submitted in the environmental fate section used radio-isotopes. Therefore according to Regulation (EU) 283/2013 methods are not necessary.

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

No methods were included in any of the efficacy studies.

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

The following methods have been submitted for the purposes of renewal and were used in the studies summarised in Section 5 (Toxicology and metabolism studies on the active substance).

Table Error! No text of specified style in document.-2 Summary of toxicology methods submitted for purposes of renewal

Matrix	Analyte	Method	Reference
Diet	Mecoprop-P	HPLC	▮▮▮ 2008
Diet	Mecoprop-P	HPLC	▮▮▮ 2003

Report:	**CA 4.1.2/01 (CA 5.5/01),** ▮▮▮ **(2008)**
Title	Mecoprop-P dietary two year carcinogenicity study in the rat Report No ▮▮▮
Guidelines:	OECD 451 OPPTS 870.4200
GLP:	Yes
Deviations	None
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012.

Measured amounts of water/trifluoroacetic acid (0.5%v/v) were added to diet samples, which were allowed to stand at room temperature, before being extracted by the addition of acetonitrile/trifluoroacetic acid (0.5% v/v). Aliquots of the supernatant were diluted with acetonitrile, as appropriate after filtration, to give sample solution concentrations within the range of the calibration standards used. Samples and standards were analysed by HPLC. The following conditions were noted:

Column: 25 cm x 4.6 mm ID Zorbax ODS (Hichrom)
Column Temperature: 50°C
Flow Rate: 1.5 mL/min
Detector wavelength   280 nm

Volume injected: 10 µl
Mobile phase: 0.1M acetic acid in water/acetonitrile, 55/45 v/v

Conclusion
No validation data supplied. The method can not be validated in accordance with SANCO/3029/99 rev. 4

Report:	**CA 4.1.2/02 (CA 5.6.1/01),** ▬▬▬▬▬ **(2003)**
Title	Mecoprop-P: oral (dietary administration) preliminary reproduction toxicity study in the rat Report No. ▬▬▬▬▬
Guidelines:	OECD 415
GLP:	Yes
Deviations	The study was modified to reduce the dietary dose during lactation to reflect increased food consumption during this period. On several occasions the humidity was outside the protocol range, the highest recording being 74%. This is not considered to adversely affect the study.
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012.

Mecoprop-P was formulated into ground diet for administration to the study animals. Mecoprop-P was measured in the diet using HPLC. No chromatographic conditions were reported in the study.

The validation data supplied are reported in Table 5.1.2-2.

Table Error! No text of specified style in document.-3 **Validation for method to determine mecoprop-P content in ground diet**

Matrix	LOQ	Linearity	Precision, %RSD (n)	Fortification levels (µg/g) and recovery (mean), %		Interference
Mecopro p-P in diet	200 µg/g [0.02 ppm]	200 – 1400 µg/g r = 0.9993 n = 6	1.05 (6) 0.39 (6) 0.24 (6) At all fortification levels %RSD < 20	200.1 600.4 1401	94 (n = 6) 95 (n = 6) 98 (n = 6) SANCO acceptable range = 70 – 110%	No significant detector response from control diet extracts.

Conclusion
The method for determining mecoprop-P in ground diet is not strictly validated in accordance with SANCO 3029/99/rev. 4. This is due to no sample preparation details, the linearity graph and specificity chromatograms not being provided. Also only the mean values for the recoveries at each fortification level were provided, not the individual values.

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 operator, worker, resident and bystander exposure studies were submitted.

B.5.1.2.5. Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies
The following methods were used in the studies summarised in Volume 3, section B.7 of the active RAR:

Table Error! No text of specified style in document.**-4 Summary of residue methods submitted for purposes of renewal**

Matrix	Analyte	Method	Reference
Whole milk Skimmed milk Cream Muscle Liver Kidney Fat	Mecoprop-P Mecoprop-P 2-ethylhexyl ester Mecoprop-P glycine conjugate 2-(2-hydroxymethyl-4-chlorophenoxy) propionic acid (HMCPP) 2-(2-carboxy-4-chlorophenoxy)propionic acid (CCPP) 4-chloro-2-methyl phenol (PCOC)	LC-MS/MS	▮▮▮ 2013 ▮▮▮ 2014
Wheat grain, straw and foliage	Mecoprop-P (as the pentafluorobenzyl ester derivative)	GC-EC	Anding, 2001
Wheat grain, straw and foliage	Mecoprop-P (as the methyl ester derivative)	GC-MS	Perny, 2002
Wheat grain, straw and foliage	Mecoprop-P (as the methyl ester derivative)	GC-MS	Gallais, 2002a
Wheat grain, straw and foliage	Mecoprop-P	LC-MS/MS	Tandy, 2014a

Both studies by ▮▮▮ 2013 and ▮▮▮ 014 shared the same method and validation data. Therefore only a single method summary is provided for these two reports.

Report:	CA 4.1.2/03 (CA 6.1/01), ▮▮▮ (2013)	
Title	Mecoprop-P livestock feeding study: magnitude of residue in milk, muscle, liver, kidney and fat of lactating dairy cattle Report No. ▮▮▮	
Guidelines:	OECD 505 OPPTS 860.1480	
GLP:	Yes	
Deviations	None	
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012.	

Report:	CA 4.1.2/04 (CA 6.1/02), ▮▮▮ (2014)	
Title	Frozen Storage Stability Study for Mecoprop-P, HMCPP, CCPP and PCOC in Bovine Specimens Report No. ▮▮▮	
Guidelines:	Not stated	
GLP:	Yes	
Deviations	N/A	
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012.	

Residues of mecoprop-P (MCPP-P), mecoprop-P 2-ethylhexyl ester (MCPP-P 2EH), mecoprop-P glycine conjugate, 2-(2-hydroxymethyl-4-chlorophenoxy) propionic acid (HMCPP), 2-(2-carboxy-4-chlorophenoxy)propionic acid (CCPP) and 4-chloro-2-methyl phenol (PCOC) were determined in animal matrices (milk and tissues) by LC-MS/MS.

Complete samples of muscle, fat, liver and kidney were homogenised in a Robot Coupe processor. Dry ice was used for fat specimens. After appropriate mixing of each sample, samples were transferred to HDPE plastic containers. No preparation was required for milk, skimmed milk or cream samples. Residues were extracted from the samples by heating overnight with a strong sodium hydroxide solution to convert esters and conjugates back to parent. 10 mL of sodium hydroxide

hydrolysis solution (47% sodium hydroxide/deionised water (15/85 v/v) and 1 mL methanol were added. The samples were left overnight to hydrolyse at 85°C. Samples were then neutralised with 15N sulphuric acid, monochloroacetic acid solution and acetonitrile, the extract was shaken with QuEChERS salts and then shaken with hexane. An aliquot of the acetonitrile layer was mixed with magnesium sulphate and aluminium oxide and, following centrifugation, an aliquot was evaporated to near dryness and reconstituted in a mixture of water plus 0.2% formic acid and methanol (70/30 v/v)to be within the appropriate concentration range. A mixture of internal standards was added to the final extract prior to analysis by LC-MS/MS. The following conditions were noted:

HPLC conditions mecoprop-P and metabolites

Column:	Onyx C18 monolithic column (3.0 x 100 mm)
Column Temperature:	Ambient
Flow Rate:	1 mL/min
Volume injected:	30 μL
Mobile phase A:	HPLC water + 0.1% formic acid
Mobile phase B:	Methanol + 0.1% formic acid

MS conditions mecoprop-P and metabolites

Ionization mode:	ESI
Probe position:	8 mm
Polarity:	Negative
Scan type:	MRM
Resolution:	Q1 – unit, Q3 - Low
Curtain Gas:	18
Collision Gas:	10
Temperature:	300°C
GS1:	30
GS2:	40
Ion spray voltage	-4500V
Dwell Time:	75.00 msec
Entrance potential:	-10
Probe position:	Horizontal 5 mm Vertical 7.5 mm

Analyte	Q1 Mass (Amu)	Q3 Mass (Amu)	Internal standard*	Approx. retention time (min)
Mecoprop-P Quantification	212.9	140.9	4-CDMAA	4.6
Mecoprop-P Confirmation	215	142.9	4-CDMAA	4.6
HMCPP Quantification	228.9	157.1	4-PB	2.2
HMCPP Confirmation	229	154.9	4-PB	2.2
CCPP Quantification	242.7	171.1	4-PB	2.0
CCPP Confirmation	243.02	127	4-PB	2.0
4-PB (Internal Standard 1)	179	93.1	N/A	2.5
4-CDMAA (Internal Standard 2)	212.9	155	N/A	4.5

* 4-CDMAA is 4-chloro-3,5-dimethylphenoxy-acetic acid and 4-PB is 4-phenoxybutyric acid

HPLC conditions PCOC

Column:	Onyx C18 monolithic column (3.0 x 100 mm)

Column Temperature: Ambient
Flow Rate: 1 mL/min
Volume injected: 50 µL
Mobile phase A: HPLC water + 0.1% acetic acid
Mobile phase B: Methanol + 0.1% acetic acid

<u>MS conditions PCOC</u>
Ionization mode: ESI
Probe position: 8 mm
Polarity: Negative
Scan type: MRM
Resolution: Q1 – unit, Q3 - Low
Curtain Gas: 18
Collision Gas: 12
Temperature: 450ºC
GS1:   25
GS2:   30
Ion spray voltage   -4500V
Dwell Time: 250.00 msec
Entrance potential: -10
Probe position: Horizontal 5 mm
                             Vertical 7.5 mm

Analyte	Q1 Mass (Amu)	Q3 Mass (Amu)	Internal standard	Approx. retention time (min)
PCOC Quantification	140.8	105.1	None	2.8
PCOC Confirmation	142.8	105.1	None	2.8

Validation data are displayed in Table 5.1.2-5:

The mecoprop-P ethylhexyl ester and glycine conjugates are expressed as mecoprop-P acid equivalents. The linear range and specificity of the method for the ester and glycine conjugate has not been reported. This is not of concern as these components are converted in mecoprop-P for analysis anyway and they do not feature in the residue definition therefore validated methods of analysis are not required. Matrix-matched solutions were used for used for calibration standards. The linear range reported for milk (whole) applies across all matrices.

Table Error! No text of specified style in document.-5 Validation for method to determine mecoprop-P, ester, conjugate and metabolite content in animal matrices

Matrix	Analyte	LOQ mg/kg	Linearity	Precision, %RSD (n)	Fortification levels (mg/kg) and recovery range / mean, %		Interference
Milk (whole)	Mecoprop-P (quant.)	0.01	0.0015 – 0.2 µg/mL	5.4 5.3	0.01 0.10	96 – 108 (103, n=5) 97 – 111 (106, n = 5)	No interference > 30% LOQ at retention time of interest. 2 mass transitions monitored with highly specific detection system (LC-MS/MS).
	Mecoprop-P (confirm.)		r = 0.9999 n = 8	4.2 7.8	0.01 0.10	102 – 114 (109, n = 5) 91 – 111 (104, n=5)	
	MCCP-P 2EH (quant.)	0.01		6.6 5.3	0.01 0.10	99 – 114 (108, n = 5) 99 – 112 (105, n = 5)	Not reported. Acceptable, as conjugates are expressed as mecoprop-P acid equivalents.
	MCCP-P 2EH (confirm.)			5.8 4.4	0.01 0.10	101 – 114 (108, n=5) 100 – 108 (106, n=5)	
	Glycine conj. (quant.)	0.01		5.1 7.8	0.01 0.10	105 – 111 (110, n=5) 103 – 119 (109, n = 5)	
	Glycine conj. (confirm.)			6.4 5.2	0.01 0.10	97 – 114 (106, n=5) 100 – 115 (106, n=5)	
	HMCPP (quant.)	0.01	0.0015 – 0.2 µg/mL	9.6 5.5	0.01 0.10	76 – 96 (87, n=5) 93 – 106 (98, n = 5)	No significant interference (> 30% LOQ) at retention time of interest. 2 mass transitions monitored with highly specific detection system (LC-MS/MS).
	HMCPP (confirm.)		r = 0.9999 n = 8	14 10.8	0.01 0.10	72 – 103 (90, n=5) 77 – 103 (93, n=5)	
	CCPP (quant.)	0.01	0.0015 – 0.2 µg/mL	6.2 2.9	0.01 0.10	90 – 106 (96, n=5) 97 – 104 (101, n = 5)	
	CCPP (confirm.)		r = 1.0000 n = 8	5.0 2.7	0.01 0.10	92 – 105 (99, n=5) 101 – 107 (104, n=5)	
	PCOC (quant.)	0.01	0.0015 – 0.2 µg/mL	5.2 3.8	0.01 0.10	84 – 95 (90, n=5) 90 – 98 (94, n = 5)	
	PCOC (confirm.)		r = 0.9998 n = 8	6.5 5.0	0.01 0.10	82 – 98 (91, n=5) 88 – 100 (95, n=5)	
Milk (skimmed)	Mecoprop-P (quant.)	0.01		4.3 2.8	0.01 0.10	96 – 106 (100, n=5) 108 – 116 (112, n=5)	No interference > 30% LOQ at retention time of interest. 2 mass transitions monitored with highly specific detection system (LC-MS/MS).
	Mecoprop-P (confirm.)			4.5 1.0	0.01 0.10	103 – 116 (111, n=5) 112 – 115 (113, n=5)	
	MCCP-P 2EH (quant.)	0.01		6.6 2.5	0.01 0.10	95 – 107 (104, n=5) 107 – 114 (111, n=5)	Not reported. Acceptable, as conjugates are expressed as mecoprop-P acid equivalents.
	MCCP-P 2EH			4.9	0.01	102 – 117 (110, n=5)	

Matrix	Analyte	LOQ mg/kg	Linearity	Precision, %RSD (n)	Fortification levels (mg/kg) and recovery range / mean, %		Interference
	(confirm.)			2.0	0.10	111 – 116 (112, n=5)	
	Glycine conj. (quant.)	0.01		4.8 2.5	0.01 0.10	112 – 118 (114, n=5) 111 – 117 (113, n=5)	
	Glycine conj. (confirm.)			3.1 4.2	0.01 0.10	108 – 118 (114, n=5) 107 – 114 (111, n=5)	
	HMCPP (quant.)	0.01		11.5 3.8	0.01 0.10	84 – 111 (99, n=5) 101 – 112 (108, n=5)	No significant interference (> 30% LOQ) at retention time of interest.
	HMCPP (confirm.)			5.9 1.7	0.01 0.10	90 – 104 (98, n=5) 102 – 106 (104, n=5)	
	CCPP (quant.)	0.01		4.6 3.7	0.01 0.10	104 – 117 (112, n=5) 105 – 114 (110, n=5)	2 mass transitions monitored with highly specific detection system (LC-MS/MS).
	CCPP (confirm.)			2.9 3.0	0.01 0.10	104 – 112 (109, n=5) 107 – 114 (110, n=5)	
	PCOC (quant.)	0.01		8.0 3.5	0.01 0.10	92 – 112 (101, n=5) 97 – 104 (101, n=5)	
	PCOC (confirm.)			8.7 1.4	0.01 0.10	94 – 117 (103, n=5) 103 – 107 (105, n=5)	
Cream	Mecoprop-P (quant.)	0.01		8.0 6.1	0.01 0.10	73 – 89 (82, n=5) 84 – 99 (91, n=5)	No interference > 30% LOQ at retention time of interest.
	Mecoprop-P (confirm.)			8.5 7.7	0.01 0.10	70 – 86 (77, n=5) 83 – 101 (91, n=5)	2 mass transitions monitored with highly specific detection system (LC-MS/MS).
	MCCP-P 2EH (quant.)	0.01		15.6 15.8	0.01 0.10	69 – 98 (78, n=5) 61 – 93 (75, n=5)	Not reported. Acceptable, as conjugates are expressed as mecoprop-P acid equivalents.
	MCCP-P 2EH (confirm.)			19.5 18.3	0.01 0.10	66 – 103 (78, n=5) 60 – 97 (76, n=5)	
	Glycine conj. (quant.)	0.01		16.0 7.8	0.01 0.10	57 – 87 (74, n=5) 76 – 93 (86, n=5)	
	Glycine conj. (confirm.)			14.2 8.0	0.01 0.10	57 – 82 (73, n=5) 75 – 93 (86, n=5)	
	HMCPP (quant.)	0.01		9.0 11.3	0.01 0.10	76 – 94 (82, n=5) 76 – 101 (87, n=5)	No significant interference (> 30% LOQ) at retention time of interest.
	HMCPP (confirm.)			3.9 10.9	0.01 0.10	79 – 84 (81, n=5) 77 – 101 (88, n=5)	
	CCPP (quant.)	0.01		7.0 9.3	0.01 0.10	83 – 97 (87, n=5) 99 – 112 (99, n=5)	2 mass transitions monitored with highly specific detection system (LC-MS/MS).
	CCPP			9.3	0.01	91 – 111 (96, n=5)	

Matrix	Analyte	LOQ mg/kg	Linearity	Precision, %RSD (n)	Fortification levels (mg/kg) and recovery range / mean, %		Interference
	(confirm.)			10.9	0.10	96 – 124 (106, n=5)	
	PCOC (quant.)	0.01		7.4	0.01	73 – 86 (80, n=5)	
				1.9	0.10	80 – 84 (82, n=5)	
	PCOC (confirm.)			15.1	0.01	73 – 105 (84, n=5)	
				2.4	0.10	78 – 83 (81, n=5)	
Muscle	Mecoprop-P (quant.)	0.01		5.9	0.01	102 (n=5)	No interference > 30% LOQ at retention time of interest.
				2.9	0.10	105 (n=5)	2 mass transitions monitored with highly specific detection system (LC-MS/MS).
	Mecoprop-P (confirm.)			1.9	0.01	110 (n=5)	
				4.0	0.10	104 (n=5)	
	MCCP-P 2EH (quant.)	0.01		7.7	0.01	105 (n=5)	Not reported. Acceptable, as conjugates are expressed as mecoprop-P acid equivalents.
				6.0	0.10	107 (n=5)	
	MCCP-P 2EH (confirm.)			7.4	0.01	104 (n=5)	
				3.6	0.10	107 (n=5)	
	Glycine conj. (quant.)	0.01		4.2	0.01	105 (n=5)	
				4.1	0.10	107 (n=5)	
	Glycine conj. (confirm.)			7.5	0.01	107 (n=5)	
				4.7	0.10	106 (n=5)	
	HMCPP (quant.)	0.01		6.6	0.01	101 (n=5)	No significant interference (> 30% LOQ) at retention time of interest.
				6.4	0.10	108 (n=5)	
	HMCPP (confirm.)			7.1	0.01	108 (n=5)	
				3.4	0.10	107 (n=5)	2 mass transitions monitored with highly specific detection system (LC-MS/MS).
	CCPP (quant.)	0.01		11.6	0.01	98 (n=5)	
				2.7	0.10	110 (n=5)	
	CCPP (confirm.)			13.2	0.01	94 (n=5)	
				3.8	0.10	104 (n=5)	
	PCOC (quant.)	0.01		7.5	0.01	86 (n=5)	
				7.6	0.10	93 (n=5)	
	PCOC (confirm.)			19.3	0.01	96 (n=5)	
				7.3	0.10	93 (n=5)	
Liver	Mecoprop-P (quant.)	0.01		2.4	0.01	91 (n=5)	No interference > 30% LOQ at retention time of interest.
				7.0	0.10	90 (n=5)	
	Mecoprop-P (confirm.)			2.2	0.01	88 (n=5)	2 mass transitions monitored with highly specific detection system (LC-MS/MS).
				7.5	0.10	88 (n=5)	
	MCCP-P 2EH (quant.)	0.01		1.9	0.01	103 (n=5)	Not reported. Acceptable, as conjugates are expressed as mecoprop-P acid equivalents.
				3.4	0.10	100 (n=5)	
	MCCP-P 2EH			2.1	0.01	101 (n=5)	

Matrix	Analyte	LOQ mg/kg	Linearity	Precision, %RSD (n)	Fortification levels (mg/kg) and recovery range / mean, %		Interference
	(confirm.)			4.8	0.10	102 (n=5)	
	Glycine conj. (quant.)	0.01		4.0 2.3	0.01 0.10	100 (n=5) 105 (n=5)	
	Glycine conj. (confirm.)			4.4 1.8	0.01 0.10	99 (n=5) 101 (n=5)	
	HMCPP (quant.)	0.01		6.7 9.7	0.01 0.10	104 (n=5) 103 (n=5)	No significant interference (> 30% LOQ) at retention time of interest.
	HMCPP (confirm.)			15.2 9.5	0.01 0.10	113 (n=5) 103 (n=5)	2 mass transitions monitored with highly specific detection system (LC-MS/MS).
	CCPP (quant.)	0.01		8.3 7.9	0.01 0.10	116 (n=5) 108 (n=5)	
	CCPP (confirm.)			7.8 9.5	0.01 0.10	110 (n=5) 102 (n=5)	
	PCOC (quant.)	0.01		14.1 8.9	0.01 0.10	83 (n=5) 80 (n=5)	
	PCOC (confirm.)			13.1 9.1	0.01 0.10	95 (n=5) 83 (n=5)	
Kidney	Mecoprop-P (quant.)	0.01		2.8 5.3	0.01 0.10	117 (n=5) 115 (n=5)	No interference > 30% LOQ at retention time of interest.
	Mecoprop-P (confirm.)			3.3 3.7	0.01 0.10	113 (n=5) 114 (n=5)	2 mass transitions monitored with highly specific detection system (LC-MS/MS).
	MCCP-P 2EH (quant.)	0.01		3.7 2.8	0.01 0.10	118 (n=5) 121 (n=5)	Not reported. Acceptable, as conjugates are expressed as mecoprop-P acid equivalents.
	MCCP-P 2EH (confirm.)			4.2 3.1	0.01 0.10	118 (n=5) 120 (n=5)	
	Glycine conj. (quant.)	0.01		3.6 2.8	0.01 0.10	119 (n=5) 113 (n=5)	
	Glycine conj. (confirm.)			3.0 3.1	0.01 0.10	115 (n=5) 113 (n=5)	
	HMCPP (quant.)	0.01		4.1 2.4	0.01 0.10	118 (n=5) 114 (n=5)	No significant interference (> 30% LOQ) at retention time of interest.
	HMCPP (confirm.)			9.4 3.3	0.01 0.10	111 (n=5) 111 (n=5)	2 mass transitions monitored with highly specific detection system (LC-MS/MS).
	CCPP (quant.)	0.01		2.3 3.3	0.01 0.10	122 (n=5) 115 (n=5)	
	CCPP			3.5	0.01	113 (n=5)	

Matrix	Analyte	LOQ mg/kg	Linearity	Precision, %RSD (n)	Fortification levels (mg/kg) and recovery range / mean, %		Interference
	(confirm.)			3.0	0.10	111 (n=5)	
	PCOC (quant.)	0.01		18.3 4.8	0.01 0.10	71 (n=5) 89 (n=5)	
	PCOC (confirm.)			19.5 5.5	0.01 0.10	70 (n=5) 88 (n=5)	
Fat	Mecoprop-P (quant.)	0.01		6.9 1.4	0.01 0.10	96 (n=5) 96 (n=5)	No interference > 30% LOQ at retention time of interest.
	Mecoprop-P (confirm.)			7.9 3.3	0.01 0.10	98 (n=5) 96 (n=5)	2 mass transitions monitored with highly specific detection system (LC-MS/MS).
	MCCP-P 2EH (quant.)	0.01		6.1 2.3	0.01 0.10	85 (n=5) 79 (n=5)	Not reported. Acceptable, as conjugates are expressed as mecoprop-P acid equivalents.
	MCCP-P 2EH (confirm.)			5.9 2.9	0.01 0.10	85 (n=5) 79 (n=5)	
	Glycine conj. (quant.)	0.01		2.4 1.8	0.01 0.10	92 (n=5) 93 (n=5)	
	Glycine conj. (confirm.)			4.4 2.9	0.01 0.10	92 (n=5) 92 (n=5)	
	HMCPP (quant.)	0.01		9.4 2.5	0.01 0.10	100 (n=5) 103 (n=5)	No significant interference (> 30% LOQ) at retention time of interest.
	HMCPP (confirm.)			6.6 2.2	0.01 0.10	93 (n=5) 104 (n=5)	2 mass transitions monitored with highly specific detection system (LC-MS/MS).
	CCPP (quant.)	0.01		9.4 2.3	0.01 0.10	107 (n=5) 112 (n=5)	
	CCPP (confirm.)			8.0 3.4	0.01 0.10	108 (n=5) 112 (n=5)	
	PCOC (quant.)	0.01		9.3 3.8	0.01 0.10	71 (n=5) 78 (n=5)	
	PCOC (confirm.)			8.9 5.8	0.01 0.10	72 (n=5) 80 (n=5)	

Conclusion

The linear range and specificity of the method for the ester and glycine conjugate has not been reported. This is not of concern as these components do not feature in the residue definition therefore validated methods of analysis are not required.

The precision values across all matrices and for all analytes are < 20% and the mean recoveries generally fall within the acceptable range 70 – 110%. Those that don't are above the upper limit, implying over-estimation of residues, which is less of a concern as this represents a worse case. Acceptable procedural recoveries in all matrices and all analytes were also reported and were comparable to validation recoveries and fell within the range 70 – 120 %. The method for detection of parent mecoprop-P, the ester, conjugate, metabolites (HMCPP and CCPP) and relevant impurity (PCOC) in animal matrices is therefore considered validated in accordance with SANCO/3029/99/rev. 4.

Report:	CA 4.1.2/05 (CA 6.1/03), Anding, C. (2001)
Title	Stability study of Mecoprop-P in soft winter wheat (grain, straw and green plant) after a nineteen months storage in a congelator at a temperature under minus 18°C Report No. AVE/00-033
Guidelines:	Not stated
GLP:	Yes
Deviations	N/A
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012.

Residues were extracted from wheat grain and straw/foliage (25 g or 20 g respectively) with a mixture of methanol/water (80/20 v/v) and then saponified at 60°C. After acidification, the extract was purified by liquid/liquid partition under acidic and basic conditions. Mecoprop-P residues in the organic extracts were esterified with pentaflourobenzyl bromide and then purified on a Florisil cartridge. Residues were dissolved in hexane before being quantified as pentafluorobenzyl ester by GC using an electron capture (EC) detector. Quantification was carried out by external standardisation.

Three different analysis conditions were used, depending on the date of analysis. All three sets of analysis conditions are described below. System #1 was used for analysis at time 0; system #2 was used for analysis at 2 and 4 months and system #3 was used for analysis at 6 months and subsequent analyses.

GC-EC system: #1 #2 #3

Instrument:	Varian 3400	Varian 3400	Varian 3400
Column:	Restek column 0.5 µm (15 m x 0.53 mm)	Supelco column 0.5 µm (15 m x 0.53 mm)	Supelco column 0.5 µm (15 m x 0.53 mm)
Stationary phase:	RTX-35	SPB-35	RTX-35
Carrier gas:	Helium	Helium	Helium
Flow Rate:	6.0 mL/min	8.0 mL/min	13.0 mL/min
Make up gas:	Nitrogen	Nitrogen	Nitrogen
Flow Rate:	22.0 mL/min	22.0 mL/min	20.0 mL/min
Volume injected:	1.0 µL	1.0 µL	2.0 µL
Injector temperature:	250°C (initial). Hold 0.1 min. 100°C/min ramp 300°C (final) Hold 1.0 min.	260°C (initial). Hold 0.1 min. 100°C/min ramp 290°C (final) Hold 1.0 min.	190°C (initial). Hold 0.2 min. 100°C/min ramp 300°C (final) Hold 1.0 min.
Column temperature:	105°C (initial). Hold 3.0 min. 4.0°C/min ramp 200°C (intermediate). Hold 1.0 min.	110°C (initial). Hold 6.0 min. 3.0°C/min ramp 185°C (intermediate) 50°C/min ramp	110°C (initial). Hold 8.0 min. 2.0°C/min ramp 170°C (intermediate)

Instrument:	Varian 3400	Varian 3400	Varian 3400
	50°C/min ramp 300°C (final) Hold 7.0 min.	280°C (final) Hold 6.0 min.	30°C/min ramp 300°C (final) Hold 5.0 min.
Detector:	ECD @ 320°C	ECD @ 320°C	ECD @ 320°C
Retention time:	24.30 – 24.40 min	27.25 – 27.35 min	30.10 – 30.30 min

Validation of the method is reported in the following table. Linearity, recovery data and chromatograms are reported for all 3 systems.

Table Error! No text of specified style in document.-6 Validation for method used in storage stability study in wheat

Matrix	LOQ	Linearity	Precision*, %RSD (n)	Fortification levels (mg/kg) and recovery range (mean), %		Interference
Wheat grain	0.2 mg/kg	0.0025 – 0.2 mg/L n = 4 x 2 #1 r = 0.9994 #2 r = 0.9975 #3 r = 0.9921	15.4	0.2 (#1) 0.2 (#2) 0.2 (#3)	70-79 (75, n = 2) 70 – 80 (75, n = 2) 71 – 103 (86, n = 4) Prior to fortification = 0.02 mg/kg	No significant interference >30% LOQ at retention time of interest observed in chromatograms at all time points.
Wheat straw	0.5 mg/kg	0.0025 – 0.2 mg/L n = 4 x 2 #1 r = 0.9994 #2 r = 0.9975 #3 r = 0.9921	10.5	0.5 (#1) 0.5 (#2) 0.5 (#3)	89 – 90 (90, n = 2) 81 – 109 (95, n = 2) 69 – 88 (78, n = 4) Prior to fortification = 0.05 mg/kg	No significant interference >30% LOQ at retention time of interest observed in chromatograms at all time points.
Wheat foliage	0.5 mg/kg	0.0025 – 0.2 mg/L n = 4 x 2 #1 r = 0.9994 #2 r = 0.9975 #3 r = 0.9921	14.3	0.5 (#1) 0.5 (#2) 0.5 (#3)	92 – 103 (98, n = 2) 99 – 103 (101, n = 2) 74 – 102 (86, n= 4) Prior to fortification = 0.05 mg/kg	No significant interference >30% LOQ at retention time of interest observed in chromatograms at all time points.

* Calculated from accuracy data at #3 system.

Conclusion

The method for determining mecoprop-P residues in wheat grain, straw and foliage is not strictly validated in accordance with SANCO/3029/99/rev.4. Insufficient determinations were conducted at each fortification level for each GC-EC method. Additionally, the level of fortification is not representative of the levels expected in the residue trials. As this storage stability study was not relied upon in the residues evaluation (Volume 3, CA B7) then the method validation is of no consequence.

Report:	CA 4.1.2/06 (CA 6.3.1/02), Perny, A. (2002)
Title	Residue decline of Mecoprop-P potassium salt in cereals in Southern Europe Report No. R A0119 / AHM R 00126
Guidelines:	Not stated
GLP:	Yes
Deviations	None
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012.

The residue trials using the method described in this study were conducted in the following study: Old, J.; Duncan, P. (2001), study no. AHM R 00 115, including the amendment Doig, A. (2011).

Cereal samples were analysed for mecoprop-P using a GC-MSD method ATM 592 (note the French translation of this method is referenced as MP 273 for extraction and MA 347 for analysis).

Frozen cereal specimens were cut into small pieces and mixed. Sub-specimens were then taken (foliage and grain 20g, straw, 10g) for analysis from the homogeneous mix. Residues were extracted from the cereal samples with alkaline methanol, followed by clean-up of the crude extract by a liquid/liquid partition and further clean-up on a solid phase extraction cartridge. The eluent from the column was methylated with methanol / sulphuric acid. The methylated sample was extracted with hexane. The methylated solutions were analysed by GC-MS. The following conditions were noted:

GC-MS
Column:	J&W Scientific column 0.25 µm (30 m x 0.25 mm)
Carrier gas:	Helium
Flow Rate:	0.6 mL/min
Volume injected:	1.0 µL
Injector temperature:	220°C
Column temperature:	60°C (initial). Hold 1.0 min.
	10°C/min ramp
	260°C (final) Hold 10.0 min.
Detector temperature:	280°C
Retention time:	13.6 min
Quantification ion:	169

The validation data are summarised in Table 5.1-7. Only one calibration curve was provided for all matrices and only procedural recoveries were conducted.

Table Error! No text of specified style in document.-7

Matrix	LOQ	Linearity	Precision*, %RSD (n)	Fortification levels (mg/kg) and mean recovery, %		Interference
Wheat grain	0.05 mg/kg	31.3 – 803.2 ng/mL n = 6 r² = 0.997		0.05 0.50	96.9, n = 1 81.8, n = 1	No significant interference >30% LOQ at retention time of interest observed in chromatograms of control samples, treated samples, spiked samples and analytical standards.
Wheat straw	0.05 mg/kg	31.3 – 803.2 ng/mL n = 6 r² = 0.997		0.05 0.50	83.6, n = 1 109.9, n = 1	No significant interference >30% LOQ at retention time of interest observed in chromatograms of control samples, treated samples, spiked samples and analytical standards.
Green plant	0.05 mg/kg	31.3 – 803.2 ng/mL n = 6		0.05 0.50	89.2, n = 1 93.7, n = 1	No significant interference >30% LOQ at retention time of interest

Matrix	LOQ	Linearity	Precision*, %RSD (n)	Fortification levels (mg/kg) and mean recovery, %		Interference
		$r^2 = 0.997$		5.5	99.5, n = 1	observed in chromatograms of control samples, treated samples, spiked samples and analytical standards.
				55	94.7, n = 1	

*Precision cannot be calculated from recoveries with single determinations at each fortification level, but the precision of all the recovery determinations were reported to be 10% (n = 8).

Conclusion

Only one determination was conducted at each fortification level and no precision data was reported. Additionally it is not clear that the linear range encompasses the sample concentration as details of final sample preparation are not provided. However, as the methods in both studies (Perny 2002 and Gallais 2002a) are fairly comparable the procedural recoveries can be combined, giving confidence that the mean recoveries are within the acceptable SANCO range of 70 – 110% and the precision of recovery is <20%. The combined validation data are displayed below in Table 5.1-8. The method can be considered fit for purpose, despite not being strictly in accordance with SANCO/3029/99/rev.4.

Table Error! No text of specified style in document.-8 Combined recovery and precision data for GC-MSD method ATM 592

Matrix	Fortification level (mg/kg)	Recovery range (mean)	%RSD
Wheat grain	0.05	96.9 – 105.5 (89.4, n=2)	11.9
	0.5	81.8 – 98.4 (90.1, n=2)	13.0
Wheat straw	0.05	73.7 – 83.6 (78.7, n=2)	8.9
	0.5	108.4 – 109.9 (109.2, n=2)	1.0
Green plant	0.05	81.7 – 89.2 (85.5, n=2)	6.2
	0.5	93.7 – 99 (96.4, n=2)	3.9

Report:	CA 4.1.2/07 (CA 6.3.1/04), Gallais, C. (2002a)
Title	Residue decline of Mecoprop-P potassium salt in cereals in Southern Europe Report No. R A1135
Guidelines:	91/414/EEC
GLP:	Yes
Deviations	None
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012.

The residue trials using the method described in this study were conducted in the following study: Wardman, JP. (2002a), study no. AHM R 01 115.

Cereal samples were analysed for mecoprop-P using the GC-MSD method ATM 592 (note the French translation of this method is referenced as MP 273 for extraction and MA 347 for analysis). Note the slightly different GC column and chromatography conditions used compared to in the previous study:

Column: Macherey Nagel column 0.5 µm (30 m x 0.25 mm)
Carrier gas: Helium
Flow Rate: 0.6 mL/min
Volume injected: 2 µL
Injector temperature: 220ºC
Column temperature: 60ºC (initial). Hold 1.0 min.

	10ºC/min ramp
	260ºC (final) Hold 10.0 min.
Detector temperature:	280ºC
Retention time:	16.9 min
Quantification ion:	169

The validation data are summarised in Table 5.1-9. Only one calibration curve was provided for all matrices and only procedural recoveries were conducted.

Table Error! No text of specified style in document.-**9**

Matrix	LOQ	Linearity	Precision*, %RSD (n)	Fortification levels (mg/kg) and mean recovery, %		Interference
Wheat grain	0.05 mg/kg	31.3 – 803.2 ng/mL n = 6 r² = 0.998		0.052 0.52	105.5, n = 1 98.4, n = 1	No significant interference >30% LOQ at retention time of interest observed in chromatograms of control samples, treated samples, spiked samples and analytical standards.
Wheat straw	0.05 mg/kg	31.3 – 803.2 ng/mL n = 6 r² = 0.998		0.052 0.52	73.7, n = 1 108.4, n = 1	No significant interference >30% LOQ at retention time of interest observed in chromatograms of control samples, treated samples, spiked samples and analytical standards.
Green plant	0.05 mg/kg	31.3 – 803.2 ng/mL n = 6 r² = 0.998		0.052 0.52 52	81.7, n = 1 99, n = 1 70.6, n = 1	No significant interference >30% LOQ at retention time of interest observed in chromatograms of control samples, treated samples, spiked samples and analytical standards.

*Cannot be calculated from recoveries with single determinations at each fortification level, but the precision of all the recovery determinations were reported to be 17% (n = 7).

Conclusion
Only one determination was conducted at each fortification level and no precision data was reported. Additionally it is not clear that the linear range encompasses the sample concentration as details of final sample preparation are not provided. However, as the methods in both studies (Perny 2002 and Gallais 2002a) are fairly comparable the procedural recoveries can be combined, giving confidence that the mean recoveries are within the acceptable SANCO range of 70 – 110% and the precision of recovery is <20%. The combined validation data are displayed in Table 5.1-8. The method can be considered fit for purpose, despite not being strictly in accordance with SANCO/3029/99/rev.4.

Report:	CA 4.1.2/08 (CA 6.3.1/05), Tandy, R. (2014a)
Title	Determination of residues of Mecoprop-P after a single application of Mecoprop-P K 600 in cereals at 4 sites in Northern Europe 2013 Report No. S13-00323
Guidelines:	EU 1999: 1607/VI/97 SANCO/3029/99 rev. 4 Guideline 7029/VI/95 (rev. 5) to Directive 91/414/EEC and Regulations (EU) 544/2011 and 545/2011 implementing Regulation (EC) 1107/2009
GLP:	Yes
Deviations	None
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012.

The method used to determine mecoprop-P residues in wheat in this study is the enforcement method, CAM-0004/001, described in section B.5.2. See section B.5.2 for method description and analysis conditions, but additional validation data including procedural recoveries are summarised below.

Some changes were made to the method for the foliage samples only. The procedures for grain and straw were not changed. 10 mL of sodium hydroxide solution was used for the hydrolysis step, instead of 20 mL. Therefore 2.5 mL of chilled 15N sulphuric acid was used to lower the pH to ~3, instead of 5 mL. Similarly only 2 mL of 1M monochloroacetic acid was used instead of 4mL. Following centrifugation a 2 mL sample of the middle solvent layer was taken, instead of 1 mL. After evaporation to dryness reconstitution was in 0.5 mL of acetonitrile and the volume was adjusted to 2.0 mL using 0.2 % formic acid in water. All other volumes remained the same.

Matrix	LOQ	Linearity	Precision*, %RSD (n)	Fortification levels (mg/kg) and mean recovery, %		Interference
Wheat grain	0.01 mg/kg	0.6 – 50 ng/L n = 7 r^2 = 0.9998		0.01 0.10 1.00	90, n = 1 86, n = 1 113, n = 1	No significant interference >30% LOQ at retention time of interest observed in chromatograms at all time points.
Wheat straw	0.01 mg/kg	0.6 – 50 ng/L n = 7 r^2 = 0.9982		0.01 0.10 1.00	93, n = 1 94, n = 1 98, n = 2	No significant interference >30% LOQ at retention time of interest observed in chromatograms at all time points.
Whole plant	0.01 mg/kg	0.6 – 200 ng/L n = 9 r^2 = 0.9984		0.01 0.5 100	96, n = 1 95, n = 2 95, n = 1	No significant interference >30% LOQ at retention time of interest observed in chromatograms at all time points.

*Cannot be calculated from recoveries with single determinations at each fortification level.

Conclusion

The method validation supplied is not strictly validated in accordance with SANCO/3029/99/rev.4 based on the validation data provided above, due to only single determinations being reported at each fortification level and no precision data. However, the mean recoveries reported are within the acceptable range 70 – 110%. Furthermore, the method CAM-0004/001 is also the enforcement method

and has been successfully validated on wheat grain, straw and foliage in accordance with SANCO/825/00 rev. 8.1 (Section B.5.2). The method can therefore be considered validated in accordance with SANCO/3029/99/rev.4 based on the validation data reported under Section B.5.2.

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

The following methods were used in the studies summarised in Section 8 (Ecotoxicological studies on the active substance).

Table Error! No text of specified style in document.-10 **Summary of ecotoxicology methods submitted for purposes of renewal**

Matrix	Analyte	Method	Reference
Avian Diet	Mecoprop-P	HPLC	▓▓▓ 1996. Study No. ▓▓▓▓▓
Algal growth (OECD medium)	Mecoprop-P	LC-MS/MS	Jenkins, 2007. Study No. ZZF0001/063120
Algal growth [f/2 medium : water (10:90 (v/v))]	Mecoprop-P	LC-MS/MS	Burke, 2007. Study No. ZZF0002/063525
Nectar, pollen and bee larvae	2,4-D	LC-MS/MS	Mack, 2012. Study No. S11-02084
Sugar solution (96% aq sugar soln., 4% acetone)	Mecoprop-P	HPLC-UV	Kleebaum, 2015. Study No. 141048023B
Aqueous media (fish eggs)	Mecoprop-P	HPLC-UV	▓▓▓ 2015. Study No. ▓▓▓

Report:	CA 4.1.2/15, ▓▓▓▓ (1996)
Title	MCPP-P-DMA Salt – Avian Dietary LC$_{50}$ test in chicks of the mallard duck (*Anas platyrhynchos* L.) Report No. ▓▓▓▓▓
Guidelines:	US EPA, Subdivision E, Series 71, §71-2
GLP:	Yes
Deviations	None
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012.

The mecoprop-P dimethylamine salt (MCPP-P-DMA) content in bird feed was determined by HPLC with UV detection. Feed samples were extracted with acetonitrile/0.5 M sulfuric acid and the extracts analysed by HPLC. The following chromatography conditions were noted:

Column: Polygosil C18 column 5μm (25 cm x 4.0 mm)
Flow Rate: 1.2 mL/min
Volume injected: 10 μL
Detection: uv 230 nm
Mobile phase A: 50% acetonitrile + 0.5M sulphuric acid (1000:5)
Mobile phase B: 50% aqua bidest+ 0.5M sulphuric acid (1000:5)

The validation data supplied are reported in the following table:

Table Error! No text of specified style in document.-**11 Validation for method to determine MCPP-P-DMA in bird feed.**

Matrix	LOQ	Linearity	Precision, %RSD (n)	Fortification levels (ppm) and recovery range (mean), %		Interference
Bird feed	97 ppm [0.0097 % w/w]	No data supplied.	1.2 (3) Calculated from recovery data.	97	90.4 - 92.4 (91.2, n=3) within SANCO acceptable range 80 – 100 %	No data supplied.

Conclusion

The method to determine MCPP-P-DMA in bird feed is not validated in accordance with SANCO/3029/99/rev.4. There were no data supplied to address the linearity or specificity of the method. Additionally, the recovery and precision data were insufficient: two fortification levels are required and a minimum of 5 determinations at each level for precision. The acceptability of the method will be determined in the relevant section of the assessment report.

Report:	CA 4.1.2/18, Jenkins, C.A. (2007)
Title	Mecoprop-P (DMA salt) algal growth inhibition assay *Navicula* Report No. ZZF0001/063120
Guidelines:	EEC C3 OECD 201
GLP:	Yes
Deviations	None
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012.

The mecoprop-P dimethylamine salt (MCPP-P-DMA) content in algal cultures was determined by an LC-MS/MS method. Samples were extracted and diluted appropriately with OECD medium (aqueous solution containing nutrients) and injected into the LC-MS/MS system. The following chromatography conditions were noted:

Instrument: Quattro LC
Column: Luna C8 column (15 cm x 2.0 mm)
Mode: Electrospray negative (ESP-)
Flow Rate: 0.2 mL/min
Volume injected: 20 μL
Retention time: *ca.* 5 min
Quantification ion: MRM m/z 213>141
Mobile phase A: Acetonitrile : water (20:80 (v/v)) containing 0.01M ammonium acetate and 0.1% acetic acid
Mobile phase B: Acetonitrile containing 0.1% acetic acid

Gradient	Time (min)	%A	%B
	0	100	0
	6	0	100
	10	0	100
	11	100	0
	15	100	0

Run time: 15 minutes

The validation data supplied are reported in the following table:

Table Error! No text of specified style in document.-**12 Validation for method to determine MCPP-P-DMA in OECD medium**

Matrix	LOQ	Linearity	Precision*, %RSD (n)	Fortification levels* (mg/L) and recovery range (mean), %		Interference
OECD medium	0.05 mg/L [0.000005 % w/w]	1 – 50 ng/mL [0.001 – 0.05 mg/L] n = 9 r = 0.999	1.4 (5) 4.2 (5) %RSD < 20 at both fortification levels	0.05 (0.000005 % w/w) 200 (0.02 % w/w)	94 - 97 (95, n=5) 94 - 103 (99, n = 5) within SANCO acceptable range 80 – 100%	Chromatograms of untreated, spiked, treated and analytical standard samples showed no interference at retention time of interest.

Conclusion

The method is validated in accordance with SANCO/3029/99/rev. 4. The acceptability of the method will be determined in the relevant section of the assessment report.

Report:	CA 4.1.2/19, Burke, J. (2007)
Title	Mecoprop-P (DMA salt) algal growth inhibition assay *Skeletonema* Report No. ZZF0002/063525
Guidelines:	EEC C3 OECD 201
GLP:	Yes
Deviations	None relating to method validation
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012.

The mecoprop-P dimethylamine salt (MCPP-P-DMA) content in algal cultures was determined by an LC-MS/MS method. Samples were extracted and diluted appropriately with F/2 medium (aqueous solution containing nutrients):water (10:90 v:v) and injected into the LC-MS/MS system. The following chromatography conditions were noted:

Instrument: Quattro LC
Column: Luna C8 column (15 cm x 2.0 mm)
Mode: Electrospray negative (ESP-)
Flow Rate: 0.2 mL/min
Volume injected: 20 µL
Retention time: *ca.* 6 min
Quantification ion: MRM m/z 213>141
Mobile phase A: Acetonitrile : water (20:80 (v/v)) containing 0.01M ammonium acetate and 0.1% acetic acid
Mobile phase B: Acetonitrile containing 0.1% acetic acid

Gradient	Time (min)	%A	%B
	0	100	0
	6	0	100
	10	0	100
	11	100	0
	15	100	0

Run time: 15 minutes

The validation data supplied are reported in the following table:

Table Error! No text of specified style in document.**-13 Validation for method to determine MCPP-P-DMA in F/2 medium**

Matrix	LOQ	Linearity*	Precision, %RSD (n)	Fortification levels (mg/L) and recovery range (mean), %		Interference
F/2 medium: water (10:90 v:v)	0.05 mg/L [0.000005 % w/w]	1 – 50 ng/mL [0.001 – 0.05 mg/L] n = 9 r = 0.999	2.8 (5) 1.8 (5) %RSD < 20 at both fortification levels	0.05 (0.000005 % w/w) 200 (0.02 % w/w)	98 - 105 (101, n=5) 97 - 101 (98, n = 5) within SANCO acceptable range 80 – 100%	Chromatograms of untreated, spiked, treated and analytical standard samples showed no interference at retention time of interest.

*Samples are diluted to appropriately fall within linear range.

Conclusion

The method is validated in accordance with SANCO/3029/99/rev. 4. The acceptability of the method will be determined in the relevant section of the assessment report.

Report:	CA 4.1.2/18, Mack, P. (2012)
Title	LAF-74: A semi-field study to investigate residues in honeybee products and honeybee larvae (*Apis mellifera carnica* L.; Hymenoptera, Apidae) in *Phacelia tanacetifolia* in Germany in 2011. Report No. S11-02084
Guidelines:	IVA (Beutel *et al.*, 1992) OEPP/EPPO (2010)
GLP:	Yes
Deviations	None
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012.

The 2,4-dichlorophenoxyacetic acid (2,4-D) content in nectar, pollen and larvae was determined by an HPLC-MS/MS method. Samples (0.2 g) were extracted at high speed with alkaline acetonitrile solution (5.0 mL of 0.5M ammonia solution in MeCN) before clean-up by mixed-mode anion exchange SPE (Waters Oasis MAX, 60mg/3mL). The samples were then reconstituted in acetonitrile: water (1:1 v:v) before analysis by HPLC-MS/MS. The following chromatography conditions were noted:

HPLC
Instrument:	Thermo Surveyor MS pump Plus with autosampler				
Column:	Aglient ZORBAX Eclipse Plus C18 column 1.8 μm (5 cm x 2.1 mm)				
Guard column:	C18 column (4 mm x 2.0 mm)				
Column temperature:	40ºC				
Volume injected:	30 μL				
Retention time:	5.8 min				
Mobile phase A:	Water				
Mobile phase B:	Methanol				
Mobile phase C:	1% formic acid in water				
Gradient	Time (min)	%A	%B	%C	Gradient
	0	78	20	2	-
	1	78	20	2	-
	4	8	90	2	Linear

6	8	90	2	-
6.01	78	20	2	-
8	78	20	2	-

<u>MS</u>
Detector:	ThermoFinnigan TSQ Quantum triple quadrupole system
Ionization mode:	ESI
Source polarity:	Negative
Spray voltage:	4,500 V
Capillary temperature:	280ºC
Capillary offset:	-35 V
Gas:	Argon
2,4-D Quantification ion:	163.1
2,4-D Confirmation ion:	160.9

The validation data supplied are reported in the following table. No residues > 30% LOQ were detected in any of the blank samples. No significant matrix effects were observed, therefore for nectar and larvae calibration was performed with standards in acetonitrile/water (1:1 v:v). For pollen calibration was performed with standards in blank pollen extracts.

Table Error! No text of specified style in document.-14 Validation for method to determine 2,4-D content in nectar, pollen and larvae

Matrix	LOQ	Linearity	Precision*, %RSD (n)	Fortification levels (mg/kg) and recovery range (mean), %		Interference
Nectar	0.01 mg/kg [0.001 % w/w]	0.2 - 100 ng/mL [0.00000002 – 0.00001 % w/w] n = 9 r > 0.99	6 (5) 3 (5) %RSD < 20 at both fortification levels	0.01 (0.001 % w/w) 10 (1.0 % w/w)	71 - 80 (73, n=5) 82 - 90 (86, n = 5) within SANCO acceptable range 70 – 110%	Chromatograms of untreated, spiked, treated and analytical standard samples showed no interference >30% LOQ at retention time of interest.
Pollen	0.01 mg/kg [0.001 % w/w]	0.2 - 100 ng/mL [0.00000002 – 0.00001 % w/w] n = 9 r > 0.99	7 (5) 4 (3) 5 (5) 2 (5) %RSD < 20 at all fortification levels	0.01 (0.001 % w/w) 10 (1.0 % w/w) 80 (8.0 % w/w) 90 (9.0 % w/w)	71 - 80 (75, n=5) 87 - 95 (98, n = 3) 76 – 86 (82, n = 5) 80 – 85 (83, n = 5) within SANCO acceptable range 70 – 110%	Chromatograms of untreated, spiked, treated and analytical standard samples showed no interference >30% LOQ at retention time of interest.
Larvae	0.01 mg/kg [0.001 % w/w]	0.2 - 100 ng/mL [0.00000002 – 0.00001 % w/w]	11 (6) 3 (5)	0.01 (0.001 % w/w) 0.2 (0.02 % w/w) 6.0 (0.6 % w/w)	70 - 90 (78, n=6) 79 (79, n = 2) 91 - 97 (94, n=5)	Chromatograms of untreated, spiked, treated and analytical standard samples showed no interference

Matrix	LOQ	Linearity	Precision*, %RSD (n)	Fortification levels (mg/kg) and recovery range (mean), %		Interference
		n = 9 r > 0.99	%RSD < 20 at both fortification levels that precision could be reported for.	w/w) 10 (1.0 % w/w)	88 (n=1) within SANCO acceptable range 70 – 110%	>30% LOQ at retention time of interest.

* Precision of recovery is reported. This is acceptable.

Conclusion

The method for determining 2,4-D in nectar, pollen and larvae is not strictly validated according to SANCO/3029/99/rev.4. Five determinations at each fortification level are required in pollen and larvae for precision. However, acceptable precision is demonstrated at all the remaining fortification levels therefore the method is likely to be fit for purpose. The acceptability of the method will be determined in the relevant section of the assessment report.

Report:	**CA 4.1.2/21 (CA 8.3.1.3-01) Kleebaum, K (2014)**
Title	Acute toxicity of Mecoprop-P technical acid to honeybee larvae Apis mellifera L. under laboratory conditions (in vitro) BioChem agrar Report No. 14 10 48 023 B Date: 08 December 2014
Guidelines:	OECD 237 Guidelines for testing chemical "Honey bee (Apis mellifera) larval toxicity test, single exposure" (2013) Analytical phase validated in accordance with SANCO/3029/99
GLP:	Yes
Deviations	None
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012.

The mecoprop-P content of sugar solutions used in the study was determined by HPLC-UV analysis. Samples of mecoprop-P in sugar solution were diluted by a factor of 500 in methanol/water solution and the mecoprop content quantified against a mecoprop-P calibration using HPLC fitted with a UV diode array detector. The following chromatography conditions were noted:

HPLC
Column: Phenomenex Kinetex C18 column 2.6µm (100mm x 2 mm)
Flow Rate: 0.4 mL/min
Detection: UV 227 nm
Mobile phase A: Water with 0.1% v/v phosphoric acid (85%)
Mobile phase B: Acetonitrile with 0.1% v/v phosphoric acid (85%)
Gradient Program:

Time (min)	% A	% B
0	50	50
6.0	10	90
8.0	10	90
8.01	50	50
10.0	Stop	

The validation data supplied are reported in Table 5.1-15.

Table Error! No text of specified style in document.-15 Validation for method to determine MCPP-P in sugar solution

Matrix	LOQ	Linearity	Precision, %RSD (n)	Fortification levels (mg/L) and recovery range (mean), %		Interference
Sugar solution (96% aq sugar soln., 4% acetone)	3512 mg/L [0.35 % w/w]	5.3 – 16.06 mg/L [80 – 120% of validation concentrations] n = 5 r = 0.999	3.1 (5) 1.2 (5) %RSD < 20 at both fortification levels	3512 mg/L (0.35 % w/w) 6676 mg/L (0.67 % w/w)	98 – 106 (101, n=5) 105 - 108 (106, n = 5) mean recoveries within SANCO acceptable range 70 – 110%	Chromatograms of analytical standard, test items and blank samples showed no interference at retention time of interest (MCPP-P *ca.* 6 min)

Conclusion
The method for determining mecoprop-P in sugar solution is validated in accordance with SANCO/3029/99/rev.4. The acceptability of the method will be determined in the relevant section of the assessment report.

Report:	CA 4.1.2/22 (CA 8.2.2.1-01) ▓▓▓▓▓ (2015)	
Title	Mecoprop-P: Toxic Effects to Rainbow Trout (Oncorhynchus mykiss) in an Early-life Stage Toxicity Test. ▓▓▓▓▓▓▓▓▓ Report No. ▓▓▓ Date: 16th June 2015
Guidelines:	OECD Guidelines for testing of chemicals, No 210, "Fish, Early-life Stage Toxicity Test" (2013)
GLP:	The study was performed in accordance with GLP; however the analytical phase was not performed to GLP.
Deviations	None
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012.

The mecoprop-P content of aqueous solutions used in the study was determined by HPLC-UV analysis. Samples were thawed and mixed to form a homogeneous solution. Aliquots were first diluted with N,N-dimethylformamide and subsequently diluted to within the calibration range with 0.1% formic acid. The following chromatography conditions were noted:

HPLC
Column: Luna C8(2): 150 mm x 4.6 mm, 3□m
Flow Rate: 0.8 mL/min
Detection: UV 230 nm
Mobile phase A: Methanol/water (v/v, 1/9) containing 10mM ammonium nitrate
Mobile phase B: Methanol/water (v/v, 9/1) containing 10mM ammonium nitrate
Gradient Program: Time (min)   % A   % B
                            0   60   40
                            7   20   80

10	20	80
10.1	60	40
16	60	40

The validation data supplied are reported in Table 5.1.2-16.

Table Error! No text of specified style in document.-16 Validation for method to determine MCPP-P aqueous medium

Matrix	LOQ	Linearity*	Precision, %RSD (n)	Fortification levels (mg/L) and recovery range (mean), %		Interference
Aqueous medium	0.124 mg/L [0.000024 % w/w]	0.0523 – 7.03 mg/L n = 8 r = 0.999	3 (5) 0.3 (5) %RSD < 20 at both 0.124 and 12.4 mg/L fortification levels	0.124 mg/L (0.000024 % w/w) 12.4 mg/L (0.00124 % w/w) 195280 mg/L (19.5 % w/w)	87 - 94 (92, n=5) 97 - 98 (98, n = 5) 104 – 117 (110, n=2) mean recoveries within SANCO acceptable range 70 – 110%	Chromatograms of analytical standard, test items and blank samples showed no interference at retention time of interest (MCPP-P *ca.* 7.8 min)

*Samples were diluted to fall within this linear range.

Conclusion

The method for determining mecoprop-P in aqueous media is validated in accordance with SANCO/3029/99/rev.4. The acceptability of the method will be determined in the relevant section of the assessment report.

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

The following method was used to determine the solubility of pure grade mecoprop-P in water and buffer solutions. The results of the test are reported in CA Volume 3, Section B-2.

Report:	CA 4.1.2/23, Comb, A.L. (2000)
Title	Mecoprop-P (pure grade) physic-chemical properties amended final report Report No. NUF004/993523
Guidelines:	EEC A6 OECD 105 OPPTS 830.7840
GLP:	Yes
Deviations	None
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012.

Samples of mecoprop-P (*ca.* 200 mg) were dissolved in buffered aqueous solutions (30 ml). The flasks were purged with nitrogen and sealed. Before analysis, the samples were filtered and a subsample (1 ml) of each filtrate was diluted to volume (50 ml for pH 4 buffer) with mobile phase. The final sample

solution was 120 mg/L. Analysis by HPLC with UV detection was conducted. The following chromatography conditions were noted:

Instrument:	Hewlett Packard 1050 Liquid Chromatograph
Column:	Spherisorb S5ODS2 (25 cm x 4 mm)
Column temperature:	30ºC
Flow Rate:	2.2 mL/min
Volume injected:	20 µL
Mobile phase:	Water : acetonitrile : glacial acetic acid (700:300:1 v/v/v)
Detector:	UV set at 220 nm
Retention time:	*ca.* 16 min

The method validation is summarised below:

Table Error! No text of specified style in document.-17 **Validation for method to determine mecoprop-P content in aqueous buffer solutions**

Matrix	Linearity	Precision, %RSD (n)	Fortification levels and recovery, %	Interference
Mecoprop-P content in aq. buffer solutions	29.98 – 149.9 mg/L [equivalent to *ca.* 25 – 120 % of nominal content] n = 5 x 2 r = 0.9999	0.34 (10) @ 98.2% w/w Acceptable Horwitz RSD = 1.34	No recovery was reported.[1]	No peaks were observed in the chromatograms of the blank solutions indicating no interference.

[1]Accuracy is not considered to be required as the pure active substance was dissolved in solvent and there are no other components present and specificity has been demonstrated. Additionally, the same method of analysis has been used in a 5-batch analysis of technical material, which demonstrates consistent levels of mecoprop-P in the technical material (96.3 – 98.9% w/w). This is considered sufficient evidence that the method is able to detect mecoprop-P in aqueous solutions.

Conclusion
The method is not validated according to SANCO/3029/99/rev.4 due to missing recovery data. However the method is considered fit for purpose.

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

The proposed residue definition for enforcement in products of plant and animal origin is mecoprop-P. A single multi-residue method has been developed for all matrices, which replaces all the previously evaluated enforcement methods.

Note the following method revisions:
CAM-0004/001 ; Method for determination of mecoprop-P and the corresponding 2-ethyl hexyl ester and glycine conjugate in cereals.
CAM-0004/002 ; Addition of animal matrices, citrus fruit and olives.
CAM-0004/003 ; Addition of surface water, soil and air.

Report:	CA 4.2/01, Allen, L. (2014a)
Title	Analytical method for the determination of phenoxy acids and their corresponding 2-ethyl hexyl esters and glycine conjugates in cereal grain, straw and foliage, bovine muscle, fat, liver

	and milk, poultry eggs, citrus fruit and olives and phenoxy acids and their corresponding 2-ethyl hexyl esters in surface water, soil and air Method No: CAM-0004/003 (stage 3-final)
Guidelines:	OECD Guideline ENV/JM/MONO(2007) 17 OPPTS 860.1340 (1996) OCSPP 850.6100 (2012) EC document SANCO/825/00 rev.8.1 EC document SANCO/3029/99 rev.4
GLP:	No GLP claim is made for the method. The method has been validated under the CEMAS GLP studies CEMS-6228, CEMS-6229 and CEMS-6230.
Deviations	N/A
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012. Replaces all previous methods.

a) Method Description

The method CAM-0004/003 is used for the determination of the total phenoxy acid present in cereal matrices (grain, straw and foliage), animal tissues, citrus fruit and olives whether present as the acid, ester (e.g. ethylhexyl) or conjugate (e.g. glycine) and the total phenoxy acid present in surface water, soil and air, whether in the form of acid or ester (e.g. ethylhexyl). During the extraction procedure, samples are hydrolysed to convert the esters and conjugates back to the parent acid for quantification. The analysis is performed using a hydrolysis reaction, QuEChERS extraction and determination by LC-MS/MS detection.

Samples (2 g of cereal matrix, 5 g of other matrices, and 100 mL of water) are hydrolysed overnight in a strong aqueous solution of sodium hydroxide to convert the ethyl-hexyl esters and glycine conjugates back to the parent acid for quantification. The hydrolysed samples are acidified and, with the exception of the water extraction procedure where QuEChERS is not required, analytes extracted into acetonitrile using QuEChERS before being concentrated for analysis. For cereal matrices, animal, acidic and oily matrices and soil the final sample concentrations were 0.2 g/mL. For water the final sample concentration was 0.2 L/mL.

The reverse phase LC-MS/MS setup with two characteristic isotopic mass transitions is considered highly specific therefore no further confirmatory conditions are required.

The following chromatography conditions were noted:

Instrumentation:	Symbiosis Pharma Liquid Chromatography System AB Sciex 4000 triple quad MS System AB Sciex Analyst 1.4.2 data system
Column:	Onyx C18 monolithic column, 3.0 x 100 mm
Guard Column: (if required)	Chromolith RP-18 end capped guard cartridge 5 x 3 mm
Column Temperature:	Ambient
Injection Volume:	40 μL
Run Time:	Approx. 9 minutes
Mobile Phase:	A: HPLC water + 0.1 % formic acid B: Methanol + 0.1 % formic acid
Flow:	1 mL/min, split 1:4 to the mass spectrometer

Gradient:	Time	A %	B %
	(min:secs)		
	0:01	55	45
	0:03	55	45
	6:00	25	75
	6:01	5	95
	7:15	5	95
	7:16	55	45
	9:00	55	45
Mecoprop-P retention time:	4.55 min		

Typical Mass Spectrometry Operating Conditions

Interface:	ESI
Source polarity:	negative
Scan type:	MRM
Resolution:	Q1- unit, Q3 - unit
Spray voltage:	-4500 V
Capillary temperature:	300 °C
GS1:	30
GS2:	40
Curtain gas:	25
Collision gas:	10
Dwell time:	75 msec
Entrance potential:	-10
Mecoprop-P quantification:	212.9 →140.9.
Mecoprop-P confirmation:	215.0 →142.9

b) Method validation
The method was validated in the following studies on wheat grain and straw (dry commodity, CEMS-6228), wheat foliage (high water commodity, CEMS-6228), olives (high oil content, CEMS-6229) and citrus (high acid and CEMS-6230).

Cereal matrices and ILV

Report:	**CA 4.2/02, Allen, L. (2013)**
Title	Validation of draft residue method CAM-0004/001 for the determination of phenoxy acids and their corresponding 2-ethyl hexyl esters and glycine conjugates in cereal grain, straw and foliage Report No. CEMR-6228
Guidelines:	OECD Guideline ENV/JM/MONO(2007) 17 UK Good Laboratory Practice Regulations 1999 (S.I. 1999/3106, as amended by S.I. 994, 2004) OPPTS 860.1340 (1996) EC document SANCO/825/00 rev.8.1 EC document SANCO/3029/99 rev.4
GLP:	Yes
Deviations	None
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012. Laboratory facility: CEM Analytical Services Ltd, Berkshire.

The method CAM-0004/001 for the determination of mecoprop-P, its corresponding 2-ethyl hexyl ester and glycine conjugate in cereal straw, grain and foliage was conducted according to the above method description. The validation data is summarised below:

Linearity	0.6 - 200 ng/mL (n = 9) [equivalent to 0.003 – 1 mg/kg] r^2 = >0.997 for all matrices and for all forms of mecoprop-P. This encompasses 30 – 120% of the LOQ.
Accuracy and Precision	Five aliquots of cereal matrix were fortified with either mecoprop-P acid, ethylhexyl ester or glycine conjugate at 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 x LOQ). Two control samples and one regent blank were analysed with each matrix. The precision of the accuracy determination is reported. No residues > 30% LOQ were found in any of the control and reagent blank samples.

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z*: 212.9 →140.9						
Wheat grain	Acid	0.01	5	113	12.1	92-126
		0.1	5	110	5.6	102-119
	2EH ester	0.01	5	110	4.3	103-116
		0.1	5	114	6.5	107-125
	Glycine conjugate	0.01	5	98	5.2	89-102
		0.1	5	106	9.6	89-115
Confirmatory transition *m/z*: 215.0 →142.9						
Wheat grain	Acid	0.01	5	110	13.7	85-124
		0.1	5	111	5.6	102-119
	2EH ester	0.01	5	108	5.3	102-117
		0.1	5	111	6.2	102-119
	Glycine conjugate	0.01	5	97	5.3	92-105
		0.1	5	105	10.1	87-115

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z*: 212.9 →140.9						
Wheat Straw	Acid	0.01	5	94	6.3	86-100
		0.1	5	86	2.6	82-87
	2EH ester	0.01	5	111	6.2	102-117
		0.1	5	101	4.4	96-107
	Glycine conjugate	0.01	5	113	5.7	103-119
		0.1	5	111	2.4	107-114
Confirmatory transition *m/z*: 215.0 →142.9						
Wheat Straw	Acid	0.01	5	102	10.3	92-119
		0.1	5	86	2.1	84-88

		0.01	5	106	9.7	92-114	
	2EH ester	0.1	5	105	5.8	96-113	
	Glycine conjugate	0.01	5	112	5.1	103-118	
		0.1	5	113	3.3	110-119	

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)	
Quantitation transition *m/z*: 212.9 →140.9							
Wheat Foliage	Acid	0.01	5	102	1.8	99-104	
		0.1	5	107	0.8	106-108	
	2EH ester	0.01	5	106	5.3	98-113	
		0.1	5	109	3.5	105-115	
	Glycine conjugate	0.01	5	100	4.5	93-105	
		0.1	5	106	1.6	104-108	
Confirmatory transition *m/z*: 215.0 →142.9							
Wheat Foliage	Acid	0.01	5	105	2.6	102-108	
		0.1	5	105	1.5	103-107	
	2EH ester	0.01	5	104	5.1	97-111	
		0.1	5	106	4.4	101-113	
	Glycine conjugate	0.01	5	103	6.2	93-109	
		0.1	5	105	0.5	104-105	

SANCO acceptable range = 70 – 120 %
Precision, %RSD ≤ 20

Specificity	Visual inspection of the chromatography showed no significant interferences (> 30% of the LOQ) at the retention time of mecoprop-P. A highly specific detection system was used for quantification (LC-MS/MS) and two mass transitions were monitored for each analyte therefore further confirmation of identity is not required. The method is considered to have the required specificity.
Matrix Effects	The effect of each cereal matrix on the LC-MS/MS response for the phenoxy acids was assessed by comparing the peak areas of a standard prepared in the presence of each matrix with the peak areas of a non-matrix standard. Significant suppression of the detector response was observed in all cereal matrices. It is recommended that matrix matched standards are prepared.
LOQ	0.01 mg/kg for all analytes in cereal matrices (grain, straw and foliage).

Conclusion
The method is validated in accordance with the EU guidance SANCO/825/00 rev. 8.1.

ILV – plant matrices

Report:	CA 4.2/03, Watson, G. (2014a)
Title	Phenoxy herbicides – independent laboratory validation of the analytical method CAM-004/001 for the determination of phenoxy acids and their corresponding 2-ethyl hexyl esters and glycine conjugates in crops Report No. S13-05322
Guidelines:	European Council Directive Regulation No 1107/2009 repealing Council Directives

	79/117/EEC and 91/414/EEC European Commission Regulations No 544/2011 and 545/2011 European Commission guidance Document on Residue Analytical Methods, SANCO/825/00 rev.8.1 OECD Guideline ENV/JM/MONO(2007) 17
GLP:	Yes
Deviations	Yes – hydrolysis conditions for grass altered.
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012. Performing laboratory: Eurofins Agroscience Services Chem Ltd, Derbyshire, UK.

The analytical method CAM-0004/001 was independently validated for the determination of mecoprop-P (total phenoxy acid) in/on cereal straw (dry/high starch) and grass (high water). These two matrices are representative and demonstrate the suitability of the method for all supported plant matrices.

Samples of matrix are hydrolysed overnight in strong aqueous sodium hydroxide to covert ethylhexyl esters and glycine conjugates back to the parent acid. Hydrolysed samples are acidified and extracted into acetonitrile using QuEChERS prior to being concentrated for analysis by LC-MS/MS. The validation data for mecoprop-P are summarised below:

Linearity	0.6 - 200 ng/mL (n = 8) r^2 = >0.995 for all matrices and for all forms of mecoprop-P
Accuracy and Precision	Five aliquots of cereals straw and grass were fortified with either the acid, ethylhexyl ester or glycine conjugate at 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 x LOQ). Two control samples and one regent blank were analysed with each matrix. The residues detected in the control samples were all < 30% LOQ. The precision of accuracy is reported. *(see table below)*

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z*: 213 →141						
Cereal straw	Acid	0.01	5	105	10.0	91-116
		0.1	5	109	8.1	98-119
	2EH ester	0.01	5	93	7.3	82-100
		0.1	5	98	4.1	92-102
	Glycine conjugate	0.01	5	97	10.0	86-111
		0.1	5	94	8.6	83-102
Confirmatory transition *m/z*: 215 →143						
Cereal straw	Acid	0.01	5	96	7.9	87-104
		0.1	5	103	7.5	92-111
	2EH ester	0.01	5	93	9.3	84-104
		0.1	5	92	6.2	83-98
	Glycine conjugate	0.01	5	95	7.5	87-106
		0.1	5	90	7.5	81-100

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z*: 213 →141						
Grass*	Acid	0.01	5	97	3.8	93-103
		0.1	5	92	2.8	89-96
	2EH ester	0.01	5	84	2.2	82-87
		0.1	5	86	3.6	81-89
	Glycine conjugate	0.01	5	95	1.6	92-96
		0.1	5	91	3.3	86-91
Confirmatory transition *m/z*: 215 →143						
Grass*	Acid	0.01	5	90	5.1	85-97
		0.1	5	92	2.1	90-95
	2EH ester	0.01	5	89	8.0	79-95
		0.1	5	85	4.0	79-87
	Glycine conjugate	0.01	5	94	5.5	90-102
		0.1	5	89	3.2	86-92

* Results obtained on second attempt using modified hydrolysis conditions using half the quantity of sodium hydroxide and acid solutions.

SANCO acceptable range = 70-120% for levels >0.01 mg/kg and 60 – 120% for ≤ 0.01 mg/kg.
Precision %RSD < 20

Specificity	Visual inspection of the chromatography showed no significant interferences (> 30% of the LOQ) at the retention time of mecoprop-P A highly specific detection system was used for quantification (LC-MS/MS) and two mass transitions were monitored for each analyte. The method is considered to have the required specificity.
Matrix Effects	The effect of each matrix on the LC-MS/MS response for the phenoxy acids was assessed by comparing the peak area of a standard prepared in the presence of matrix with the peak areas of a non-matrix standard. Significant suppression of the detector response was observed in both cereal straw and grass matrices, therefore it is recommended that matrix matched standards are used for all of these, and similar, matrices.
LOQ	0.01 mg/kg for all analytes in matrices (cereal straw and grass).

Conclusion

The method CAM-0004/001 was successfully independently validated for the determination of residues of mecoprop-P (as total phenoxy acid) in plant matrices (dry commodity, wheat and high water content, grass) by LC-MS/MS with a limit of quantification of 0.01 mg/kg in all tested material. The method is successfully validated in accordance with SANCO/825/00 rev. 8.1.

Animal, orange and olive matrices and ILV

Report:	**CA 4.2/04, Allen, L. (2014b)**
Title	Validation of draft residue method CAM-0004/002 for the determination of phenoxy acids and their corresponding 2-ethyl hexyl esters and glycine conjugates in animal matrices (egg, milk, muscle, fat, liver, kidney), orange and olives Report No. CEMR-6229
Guidelines:	OECD Guideline ENV/JM/MONO(2007) 17 UK Good Laboratory Practice Regulations 1999 (S.I. 1999/3106, as amended by S.I. 994, 2004) OPPTS 860.1340 (1996) EC document SANCO/825/00 rev.8.1 EC document SANCO/3029/99 rev.4
GLP:	Yes
Deviations	None
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012. Performing laboratory: CEMAS, Berkshire, UK.

The analytical method CAM-0004/002 for the determination of mecoprop-P (total phenoxy acid) in/on animal matrices (egg, milk, muscle, fat, liver, kidney), orange (high acid) and olives (high oil) whether present as the acid, ester (e.g. ethylhexyl) or conjugate (e.g. glycine) was conducted according to the above method described in part a. The validation data is summarised below:

Linearity	0.6 ng/mL - 200 ng/mL [equivalent to 0.003 – 1 mg/kg] $r^2 = >0.990$ for all animal, acidic and oily matrices and for all forms of mecoprop-P; n = 9 The linear range accommodates the LOQ.
Accuracy and Precision	Five aliquots of animal matrix (egg, milk, muscle, fat, liver, kidney), orange and olives were fortified with either the acid, ethylhexyl ester or glycine conjugate at 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 x LOQ). Two control samples and one regent blank were analysed with each matrix. Precision of accuracy determinations was reported. The residues detected in the control samples were all < 30% LOQ.

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z*: 212.9 →140.9						
Muscle (Bovine)	Acid	0.01	5	86	3.8	81-89
		0.1	5	87	8.8	78-93
	2EH ester	0.01	5	80	3.6	75-82
		0.1	5	85	3.3	83-90
	Glycine conjugate	0.01	5	94	4.4	89-100
		0.1	5	91	2.4	88-94
Confirmatory transition *m/z*: 215.0 →142.9						
Muscle (Bovine)	Acid	0.01	5	81	4.5	76-86
		0.1	5	86	8.2	78-93
	2EH ester	0.01	5	80	5.7	72-83
		0.1	5	84	2.8	80-86

		0.01	5	93	5.6	85-98
	Glycine conjugate	0.1	5	92	5.4	87-99

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z:* 212.9 →140.9						
Fat (Bovine)	Acid	0.01	5	101	6.7	90-107
		0.1	4	99	1.6	97-197*
	2EH ester	0.01	5	99	7.3	87-106
		0.1	5	106	5.6	99-112
	Glycine conjugate	0.01	5	103	5.5	97-110
		0.1	5	103	5.4	95-109
Confirmatory transition *m/z:* 215.0 →142.9						
Fat (Bovine)	Acid	0.01	5	105	9.7	88-113
		0.1	4	97	2.2	94-204*
	2EH ester	0.01	5	97	6.7	87-104
		0.1	5	104	2.4	102-107
	Glycine conjugate	0.01	5	103	9.2	92-117
		0.1	5	101	6.7	91-109

* 197 and 204 % recovery values were anomalies caused by fortifying the sample vial twice in error. These were excluded from the statistical calculations.

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z:* 212.9 →140.9						
Liver (Bovine)	Acid	0.01	5	88	12.5	69-98
		0.1	5	101	5.9	95-111
	2EH ester	0.01	5	95	8.5	81-101
		0.1	5	101	1.1	99-102
	Glycine conjugate	0.01	5	89	10.6	74-98
		0.1	5	98	3.3	95-103
Confirmatory transition *m/z:* 215.0 →142.9						
Liver (Bovine)	Acid	0.01	5	87	11.6	70-95
		0.1	5	99	6.3	92-109
	2EH ester	0.01	5	103	7.8	92-111
		0.1	5	101	2.0	98-103
	Glycine conjugate	0.01	5	90	10.9	73-97
		0.1	5	95	3.0	90-97

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z*: 212.9 →140.9						
Kidney (Bovine)	Acid	0.01	5	85	8.8	76-94
		0.1	5	96	6.2	86-102
	2EH ester	0.01	5	95	5.5	87-99
		0.1	5	92	8.2	79-98
	Glycine conjugate	0.01	5	98	2.1	95-100
		0.1	5	92	6.9	85-98
Confirmatory transition *m/z*: 215.0 →142.9						
Kidney (Bovine)	Acid	0.01	4*	92	10.6	79-102
		0.1	5	95	5.9	86-100
	2EH ester	0.01	5	87	5.0	83-93
		0.1	5	91	7.2	80-96
	Glycine conjugate	0.01	5	97	7.7	88-105
		0.1	5	92	8.8	81-99

* Poor chromatography of one of the samples prevented recovery determination therefore the mean and precision are calculated from 4 values. This is not of concern as sufficient information has been provided to consider the ILV acceptable.

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z*: 212.9 →140.9						
Milk (Bovine)	Acid	0.01	5	95	3.1	91-99
		0.1	5	101	4.3	96-107
	2EH ester	0.01	5	90	4.0	85-93
		0.1	5	101	2.3	99-105
	Glycine conjugate	0.01	5	95	3.3	92-100
		0.1	5	98	2.8	95-102
Confirmatory transition *m/z*: 215.0 →142.9						
Milk (Bovine)	Acid	0.01	5	95	4.8	91-102
		0.1	5	97	3.7	94-101
	2EH ester	0.01	5	91	8.1	83-100
		0.1	5	99	2.5	96-101
	Glycine conjugate	0.01	5	96	2.5	93-99
		0.1	5	97	3.8	91-100

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z*: 212.9 →140.9						
Eggs (Poultry)	Acid	0.01	5	87	4.9	82-94
		0.1	5	99	4.6	95-106
	2EH ester	0.01	5	94	4.0	89-99
		0.1	5	95	2.9	92-98
	Glycine conjugate	0.01	5	88	3.1	84-91
		0.1	5	91	3.6	86-94
Confirmatory transition *m/z*: 215.0 →142.9						
Eggs (Poultry)	Acid	0.01	5	90	6.4	83-98
		0.1	5	97	5.5	92-105
	2EH ester	0.01	5	89	4.6	82-92
		0.1	5	95	3.1	91-98
	Glycine conjugate	0.01	5	96	2.4	93-98
		0.1	5	92	1.7	90-94

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z*: 212.9 →140.9						
Orange (whole)	Acid	0.01	5	92	5.4	85-98
		0.1	5	99	1.5	97-100
	2EH ester	0.01	5	92	4.3	89-99
		0.1	5	97	3.2	94-102
	Glycine conjugate	0.01	5	96	1.7	94-98
		0.1	5	98	0.9	97-99
Confirmatory transition *m/z*: 215.0 →142.9						
Orange (whole)	Acid	0.01	5	95	4.0	91-100
		0.1	5	96	1.9	93-98
	2EH ester	0.01	5	96	6.6	88-103
		0.1	5	96	3.6	93-101
	Glycine conjugate	0.01	5	96	4.5	89-100
		0.1	5	97	3.1	95-102

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z:* 212.9 →140.9						
Olives	Acid	0.01	5	102	3.6	96-106
		0.1	5	101	3.7	99-108
	2EH ester	0.01	5	89	3.4	86-93
		0.1	5	91	7.4	86-101
	Glycine conjugate	0.01	5	102	3.6	96-106
		0.1	5	106	5.5	100-114
Confirmatory transition *m/z:* 215.0 →142.9						
Olives	Acid	0.01	5	97	3.1	94-101
		0.1	5	100	3.0	96-104
	2EH ester	0.01	5	87	7.7	79-96
		0.1	5	94	10.0	85-108
	Glycine conjugate	0.01	5	104	4.9	96-109
		0.1	5	104	4.7	98-109

All mean recoveries within the range 60% - 120% with a relative standard deviation of <30% at the 0.01 mg/kg level

All mean recoveries within the range 70% - 120% with a relative standard deviation of <20% at the 0.1 mg/kg level

No residues of mecoprop-P (or any other phenoxy acid) were found at >30% of the LOQ in any of the control or reagent blank samples.

Specificity	Visual inspection of the chromatography showed no significant interferences (> 30% of the LOQ) at the retention time of mecoprop-P. A highly specific detection system was used for quantification (LC-MS/MS) and two mass transitions were monitored for each analyte therefore further confirmation methods are not required. The method is considered to have the required specificity.
Matrix Effects	The effect of each matrix on the LC-MS/MS response for the phenoxy acids was assessed by comparing the peak area of a standard prepared in the presence of matrix with the peak areas of a non-matrix standard. Significant suppression of the detector response was observed in most of the animal matrices (egg, milk, muscle, liver, and kidney) and orange. In the presence of olives, significant suppression of the detector response was seen in some of the analytes while significant enhancement of the detector response was seen in some of the analytes. In the presence of bovine fat, significant enhancement of the detector response was seen in all of the analytes. Therefore, It is recommended that matrix matched standards are used for all of these, and similar, matrices.
LOQ	0.01 mg/kg for all analytes in animal matrices (egg, milk, muscle, fat, liver, kidney), orange and olives.

Conclusion

The method for the determination of mecoprop-P on animal matrices (egg, milk, muscle, fat, liver, kidney) orange and olives is validated in accordance with the EU guidance SANCO/825/00 rev. 8.1.

ILV – animal matrices

Report:	CA 4.2/05, Watson, G. (2014b)
Title	Phenoxy herbicides – independent laboratory validation of the analytical method CAM-004/002 for the determination of phenoxy acids and their corresponding 2-ethyl hexyl esters and glycine conjugates in six matrices by LC-MS/MS Report No. S14-00286
Guidelines:	European Council Directive Regulation No 1107/2009 repealing Council Directives 79/117/EEC and 91/414/EEC European Commission Regulations No 544/2011 and 545/2011 European Commission guidance Document on Residue Analytical Methods, SANCO/825/00 rev.8.1 OECD Guideline ENV/JM/MONO(2007) 17
GLP:	Yes
Deviations	None
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012. Performing laboratory: Eurofins Agroscience Services Chem Ltd, Derbyshire, UK.

The analytical method CAM-0004/002 was independently validated for the determination of mecoprop-P (total phenoxy acid) in/on animal matrices (egg, muscle, fat, liver), orange (high acid) and olives (high oil) whether present as the acid, ester (e.g. ethylhexyl) or conjugate (e.g. glycine).

Bovine fat, muscle and liver were taken as representative animal matrices, citrus fruit and olive were also validated in the study. Samples of matrix were hydrolysed overnight in strong aqueous sodium hydroxide to convert ethylhexyl esters and glycine conjugates back to the parent acid. Hydrolysed samples are acidified and extracted into acetonitrile using QuEChERS prior to being concentrated for analysis by LC-MS/MS. The validation data are summarised below:

Linearity	0.6 ng/mL - 200 ng/mL [equivalent to 0.003 – 1 mg/kg] r^2 = >0.990 for all animal, acidic and oily matrices and for all forms of mecoprop-P; n = 9 The linear range accommodates the LOQ.
Accuracy	Five aliquots of animal matrix (egg, muscle, fat, liver), orange and olives were fortified with either the acid, ethylhexyl ester or glycine conjugate at 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 x LOQ). Two control samples and one regent blank were analysed with each matrix. The results are summarised below.

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
		Quantitation transition *m/z*: 213 →143				
Muscle (Bovine)	Acid	0.01	5	80	8.6	72 - 89
		0.1	5	100	4.3	95 - 106
	2EH ester	0.01	5	83	9.6	73 - 93
		0.1	5	103	6.5	96 - 114
	Glycine conjugate	0.01	5	84	5.5	80 - 92
		0.1	5	98	5.6	90 - 105
		Confirmatory transition *m/z*: 215 →143				
Muscle	Acid	0.01	5	83	8.4	77 - 94

(Bovine)		0.1	5	90	5.7	84 - 97
	2EH ester	0.01	5	82	9.4	74 - 92
		0.1	5	95	6.3	90 - 105
	Glycine conjugate	0.01	5	86	4.8	81 - 90
		0.1	5	91	5.2	85 - 98

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z*: 213 →141						
Fat (Bovine)	Acid	0.01	5	89	12.2	73 - 101
		0.1	5	98	5.5	92 - 104
	2EH ester	0.01	5	87	6.1	79 - 92
		0.1	5	106	4.5	100 - 111
	Glycine conjugate	0.01	5	87	4.8	82 - 93
		0.1	5	96	3.4	92 - 100
Confirmatory transition *m/z*: 215 →143						
Fat (Bovine)	Acid	0.01	5	84	15.4	63 - 98
		0.1	5	94	4.5	89 - 99
	2EH ester	0.01	5	87	12.1	70 - 96
		0.1	5	100	4.3	96 - 107
	Glycine conjugate	0.01	5	85	7.3	78 - 93
		0.1	5	90	4.9	84 - 96

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z*: 213 →141						
Liver (Bovine)	Acid	0.01	5	80	8.2	71 - 89
		0.1	5	91	4.8	87 - 97
	2EH ester	0.01	5	78	8.2	70 - 85
		0.1	5	100	1.5	98 - 102
	Glycine conjugate	0.01	5	72	6.3	67 - 77
		0.1	5	97	3.7	92 - 100
Confirmatory transition *m/z*: 215 →143						
Liver (Bovine)	Acid	0.01	5	81	4.6	76 - 86
		0.1	5	83	7.4	75 - 92
	2EH ester	0.01	5	80	2.8	77 - 83
		0.1	5	87	3.4	82 - 89
	Glycine conjugate	0.01	5	73	7.1	66 - 80
		0.1	5	87	4.3	84 - 93

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z*: 213→141
Eggs (Poultry)	Acid	0.01	5	81	6.4	73 - 87
		0.1	5	90	5.3	83 - 95
	2EH ester	0.01	5	93	3.9	88 - 98
		0.1	5	102	3.7	97 - 106
	Glycine conjugate	0.01	5	87	3.3	84 - 91
		0.1	5	96	9.3	87 - 111
Confirmatory transition *m/z*: 215 →143
Eggs (Poultry)	Acid	0.01	5	86	3.5	83 - 90
		0.1	5	85	6.1	80 - 93
	2EH ester	0.01	5	92	7.7	83 - 98
		0.1	5	96	1.9	93 - 97
	Glycine conjugate	0.01	5	90	3.5	85 - 93
		0.1	5	90	8.0	80 - 100

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z*: 213 →141
Citrus fruit (Orange)	Acid	0.01	5	89	3.5	85 - 92
		0.1	5	87	3.1	84 - 91
	2EH ester	0.01	5	78	7.2	71 - 86
		0.1	5	92	4.9	84 - 95
	Glycine conjugate	0.01	5	83	3.7	80 - 88
		0.1	5	91	2.3	89 - 94
Confirmatory transition *m/z*: 215→143
Citrus fruit (Orange)	Acid	0.01	5	92	3.1	90 - 97
		0.1	5	85	2.5	82 - 87
	2EH ester	0.01	5	75	8.3	67 - 84
		0.1	5	92	3.8	88 - 96
	Glycine conjugate	0.01	5	87	5.1	80 - 91
		0.1	5	86	2.4	84 - 88

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z*: 213 →141
Olives	Acid	0.01	4*	95	7.0	86 - 102

		0.1	5	113	6.2	107 - 124
	2EH ester	0.01	5	102	2.4	100 - 106
		0.1	5	104	4.8	97 - 109
	Glycine conjugate	0.01	5	97	4.7	93- 104
		0.1	5	104	6.6	93 - 111
Confirmatory transition *m/z*: 215→143						
Olives	Acid	0.01	4*	99	8.6	86 - 105
		0.1	5	109	7.9	100 - 123
	2EH ester	0.01	5	97	5.6	91 - 104
		0.1	5	98	6.7	89 - 104
	Glycine conjugate	0.01	5	104	5.7	100 - 114
		0.1	5	101	6.3	91 - 107

*One result rejected based on the results of a Dixon test, therefore mean and RSD based on 4 results.

All mean recoveries were within the range 70% - 120% with a relative standard deviation of <20% at both the 0.01 mg/kg level and 0.1 mg/kg levels. No residues of mecoprop-P (or any other phenoxy acid) were found at >30% of the LOQ in any of the control or reagent blank samples.

Repeatability	n = 5 for each extract and for each of acid, ethylhexyl ester and glycine conjugate at 2 validation levels. % RSD = <20%
Specificity	Visual inspection of the chromatography showed no significant interferences (> 30% of the LOQ) at the retention time of mecoprop-P. A highly specific detection system was used for quantification (LC-MS/MS) and two mass transitions were monitored for each analyte.
Matrix Effects	The effect of each matrix on the LC-MS/MS response for the phenoxy acids was assessed by comparing the peak area of a standard prepared in the presence of matrix with the peak areas of a non-matrix standard. Significant suppression of the detector response was observed during the validation therefore matrix matched standards were used for quantification.
LOQ	0.01 mg/kg for all analytes in animal matrices (egg, muscle, fat, liver), orange and olives.

Conclusion
The method CAM-0004/002 was successfully independently validated for the determination of residues of mecoprop-P (as total phenoxy acid) in animal (egg, muscle, fat, liver), orange (high acid) and olives (high oil) by LC-MS/MS with a limit of quantification of 0.01 mg/kg in all tested material. The method is successfully validated in accordance with SANCO/825/00 rev. 8.1.

Soil, water and air

The enforcement/monitoring residue definition for soil, water and air is mecoprop-P. O-cresol has been identified in surface water at a maximum of 30.2%, but is not ecotoxicologically relevant and therefore does not need to be included in the residue definition for monitoring (see CP B.9.4.5).

Report:	CA 4.2/06, Allen, L. (2014c)
Title	Validation of draft residue method CAM-0004/003 for the determination of phenoxy acids and their corresponding 2-ethyl hexyl esters in surface water, soil and air Report No: CEMR-6230
Guidelines:	OECD Guideline ENV/JM/MONO(2007) 17 OPPTS 860.1340 (1996) OCSPP 850.6100 (2012) EC document SANCO/825/00 rev.8.1 EC document SANCO/3029/99 rev.4
GLP:	Yes
Deviations	None
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012. Performing laboratory: CEMAS, Berkshire, UK.

The method CAM-0004/003 for the determination of mecoprop-P and it's corresponding 2-ethyl hexyl ester in surface water, soil (sandy and clay) and air was conducted according to the method described in part a.

Samples of matrix are hydrolysed overnight in strong aqueous sodium hydroxide to convert ethylhexyl esters back to the parent acid. Hydrolysed samples are acidified and, with the exception of the water extraction procedure where QuEChERS is not required, extracted into acetonitrile using QuEChERS prior to being concentrated for analysis by LC-MS/MS. The validation data is summarised below:

Linearity	Soil (sandy and clay): 0.6 - 200 ng/mL [equivalent to 0.003 – 1 mg/kg] (n = 9), $r^2 \geq 0.99$. Water: 0.6 - 200 ng/mL [equivalent to 0.003 – 2.5 µg/L] (n = 9), $r^2 \geq 0.99$. Air: 0.6 - 200 ng/mL [equivalent to 0.015 – 5 µg/tube] (n = 9), $r^2 \geq 0.99$. The linear ranges accommodate the LOQ.
	Five aliquots of soil (sandy and clay) and air were fortified with either the acid, ethylhexyl ester or glycine conjugate at 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 x LOQ). Five aliquots of surface water were fortified at 0.02 and 0.1 µg/L. Two control samples and one regent blank were analysed with each matrix. Precision of accuracy determinations was reported. The residues detected in the control samples were all < 30% LOQ. {table below}

Matrix		Fortification level (µg/L)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z*: 212.9 →140.9						
Surface water	Acid	0.02	5	116	4.5	109 - 122
		0.1	5	107	8.1	97 - 116
	2EH ester	0.02	5	96	8.1	82 - 101
		0.1	5	109	13.3	87 - 124
Confirmatory transition *m/z*: 215.0 →142.9						

Surface water	Acid	0.02	5	104	4.7	98 - 111
		0.1	5	111	14.5	95 - 136
	2EH ester	0.02	5	88	9.0	75 - 94
		0.1	5	104	7.3	91 - 110

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z:* **212.9 →140.9**						
Soil (Sandy type)	Acid	0.01	5	103	4.0	99-108
		0.1	5	108	3.1	103-112
	2EH ester	0.01	5	106	3.9	102-112
		0.1	5	106	3.9	101-110
Confirmatory transition *m/z:* **215.0 →142.9**						
Soil (Sandy type)	Acid	0.01	5	104	4.5	99-111
		0.1	5	105	4.1	99-110
	2EH ester	0.01	5	102	2.4	100-106
		0.1	5	102	4.3	97-107

Matrix	Component	Fortification level (mg/kg)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z:* **212.9 →140.9**						
Soil (Clay type)	Acid	0.01	5	100	3.1	95-103
		0.1	5	104	3.9	98-109
	2EH ester	0.01	5	102	2.5	99-105
		0.1	5	100	4.4	95-107
Confirmatory transition *m/z:* **215.0 →142.9**						
Soil (Clay type)	Acid	0.01	5	101	1.5	99-102
		0.1	5	100	3.2	96-104
	2EH ester	0.01	5	101	3.7	96-105
		0.1	5	100	5.2	91-104

Matrix	Component	Fortification level (µg/tube)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z:* **212.9 →140.9**						
Air	Acid	0.05	5	84	7.5	75-92
		0.5	5	91	9.8	79-104
	2EH ester	0.05	5	73	3.8	82-89
		0.5	5	87	4.3	85-94
Confirmatory transition *m/z:* **215.0 →142.9**						
	Acid	0.05	5	84	10.7	72-94

Accuracy and Precision (row label, leftmost column)

Air		0.5	5	89	9.7	80-103
	2EH ester	0.05	5	77	6.9	81-94
		0.5	5	86	4.4	82-90

For soil (sand and clay), air and surface water the recoveries are within the SANCO acceptable ranges = 70 – 120 % for > 0.01 ≤ 0.1 mg/kg and 70 – 110% for > 0.1 ≤ 1.0 mg/kg. The precision %RSD are all < 20.

Specificity	Visual inspection of the chromatography showed no significant interferences (> 30% of the LOQ) at the retention time of mecoprop-P. A highly specific detection system was used for quantification (LC-MS/MS) and two mass transitions were monitored for each analyte therefore further confirmation methods are not required.
Matrix Effects	Significant suppression of the detector response was observed in the presence of sandy soil. In the presence of surface water, air and clay soil, no significant suppression of the detector response was observed. Therefore, it is recommended that matrix matched standards are used for soil matrices and batch standards are used for water and air matrices.
LOQ	Soil (sand and clay): 0.01 mg/kg Air: 0.05 µg/tube Water: 0.02µg/L

Conclusion

The method for the determination of mecoprop-P and its ester in water, soil (sand and clay) and air is validated in accordance with the EU guidance document SANCO/825/00 rev. 8.1. This method is also suitable for drinking water with no further validation required, as the LOQ for drinking water, 0.1 µg/L, is accommodated.

Drinking water ILV

Report:	CA 4.2/07, Weir, A. (2014)
Title	Phenoxy herbicides – independent laboratory validation of the analytical method CAM-004/003 for the determination of phenoxy acids and their corresponding 2-ethyl hexyl esters in drinking water by LC-MS/MS Report No. S14-01199
Guidelines:	European Council Directive Regulation No 1107/2009 repealing Council Directives 79/117/EEC and 91/414/EEC European Commission Regulations No 544/2011 and 545/2011 European Commission guidance Document on Residue Analytical Methods, SANCO/825/00 rev.8.1 OECD Guideline ENV/JM/MONO(2007) 17
GLP:	Yes
Deviations	None
Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012. Performing laboratory: Eurofins, Derbyshire, UK.

The analytical method CAM-0004/003 was independently validated for the determination of mecoprop-P (total phenoxy acid) in drinking water. Samples of matrix are hydrolysed overnight in strong aqueous sodium hydroxide to convert ethylhexyl esters back to the parent acid. Hydrolysed samples are acidified and purified/concentrated prior to analysis by LC-MS/MS. The validation data are summarised below:

Linearity	0.6 µg/mL - 200 ng/mL r^2 = >0.990 for all matrices and for all forms of mecoprop-P n = 9
Accuracy	Five aliquots of drinking water were fortified with either the acid or ethylhexyl ester at 0.02 µg/L and 0. 1µg/L mecoprop-P in water. Two control samples and one regent blank were analysed with each matrix. The results are summarised below. *(see table below)* All mean recoveries were in the range 70 -120% at both fortification levels, with RSD the required 20%.
Repeatability	n = 5 for each extract and for each of acid and ethylhexyl ester at 2 validation levels. % RSD = <10%
Specificity	Visual inspection of the chromatography showed no significant interferences (> 30% of the LOQ) at the retention time of mecoprop-P A highly specific detection system was used for quantification (LC-MS/MS) and two mass transitions were monitored for each analyte. The method is considered to have the required specificity.
Matrix Effects	The effect of drinking water on the LC-MS/MS response for the phenoxy acids was assessed by comparing the peak area of a standard prepared in the presence of matrix with the peak areas of a non-matrix standard. No significant suppression of the detector response was observed in drinking water, therefore solvent standards are used for quantitation throughout the validation.
LOQ	0.02 µg/L in water

Accuracy table:

Matrix		Fortification level (µg/L)	n	Mean (%)	RSD (%)	Range (%)
Quantitation transition *m/z*: 213 →141						
Surface water	Acid	0.02	5	85	5.7	79 - 89
		0.1	5	91	3.1	87 - 94
	2EH ester	0.02	5	96	7.0	88 - 106
		0.1	5	102	8.0	96 - 116
Confirmatory transition *m/z*: 215 →143						
Surface water	Acid	0.02	5	84	5.8	80 - 90
		0.1	5	89	3.6	84 - 92
	2EH ester	0.02	5	93	7.4	83 - 102
		0.1	5	97	7.6	92 - 110

Conclusion

The method CAM-0004/003 was successfully independently validated for the determination of residues of mecoprop-P (as total phenoxy acid) in drinking water with a limit of quantification of 0.02 µg/L. The method is successfully validated in accordance with SANCO/825/00 rev. 8.1.

Body fluids and tissues
An enforcement method for the analysis of residues in body fluids and tissues is not required. According to SANCO/825/00 rev. 8.1 methods are not required for substances not classified as T+, T or Acute tox (cat. 1-3), CMR (cat. 1) or STOT (cat. 1). Mecoprop-P does not meet any of these classification criteria and therefore a method is not required.

B.5.3. REFERENCES RELIED ON

Regarding the literature search undertaken by the applicant (report dated 15/07/2015). It is considered that the search is acceptable in terms of databases searched and the search criteria applied. The search did not reveal any references of relevance to this section.

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte brate study Y/N	Data protec tion claime d Y/N	Justification if data protection is claimed	Owner	Previous evaluation
CA 1.11/01	Mahmood, T.	2014	The Analysis of Seven Batches of Mecoprop-P TGAI. Report No. 14/0861	N	Y	New data submitted (replaces old batch data)	Nufarm	Submitted for the purposes of renewal.
CA 4.1.2/03	■	2013	Mecoprop-P livestock feeding study: magnitude of residue in milk, muscle, liver, kidney and fat of lactating dairy cattle Report No. ■ GLP Not published	Y	Y	New data submitted	Nufarm	Submitted for the purposes of renewal.
CA 4.1.2/04	■	2014	Frozen Storage Stability Study for Mecoprop-P, HMCPP, CCPP and PCOC in Bovine Specimens Report No. ■ GLP Not published	Y	Y	New data submitted	Nufarm	Submitted for the purposes of renewal.
CA 4.1.2/06	Perny, A.	2002	Residue decline of Mecoprop-P potassium salt in cereals in Southern Europe R A0119 Anadiag GLP Not published	N	N	N/A	Nufarm	Submitted for the purposes of renewal.
CA 4.1.2/07	Gallais, C.	2002a	Residue decline of Mecoprop-P potassium salt in cereals in Southern Europe R A1135 Anadiag GLP Not published	N	N	N/A	Nufarm	Submitted for the purposes of renewal.
CA 4.1.2/08 (CA 6.3.1/05)	Tandy, R.	2014a	Determination of residues of Mecoprop-P after a single application of Mecoprop-P K 600 in cereals at 4 sites in Northern Europe 2013 S13-00323	N	Y	Replaces previous (DAR) residue trials which are now	Nufarm	Submitted for the purposes of renewal.

Data Point	Author (s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte brate study Y/N	Data protec tion claime d Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			Eurofins Agroscience Services GLP Not published			unacceptable		
CA 4.1.2/18	Jenkins, C.A.	2007	Mecoprop-P (DMA salt) algal growth inhibition assay *Navicula* ZZF0001/063120 Huntingdon Life Sciences Ltd GLP Not published	N	N	N/A	Nufarm	Submitted for the purposes of renewal.
CA 4.1.2/19	Burke, J.	2007	Mecoprop-P (DMA salt) algal growth inhibition assay *Skeletonema* ZZF0002/063525 Huntingdon Life Sciences Ltd GLP Not published	N	N	N/A	Nufarm	Submitted for the purposes of renewal.
CA 4.1.2/21	Kleebau m	2014	Acute toxicity of Mecoprop-P technical acid to honeybee larvae Apis mellifera L. under laboratory conditions (in vitro) BioChem agrar Report No. 14 10 48 023 B GLP Not published	N	Y	New data submitted	Nufarm	Submitted for the purposes of renewal.
CA 4.1.2/22	▮▮▮	2015	Mecoprop-P: Toxic Effects to Rainbow Trout (Oncorhynchus mykiss) in an Early-life Stage Toxicity Test. ▮▮▮▮▮▮ Report No. ▮▮▮ GLP Not published	Y	Y	New data submitted	Nufarm	Submitted for the purposes of renewal.
CA 4.1.2/23	Comb. A.L.	2000	Mecoprop-P (pure grade) physic-chemical properties NUF004/993523 Huntingdon Life Sciences Ltd GLP Not published	N	N	N/A	Nufarm	Submitted for the purposes of renewal.
CA 4.2/01	Allen, L	2014a	Analytical method for the determination of phenoxy acids and their corresponding 2-ethyl hexyl esters and glycine conjugates in cereal grain, straw and foliage, bovine muscle, fat, liver and milk, poultry eggs, citrus fruit and olives and phenoxy acids and their corresponding 2-ethyl hexyl esters in surface water, soil and air CAM-0004/003	N	Y	New study replaces all previous monitoring methods of analysis.	Nufarm	Submitted for the purposes of renewal.

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte brate study Y/N	Data protec tion claime d Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			CEM Analytical Services Limited (CEMAS) GLP Not published					
CA 4.2/02	Allen, L	2013	Validation of draft residue method CAM-0004/001 for the determination of phenoxy acids and their corresponding 2-ethyl hexyl esters and glycine conjugates in cereal grain, straw and foliage CEMR-6228 CEM Analytical Services Limited (CEMAS) GLP Not published	N	Y	New study replaces all previous monitoring methods of analysis.	Nufarm	Submitted for the purposes of renewal.
CA 4.2/03	Watson, G.	2014a	Phenoxy herbicides – independent laboratory validation of the analytical method CAM-004/001 for the determination of phenoxy acids and their corresponding 2-ethyl hexyl esters and glycine conjugates in crops by LC-MS/MS S13-05322 Eurofins Agroscience Services GLP Not published	N	Y	New study replaces all previous monitoring methods of analysis.	Nufarm	Submitted for the purposes of renewal.
CA 4.2/04	Allen, L.	2014 b	Validation of draft residue method CAM-0004/002 for the determination of phenoxy acids and their corresponding 2-ethyl hexyl esters and glycine conjugates in animal matrices (egg, milk, muscle, fat, liver, kidney), orange and olives CEMS-6229 CEM Analytical Services Limited (CEMAS) GLP Not published	N	Y	New study replaces all previous monitoring methods of analysis.	Nufarm	Submitted for the purposes of renewal.
CA 4.2/05	Watson, G.	2014 b	Phenoxy herbicides – independent laboratory validation of the analytical method CAM-004/002 for the determination of phenoxy acids and their corresponding 2-ethyl hexyl esters and glycine conjugates in six matrices by LC-MS/MS Report No. S14-00286 GLP	N	Y	New study replaces all previous monitoring methods of analysis.	Nufarm	Submitted for the purposes of renewal.

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte brate study Y/N	Data protec tion claime d Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			Not published					
CA 4.2/06	Allen, L.	2014c	Validation of draft residue method CAM-0004/003 for the determination of phenoxy acids and their corresponding 2-ethyl hexyl esters in surface water, soil and air CEMS-6230 CEM Analytical Services Limited (CEMAS) GLP Not published	N	Y	New study replaces all previous monitoring methods of analysis.	Nufarm	Submitted for the purposes of renewal.
CA 4.2/07	Weir, A	2014	Phenoxy herbicides – independent laboratory validation of the analytical method CAM-004/003 for the determination of phenoxy acids and their corresponding 2-ethyl hexyl esters in drinking water by LC-MS/MS Report No. S14-01199 GLP Not published	N	Y	New study replaces all previous monitoring methods of analysis.	Nufarm	Submitted for the purposes of renewal.