1 SUMMARY

The draft analytical method described in CAM-0004/003 for the determination of 2,4-D, 2,4-DB, MCPA, MCBP, Mecoprop-P and Dichloroprop-P was successfully validated to a limit of quantitation (LOQ) of 0.01 mg/kg for soil, 0.05 µg/tube for air and 0.01 µg/L for surface water (except for Mecoprop-P which has an LOQ of 0.02 µg/L in surface water due to chromatographic interference).

2 STUDY OBJECTIVE

The purpose of this study was to validate the draft analytical method CAM-0004/003, which has been developed at CEMAS for the analysis of 2,4-D, 2,4-DB, MCPA, MCPB, Mecoprop-P and Dichloroprop-P in soil, surface water and air to a limit of quantitation (LOQ) of 0.01 mg/kg for soil, 0.05 µg/tube for air and 0.01 µg/L for surface water (except for Mecoprop-P which has an LOQ of 0.02 µg/L due to chromatographic interference).

3 MATERIALS / TEST SYSTEM

3.1 REFERENCE ITEMS

Identity:	2,4-D
Batch no:	SP547-55
Purity:	99.98%
Expiry date:	19 Dec 2017
Storage:	Ambient
Identity:	2,4-D (2EH Ester)
Batch no:	S1198
Purity:	99.19%
Expiry date:	13 Feb 2015
Storage:	Refrigerated

Identity:	2,4-DB
Batch no:	AC501/015
Purity:	100.0%
Expiry date:	14 May 2014*
Storage:	Ambient
Identity:	2,4-DB (2EH Ester)
Batch no:	S1167
Purity:	99.51%
Expiry date:	21 Aug 2015
Storage:	Refrigerated

*The air breakthrough assessment was carried out after the expiry date but the standard stability data produced with this study indicate that this standard was acceptable for use during the time frames used.

Identity:	MCPA
Batch no:	CN/572/48
Purity:	99.28%
Expiry date:	02 May 2017
Storage:	Ambient
Identity:	MCPA (2EH Ester)
Batch no:	S1210
Purity:	99.45%
Expiry date:	17 Apr 2015
Storage:	Refrigerated

Identity:	MCPB
Batch no:	S1163
Purity:	99.77%
Expiry date:	25 Jul 2017
Storage:	Ambient
Identity:	MCPB (2EH Ester)
Batch no:	S1188
Purity:	95.18%
Expiry date:	17 Nov 2015
Storage:	Refrigerated

Identity:	Mecoprop-P
Batch no:	DC/532/22
Purity:	99.87% (as Mecoprop)
Expiry date:	30 May 2015
Storage:	Ambient
Identity:	Mecoprop-P (2EH Ester)
Batch no:	S1178
Purity:	99.58% (as Mecoprop 2EH ester)
Expiry date:	21 Aug 2015
Storage:	Refrigerated

Identity:	Dichloroprop-P
Batch no:	SP547-96
Purity:	99.98%
Expiry date:	06 May 2016
Storage:	Ambient
Identity:	Dichloroprop-P (2EH Ester)
Batch no:	S1209
Purity:	99.86%
Expiry date:	23 Apr 2015
Storage:	Refrigerated

Identity:	(4-chloro-3,5-dimethyl-phenoxy) acetic acid
Batch no:	S236071-25MG
Purity:	100%
Expiry date:	01 Aug 2014
Storage:	Refrigerated
Identity:	(2,4,6-trimethyl-phenoxy) acetic acid
Batch no:	S236055-25MG
Purity:	100%
Expiry date:	01 Aug 2014
Storage:	Refrigerated

See Appendix 1 for the certificates of analyses.
The test / reference item will be retained until expiry and then disposed of.

3.2 TEST SYSTEM

The validation study was carried out using control samples of surface water, soil (sandy type), soil (clay type) and air tubes sourced by CEMAS.

Matrix	CEMAS Identification Number	Expiry Date
Surface water	CCON/037/005	12 Aug 2018
Surface water	CCON/037/007	14 Feb 2019
Surface water	CCON/037/008	24 Feb 2019
Soil (sandy type)	CCON/073/002	24 Jun 2018
Soil (clay type)	CCON/073/008	27 Jan 2018

Air Tubes: Tenax (Lot No. 8508)

4 ANALYTICAL PROCEDURES

4.1 ANALYTICAL METHOD

Each matrix was analysed according to the procedures in the draft CEMAS analytical method CAM-0004/003 "Analytical Method for the Determination of Phenoxy Acids and their corresponding 2 Ethyl-Hexyl Esters in Surface Water, Soil and Air" This validated procedure was issued as CAM-0004/003.

Principle of the method: Samples are hydrolysed overnight in a strong aqueous solution of sodium hydroxide to convert the ethyl-hexyl esters 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 are extracted into acetonitrile using QuEChERS before being concentrated for analysis. The reverse phase LC-MS/MS setup uses a monolithic column and flow split to optimise sensitivity.

4.2 FORTIFICATION RATES

Recovery of 2,4-D, 2,4-DB, MCPA, MCPB, Mecoprop-P and Dichloroprop-P through the analytical procedure was assessed by fortifying 5 aliquots of each matrix with a mixed standard containing 2,4-D, 2,4-DB, MCPA, MCPB, Mecoprop-P and Dichloroprop-P at the LOQ level (0.01 mg/kg for soil, 0.05 µg/tube for air and 0.01 µg/L for surface water (except for Mecoprop-P which has an LOQ of 0.02 µg/L due to chromatographic interference)) and 5 aliquots of each matrix with a mixed standard containing 2,4-D, 2,4-DB, MCPA, MCPB, Mecoprop-P and Dichloroprop-P at a level of 0.1 mg/kg for soil, 0.5 µg/tube for air and 0.1µg/L for surface water.

In addition, two control samples and one reagent blank were analysed with each matrix.

Matrix	Untreated Replicates	Replicates at Fortification Level (LOQ)	Replicates at Fortification Level (LOQ x 10) *(LOQ x 5 for Mecoprop-P in water)
Surface water (for 2,4-D, 2,4-DB, MCPA, MCPB, Dichloroprop-P)	2	5 at 0.01 µg/L	5 at 0.1 µg/L
Surface water (for Mecoprop-P)	2	5 at 0.02 µg/L	5 at 0.1 µg/L*
Soil (sandy type)	2	5 at 0.01 mg/kg	5 at 0.1 mg/kg
Soil (clay type)	2	5 at 0.01 mg/kg	5 at 0.1 mg/kg
Air tubes	2	5 at 0.05 µg/tube	5 at 0.5 µg/tube

After fortification of the front sections, air sampling tubes were subjected to the following conditions prior to extraction: air drawn through at 0.5 L/min for 6 hours at 35°C and at 80% relative humidity. The air concentration at the LOQ level (0.05 µg/tube) under these sampling conditions is calculated by:

0.5 L/min (500 mL/min) for 6 h (360 min) = 500 mL/min x 360 min =180000 mL (180 L)

180 L = 0.180 m^3

$$\frac{0.05\ \mu g/tube}{0.180\ m^3} = 0.28\ \mu g/m^3$$

4.3 MATRIX EFFECTS

The effect of each matrix on the LC-MS/MS response for 2,4-D, 2,4-DB, MCPA, MCPB, Mecoprop-P and Dichloroprop-P 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 (i.e. standards prepared in HPLC water (0.2% formic acid)/acetonitrile (60/40, v/v)). The comparison was made using standards at the concentration of 0.01 µg/mL.

Percent matrix effects were determined by: $((B/A)\times 100)-100$
where A is the non-matrix standard response and B is the matrix-matched standard response.

The results are presented in Table 40.

4.4 EXTRACT STABILITY

The stability of 2,4-D, 2,4-DB, MCPA, MCPB, Mecoprop-P and Dichloroprop-P in final extracts was assessed by storing extracts refrigerated at between 2-8°C. The extracts were then re-analysed after 7 days (10 days for soil (clay type) acid, see deviation 1) of storage against freshly prepared calibration standards. The recovery data obtained are summarised in Tables 31 to 36.

4.5 STANDARD STABILITY

The stability of mixed 2,4-D, 2,4-DB, MCPA, MCPB, Mecoprop-P and Dichloroprop-P standard solutions in acetonitrile was assessed by comparing a batch standard made from a freshly prepared mixed standard solution to a batch standard made from stored mixed standard solution after 149 days storage at between 2-8°C.

The comparison was determined by: $((B/A)\times 100)-100$
where A is the stored standard response and B is the freshly prepared standard response.

The results are presented in Tables 41 to 46.

4.6 AIR BREAKTHROUGH ASSESSMENT

Breakthrough of 2,4-D, 2,4-DB, MCPA, MCPB, Mecoprop-P and Dichloroprop-P on the air tubes was assessed by fortifying the front section of the air tubes as detailed in the table in section 4.2, drawing air through at 0.5 L/min for 6 hours at 35°C and 80% relative humidity. The front and back sections of the air tubes were then extracted using the procedures described in the analytical method CAM-0004/003.

The results are presented in Tables 25 to 30. Example chromatograms are presented in Figures 217 to 276.

Limit of Detection (LOD)

TABLE 37: Limit of Detection in Surface Water

	2,4-D		2,4-DB	
	Quantitation Transition (*m/z* 218.8 → 161.0)	Confirmatory Transition (*m/z* 220.8 → 162.9)	Quantitation Transition (*m/z* 247.0 → 161.0)	Confirmatory Transition (*m/z* 249.0 → 163.0)
Estimated Baseline noise of control sample (cps)	43	115	135	80
Estimated baseline noise x 3 (cps)	129	345	405	240
Height of standard at 3 ng/mL (cps)	13500	9800	5720	3580
Estimated LOD (µg/L)	0.000143	0.000528	0.001062	0.001006

	MCPA		MCPB	
	Quantitation Transition (*m/z* 199.0 → 140.9)	Confirmatory Transition (*m/z* 200.9 → 142.9)	Quantitation Transition (*m/z* 227.0 → 140.9)	Confirmatory Transition (*m/z* 229.0 → 142.9)
Estimated Baseline noise of control sample (cps)	105	260	26	40
Estimated baseline noise x 3 (cps)	315	780	78	120
Height of standard at 3 ng/mL (cps)	24200	7210	9100	2900
Estimated LOD (µg/L)	0.000195	0.001623	0.000129	0.000621

	Mecoprop-P		Dichloroprop-P	
	Quantitation Transition (*m/z* 212.9 → 140.9)	Confirmatory Transition (*m/z* 215.0 → 142.9)	Quantitation Transition (*m/z* 232.9 → 160.8)	Confirmatory Transition (*m/z* 234.9 → 162.8)
Estimated Baseline noise of control sample (cps)	195	265	230	420
Estimated baseline noise x 3 (cps)	585	795	6900	1260
Height of standard at 3 ng/mL (cps)	22800	8400	21500	14100
Estimated LOD (µg/L)	0.000385	0.001420	0.000481	0.001340

TABLE 38: Limit of Detection in Soil (Clay type)

	2,4-D		2,4-DB	
	Quantitation Transition (*m/z* 218.8 → 161.0)	Confirmatory Transition (*m/z* 220.8 → 162.9)	Quantitation Transition (*m/z* 247.0 → 161.0)	Confirmatory Transition (*m/z* 249.0 → 163.0)
Estimated Baseline noise of control sample (cps)	60	60	42	37
Estimated baseline noise x 3 (cps)	180	180	126	111
Height of standard at 3 ng/mL (cps)	7990	6120	2870	1870
Estimated LOD (mg/kg)	0.000338	0.000441	0.000659	0.000890

	MCPA		MCPB	
	Quantitation Transition (*m/z* 199.0 → 140.9)	Confirmatory Transition (*m/z* 200.9 → 142.9)	Quantitation Transition (*m/z* 227.0 → 140.9)	Confirmatory Transition (*m/z* 229.0 → 142.9)
Estimated Baseline noise of control sample (cps)	160	45	32	28
Estimated baseline noise x 3 (cps)	480	135	96	84
Height of standard at 3 ng/mL (cps)	15000	4350	4530	1380
Estimated LOD (mg/kg)	0.000480	0.000466	0.000318	0.000913

	Mecoprop-P		Dichloroprop-P	
	Quantitation Transition (*m/z* 212.9 → 140.9)	Confirmatory Transition (*m/z* 215.0 → 142.9)	Quantitation Transition (*m/z* 232.9 → 160.8)	Confirmatory Transition (*m/z* 234.9 → 162.8)
Estimated Baseline noise of control sample (cps)	70	35	90	60
Estimated baseline noise x 3 (cps)	210	105	270	180
Height of standard at 3 ng/mL (cps)	14200	4720	12100	8120
Estimated LOD (mg/kg)	0.000222	0.000334	0.000335	0.000333

TABLE 39: Limit of Detection in Air

	2,4-D		2,4-DB	
	Quantitation Transition (*m/z* 218.8 → 161.0)	Confirmatory Transition (*m/z* 220.8 → 162.9)	Quantitation Transition (*m/z* 247.0 → 161.0)	Confirmatory Transition (*m/z* 249.0 → 163.0)
Estimated Baseline noise of control sample (cps)	55	60	35	23
Estimated baseline noise x 3 (cps)	165	180	105	69
Height of standard at 3 ng/mL (cps)	9540	7550	3530	2260
Estimated LOD (µg/tube)	0.000259	0.000358	0.000446	0.000458

	MCPA		MCPB	
	Quantitation Transition (*m/z* 199.0 → 140.9)	Confirmatory Transition (*m/z* 200.9 → 142.9)	Quantitation Transition (*m/z* 227.0 → 140.9)	Confirmatory Transition (*m/z* 229.0 → 142.9)
Estimated Baseline noise of control sample (cps)	110	70	24	15.5
Estimated baseline noise x 3 (cps)	330	210	72	46.5
Height of standard at 3 ng/mL (cps)	18900	5770	5260	1760
Estimated LOD (µg/tube)	0.000262	0.000546	0.000205	0.000396

	Mecoprop-P		Dichloroprop-P	
	Quantitation Transition (*m/z* 212.9 → 140.9)	Confirmatory Transition (*m/z* 215.0 → 142.9)	Quantitation Transition (*m/z* 232.9 → 160.8)	Confirmatory Transition (*m/z* 234.9 → 162.8)
Estimated Baseline noise of control sample (cps)	54	45	50	34
Estimated baseline noise x 3 (cps)	162	135	150	102
Height of standard at 3 ng/mL (cps)	17600	6510	14500	9590
Estimated LOD (µg/tube)	0.000138	0.000311	0.000155	0.000160

Matrix Effects

TABLE 40: Matrix Effects of Phenoxyacids

Matrix	% Matrix Effects for 2,4-D	
	(Quantitation transition *m/z* 218.8→161.0)	(Confirmatory transition *m/z* 220.8→162.9)
Surface Water	+9.9	+11.2
Soil (Sandy type)	+35.9	+38.4
Soil (Clay type)	+5.4	+6.4
Air	+14.5	+14.3
Matrix	% Matrix Effects for 2,4-DB	
	(Quantitation transition *m/z* 247.0→161.0	(Confirmatory transition *m/z* 249.0→163.0)
Surface Water	-15.1	-15.1
Soil (Sandy type)	+52.9	+55.7
Soil (Clay type)	-4.5	-9.2
Air	+11.1	+14.8
Matrix	% Matrix Effects for MCPA	
	(Quantitation transition *m/z* 199.0→140.9)	(Confirmatory transition *m/z* 200.9→142.9)
Surface Water	-5.2	-5.5
Soil (Sandy type)	+34.1	+39.8
Soil (Clay type)	+4.3	+4.4
Air	+13.4	+8.5
Matrix	% Matrix Effects for MCPB	
	(Quantitation transition *m/z* 227.0→140.9)	(Confirmatory transition *m/z* 229.0→142.9)
Surface Water	-9.5	-13.3
Soil (Sandy type)	+58.2	+60.2
Soil (Clay type)	-1.2	+6.4
Air	+15.4	+4.9

TABLE 40: Matrix Effects of Phenoxyacids (Continued)

Matrix	% Matrix Effects for Mecoprop-P	
	(Quantitation transition *m/z* 212.9→140.9)	(Confirmatory transition *m/z* 215.0→142.9)
Surface Water	+13.6	+15.4
Soil (Sandy type)	+44.8	+49.3
Soil (Clay type)	+3.9	+7.2
Air	+11.5	+12.3
Matrix	% Matrix Effects for Dichloroprop-P	
	(Quantitation transition *m/z* 232.9→160.8)	(Confirmatory transition *m/z* 234.9→162.8)
Surface Water	-3.7	-5.0
Soil (Sandy type)	+53.7	+50.6
Soil (Clay type)	+4.6	+6.2
Air	+18.5	+17.3

Negative % difference in signal indicates matrix suppression
Positive % difference in signal indicates matrix enhancement
Suppression or enhancement is seen as significant if it is > ± 20%