Draft Renewal Assessment Report
under Regulation (EC) 1107/2009

CLOPYRALID

Volume 3 – B.5 (AS)

RMS: Finland
Co-RMS: Poland

May 2017

Version History

When	What
2017/ May	DRAR- First version submitted to EFSA

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B.5. METHODS OF ANALYSIS

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

The following table provides a justification for the use of a different method to that evaluated for the Active Approval.

Data Point/Method	
CA 4.1.1(a)/1	No new method was submitted

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

(a) Determination of the pure active substance in the active substance as manufactured and specified in the dossier submitted in support of approval under Regulation (EC) No 1107/2009

CA 4.1.1(a)/1 Method Validation for the Determination of Assay in Clopyralid

Reference	**CA 4.1.1(a)/1**, Liang, Y.Y. (1995)
Report title	Analytical Method for the Determination of Clopyralid in Technical Grade Produced by the Penta Process
DAS Study number	DECO-GP-AR-94-47020A
Guidelines	SANCO 3030/99 rev. 4
GLP	Yes

Description of the method:	Method for determination of active ingredient in technical Clopyralid. Technical material is dissolved in internal standard solution and solutions are analyzed by gas chromatography (GC) using a DB-1 capillary column with a split injection and a flame ionization detector (FID). Quantitation was made by peak area measurements with internal standardization.
Specificity:	The GC/FID technique is used. Confirmation of the presence of the active ingredient is obtained by comparison of the analyte in the sample with the retention time of the analyte in the calibration standards.
Interference by other substances:	The method has no known interferences at typical concentrations.
Explanation of interferences contributing more than ±3%:	None noted

Linearity and range, equation and r^2:

All correlation coefficients (r^2) were above 0.99, demonstrating good linearity. Results presented in **Table 5.1.1-1**.

Table 5.1.1-1: Linearity of the method for the determination of active ingredient in Clopyralid technical

Component	Concetration range (mg/10 mL)	Linear Regression		
		Slope	Intercept	r^2
Clopyralid	95.99 – 331.6	1.003	0.06	0.99

Accuracy:

Not applicable

Repeatability:

The repeatability (precision) of the method for active ingredient has been tested by determination of the overall Relative Standard Deviation (RSD%) on the results collected over two days. In general, RSD values confirm the repeatability of the method. The RSD values are presented in **5.1.1-2**.

Table 5.1.1-2: RSD of the method for the determination of active ingredient in Clopyralid technical

Component	% RSD
Clopyralid	0.76

Applicability of existing CIPAC methods:

There is currently no CIPAC method

Study Comments: 4.1.1(a)/1	The method is not acceptably validated. According to SANCO/3030/99 rev. 4, confirmatory techniques are required to support identification of the a.s when the primary method of determination is not highly specific
Agreed endpoint: 4.1.1(a)/1	GC/FID

(b) Determination of significant and relevant impurities and additives (such as stabilisers) in the active substance as manufactured

Relevant impurities

There are no relevant impurities in clopyralid therefore no analytical methods are required.

Significant impurities and additives (such as stabilisers)

This is considered CONFIDENTIAL information and can be found in Volume 4.

B.5.1.2. Methods for risk assessment

Rationale for Submission of Analytical Summaries for Data Generation Methods:

Analytical methods used for the generation of pre-approval data are summarized below. Only analytical methods used in non-isotope labelled studies that were not previously reviewed and accepted by the RMS and EFSA, were summarized and compiled.

The analytical method summaries include the recovery, precision, limit of quantitation, specificity and linearity of the method as outlined in SANCO/3029/99 rev.4 for validation of a data generation method.

Table 5.1.2.1 – Summary of Validated Methods for the Generation of Pre-Authorization Data

Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Environmental Fate (CA 4.1.2(a))			
Soil	0.05 µg/kg	LC-MS/MS 120612	**CA 4.2 (b)/1** Vincent, T.P. 2013 Used for studies 130673 and 150672 **Submitted under: CA 7.1.2.2.1/3 and CA 7.1.2.2.1/4**
Efficacy (CA 4.1.2(b))			
-	-		-
Toxicology (CA 4.1.2(c))			
-	-		-
Exposure (CA 4.1.2(d))			
-	-		-
Residues (CA 4.1.2(e))			
Aqueous, Dry, Oily and Acidic Crops	0.01 mg/kg	LC-MS/MS 120610	**CA 4.2 (a)/1** Vogl, E. 2012 Used for studies 120939, 140653 and 140655 **Submitted under: CA 6.1.1/1, 6.3.1/1 and 6.3.2/1**
Bovine and Poultry Tissues	0.01 mg/kg	LC-MS/MS 120483	**CA 4.2 (a)/3** [redacted] Used for studies 120602, 150031 and 150030 **Submitted under: CA 6.1.2/1, CA 6.4.1/3 and CA 6.4.2/4**
Bovine and Poultry Tissues	0.01 mg/kg	GC-MS GRM 02.14	**CA 4.1.2(e)/1** [redacted] (2002) Used for study 020120 **Submitted under: CA 6.1.2/2**
Grass, Cereal Grain and Straw	0.20 mg/kg in grass and straw; 0.05 mg/kg in grain	GC-MS ERC 97.10 and	**CA 4.1.2(e)/2** Clements, B,; Harrington, R. (1997)

Matrix type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
		R-T-M 76	Used for Studies: R96-138, R97-104, S04DAR.BREGG39,JL34, S04DAR,BREGG40,JL35, R96-138, R97-103, S03DAHBOFIX, R97-105 and S03AHBOFIX **Submitted under: CA 6.3.2/2, CA 6.3.2/3, CA 6.3.2/4, CA 6.3.2/5, CA 6.3.3/3, CA 6.3.3/4, CA 6.3.3/5, CA 6.3.3/6 and CA 6.5.3/1**
Wet and Dry Crops	0.01 mg/kg	GC-MS GRM 01.16	**CA 4.1.2(e)/3** Hastings, M (2002) Used for Studies 14SRFR12R03, GHE-P-11274, GHE-P-11274, and GHE-P-11684 **Submitted under: CA 6.3.3/1, CA 6.3.3/2, CA 6.5.3/2, and CA 6.5.3/3**
Ecotoxicology (CA 4.1.2(f))			
AAP Medium	0.100 mg/L	LC-UV	**CA 4.1.2(f)/1** Hoberg, J. R.(2006) Used for study: 060246 **Submitted under: CA 8.2.6.2-1**
Fresh Water Algal Medium	1.20 mg/L	LC-UV	**CA 4.1.2(f)/2** Aufderheide (2015) Used for study (140515) **Submitted under: CA 8.2.6.2-2**
Hard Water	2.0 ppb	LC-MS/MS	**CA 4.1.2(f)/3** Banman, C.S., Moore, S. (2015) Used for study 140735 **Submitted under CA 8.2.7-2**
Physical and Chemical Properties (CA 4.1.2 (g))			
-	-		-

(a) Methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies

See Chapter B.5.2 (b) (soil, water, sediment) and Chapter B.5.2 (c) (air).

(b) Methods in soil, water and any additional matrices used in support of efficacy studies

See Chapter B.5.2 (b) (soil, water).

No new studies were submitted

(c) Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

See Chapter B.5.2 (a) (feed), Chapter B.5.2 (d) (body fluids and tissues) and Chapter B.5.2 (c) (air).

No new studies were submitted

(d) Methods in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies

See Chapter B.5.2 (d) (body fluids) and Chapter B.5.2 (c) (air).

No new studies were submitted

(e) 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

See Chapter B.5.2 (a) (plants, plant products, processed food commodities, food of plant and animal origin, feed).

Report	**CA 4.1.2(e)/1** [redacted] (2002) Studies submitted under **CA 6.1.2/2 (020120)**
Title	Determination of Residues of Clopyralid in Animal Tissues by Gas Chromatography with Negative-Ion Chemical Ionization Mass Spectrometry **Additional information:** Assessment of the analytical method
Analytical Method Study ID	Dow AgroSciences Study Number: GRM 02.14
Performing Laboratory	[redacted]
Guidelines	SANCO/3029/99 rev. 4
GLP	Yes

Method Principle	Residues of clopyralid are extracted from the tissue sample by heating with a 2.5 N sodium hydroxide solution. The sample is allowed to cool before a 1.0-mL aliquot is diluted with 1.0 N hydrochloric acid and purified using an HLB solid-phase extraction (SPE) column. The SPE column is washed with a 1.0-N hydrochloric acid:methanol solution (85: 15 v/v) and eluted with dichloromethane. The eluate is evaporated to dryness and derivatized at 105 ·C with a 1-propanol:concentrated sulfuric acid solution (96:4 v/v). The derivatizing reagent is evaporated and the clopyralid propyl ester is partitioned into hexane containing 0.01 ug/ml, clopyralid butyl ester as an internal standard. The hexane extract is then analyzed by capillary gas chromatography with negative-ion chemical ionization mass spectrometry (GC/NCI-MS). GC Column: Agilent HP-5MS fused silica capillary, HP-5MS liquid phase 30 m x 0.25 mm i.d., 1.0-µm film thickness Carrier Gas: Helium, 15 psi head pressure, 43 cm/s Ions Monitored: Clopyralid propyl ester *m/z* 233 (quantitation ion) *m/z* 235 (confirmation ion)
Recovery	Matrix samples were fortified at 0.01, 0.10, 0.50 and 1 mg/kg. Mean recovery values at each fortification concentration were within the acceptance range of 70-110% (**Tables 1, 2 and 3**). **Table 1. Recovery of Clopyralid in Bovine Tissues**

Matrix	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n
Beef Kidney	0.01	90	NA	2
	0.10	93	NA	1
	0.50	95	NA	1
	1.0	87	NA	1
Beef Liver	0.01	89	NA	2
	0.10	88	NA	1
	0.50	82	NA	1
	1.0	85	NA	1
Beef Fat	0.01	84	NA	2
	0.10	70	NA	1
	0.50	75	NA	1
	1.0	85	NA	1
Milk	0.01	94	NA	2
	0.10	84	NA	1
	0.50	87	NA	1
	1.0	84	NA	1
Beef Tissues	0.01-1.00	86	9	20

Recovery

Table 2. Recovery of Clopyralid in Poultry Tissues

Matrix	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n
Chicken Fat	0.01	93	NA	2
	0.10	86	NA	1
	0.50	92	NA	1
	1.0	83	NA	1
Chicken Muscle	0.01	86	NA	2
	0.10	77	NA	1
	0.50	80	NA	1
	1.0	78	NA	1
Chicken Eggs	0.01	74	NA	2
	0.10	86	NA	1
	0.50	83	NA	1
	1.0	86	NA	1
ChickenTissues Combined	0.01-1.00	84	8	15

Table 3. Recovery of Clopyralid in Bovine and Poultry Tissues

Matrix	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n
Animal Tissues Bovine and Poultry Combined	0.01	88	10	14
	0.10	83	9	7
	0.50	85	8	7
	1.00	84	4	7

Repeatability

Relative standard deviations at each fortification level cannot been defined due to the inadequate number of determinations.

Specificity

The method is selective for the determination of clopyralid by virtue of the chromatographic separation and detection system used. In addition, no control matrix samples had interfering peaks above the LOD of the method.

Limit of Quantitation

The limit of quantitation for clopyralid is 0.01 mg/kg. in animal tissues

Linearity

For the linear least squares regression equations describing the detector response as a function of the standard calibration curve concentrations. The calibration curve was constructed using 8 standards across the range from 0.0001 to 0.05 µg/mL. The correlation coefficients (r^2) were greater than 0.999 for all of the calibration curve determinations during the method validation.

Study Comments: 4.1.2(e)/1	The method is not acceptably validated for the determination of clopyralid in animal matrices. According to SANCO/3029/99 rev. 4, 5 determinations should be made at each fortification level. Here only 1-2 determinations have been performed.
Agreed endpoint: 4.1.2(e)/1	GC/NCI-MS

Report	**CA 4.1.2(e)/2** Clements, B,; Harrington, R. (1997) Studies submitted under **CA 6.3.2/2 (R96-138) CA 6.3.2/3 (R97-104) CA 6.3.2/4 (S04DAR.BREGG39,JL34), CA 6.3.2/5 (S04DAR.BREGG40,JL35), CA 6.3.3/3 (R96-138), CA 6.3.3/4 (R97-103), CA 6.3.3/5 (S03DAHBOFIX), CA 6.3.3/6 (R97-105) and CA 6.5.3/1 (S03AHBOFIX)**
Title	Determination of Residues of MCPA. Clopyralid and Fluroxypyr in Grass and Cereal Grain and Straw **Additional information:** Assessment of the analytical method
Analytical Method Study ID	Dow AgroSciences Study Number: ERC 97.10
Performing Laboratory	Dow AgroSciences, LLC, Letcombe UK
Guidelines	SANCO/3029/99 rev. 4
GLP	Yes
Method Principle	MCPA, clopyralid and fluroxypyr are extracted from grass, grain and straw by macerating and shaking with caustic methanol. An aliquot is acidified and the analytes are partitioned into methyl-tertiary-butyl ether (MTBE) then into aqueous sodium bicarbonate, which is acidified and the analytes are extracted back into MTBE. The organic phase is evaporated to dryness and the residuum treated with 4% v/v concentrated sulphuric acid/n-butanol to form the butyl esters of MCPA, clopyralid and fluroxypyr. Following the addition of water, MCPA, clopyralid and fluroxypyr butyl esters are partitioned into hexane. The hexane extract is then analysed by capillary gas chromatography using mass selective detection. GC column: Hewlett Packard ULTRA 2 capillary column (5% phenyl methyl silicone). Dimensions: 12m x 0.2 rom i.d. and 0.33.um film thickness. Carrier gas: High purity Helium @ 15 psi head pressure Ions Monitored: Quantitation Confirmation MCPA 256.0 258.0 Clopyralid 174.0 176.0 Fluroxypyr 310.0 312.0

Recovery	Matrix samples were fortified at four or more concentrations. Mean recovery values at each fortification concentration were within the acceptance range of 70-110% (**Table 1**). **Table 1. Recovery of Clopyralid (m/z 174.0)**

Matrix	Fortification Level (mg/kg)	Mean Recovery (%)	RSD (%)	n
Grass	0.20	100	7.7	8
	0.50	106	3.1	4
	1.0	97	4.8	4
	2.0	92	7.1	4
	5.0	92	1.9	4
Barley and Wheat Grain	0.05 (barley,wheat)	92	6.9	8
	0.10 (barley)	90	3.9	4
	0.25 (barley,wheat)	100	4.9	4
	0.50 (barley)	92	6.2	4
Barley and Wheat Straw	0.2 (barley,wheat)	92	3.7	8
	0.5 (barley)	91	1.6	4
	1.0 (barley,wheat)	92	2.7	4
	2.0 (barley)	91	1.7	4

Repeatability	Relative standard deviations at each fortification level were below the 20% criterion, demonstrating good repeatability of the method.
Specificity	GC/MS affords a highly specific method for quantitation and confirmation of clopyralid by retention time matching with standards in conjunction with monitoring compound specific-ions. The control matrix samples had no interfering peaks above the LOQ of the method.
Limit of Quantitation	The limit of quantitation for clopyralid is: 0.20 mg/kg in grass and straw 0.05 mg/kg in grain
Linearity	Calibration curves for clopyralid butyl ester were calculated by linear regression of 7 data points over the concentration range of 0.005 - 0.125 µg/rnL. The coefficient of determination (R^2) was 0.9993.

Study Comments: 4.1.2(e)/2	According to SANCO/3029/99 rev. 4, 5 determinations should be made at each fortification level. Although this criteria is not quite fulfilled, the method can be considered to be fit for purpose.
Agreed endpoint: 4.1.2(e)/2	GC/MS

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Report	**CA 4.1.2(e)/3** Hastings, M (2002) Studies submitted under CA 6.3.3/1 (14SRFR12R03) CA 6.3.3/2 (GHE-P-11274), CA 6.5.3/2 (GHE-P-11274) and CA 6.5.3/3 (GHE-P-11684)
Title	Determination of Residues of Clopyralid on Agricultural Crops by Gas Chromatography with Negative-Ion Chemical Ionization Mass Spectrometry **Additional information:** Assessment of the analytical method
Analytical Method Study ID	Dow AgroSciences Study Number: GRM 01.16
Performing Laboratory	Dow AgroSciences, LLC 9330 Zionsville Road Indianapolis, Indiana, 46268, USA
Guidelines	SANCO/3029/99 rev. 4
GLP	Yes
Method Principle	Residues of clopyralid are extracted from the crop sample by homogenizing and shaking with a methanol: 10 N sodium hydroxide solution (100: 1 v/v). The sample is then allowed to stand for 12 hours before a 1.0-mL aliquot is diluted with 1.0 N hydrochloric acid and purified using an HLB solid-phase extraction (SPE) column. The SPE column is washed with a 1.0-N hydrochloric acid:methanol solution (85: 15 v/v) and eluted with dichloromethane. The eluate is evaporated to dryness and derivatized at 105 °C with a l-propanol:concentrated sulfuric acid solution (96:4 v/v). The derivatizing reagent is evaporated and the clopyralid propyl ester is partitioned into hexane containing 0.01 ug/rnl, clopyralid butyl ester as an internal standard. The hexane extract is then analyzed by capillary gas chromatography with negative-ion chemical ionization mass spectrometry (GC/NCI-MS). GC Column: Agilent HP-5MS fused silica capillary, HP-5MS liquid phase 30 m x 0.25 mm i.d., 1.0-µm film thickness Carrier Gas: Helium, 15 psi head pressure, 43 cm/s Ions Monitored: Clopyralid propyl ester *m/z* 233 (quantitation ion) *m/z* 235 (confirmation ion

Recovery	Matrix samples were fortified at 0.01, 0.10 and 1.00 mg/kg. Mean recovery values at each fortification concentration were within the acceptance range of 70-110% (**Table 1**). **Table 1. Recovery of Clopyralid in Crops**

Matrix	Fortification level (mg/kg)	Mean Recovery (%)	RSD (%)	n
Wet	0.01	83	6.6	13
	0.10	84	6.9	13
	1.00	84	6.9	13
Dry	0.01	85	11.7	11
	0.10	79	9.9	11
	1.00	81	8.1	11
High Starch	0.01	86	4.0	3
	0.10	84	6.0	3
	1.00	76	1.3	3
Acidic	0.01	70	NA	1
	0.10	81	NA	1
	1.00	75	NA	1
Oily	0.010	101	NA	1
	0.10	109	NA	1
	1.00	89	NA	1

Repeatability	Relative standard deviations at each fortification level cannot been defined due to the inadequate number of determinations.
Specificity	GC/NCI-MS affords a highly specific method for both quantitation and confirmation of residue identity by retention time matching in conjunction with monitoring two characteristic ions, *m/z* 233 and 235. The control matrix samples had no interfering peaks above the limit of quantitation.
Limit of Quantitation	The limit of quantitation for clopyralid is 0.01 mg/kg.
Linearity	A linear calibration curve resulting from the injection of 8 standard concentrations across the range of 0.0001 to 0.05 µg/mL demonstrates linearity with a correlation coefficient (r^2) of 0.9996.

Study Comments: 4.1.2(e)/3	The method is not acceptably validated for the determination of clopyralid in crop matrices. According to SANCO/3029/99 rev. 4, 5 determinations should be made at each fortification level. Here only 1 determination has been performed for every crop.
Agreed endpoint: 4.1.2(e)/3	GC/NCI-MS

(f) Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

See Chapter B.5.2 (b) (soil, water, sediment) and Chapter B.5.2 (a) (feed).

Report	**CA 4.1.2(f)/1** Hoberg, J.R. (2006) Study submitted under CA 8.2.6.2/1 (060246)
Title	Clopyralid Technical Grade - Growth Inhibition Test with Freshwater Blue-Green Alga (*Anabaena flos-aquae*) **Additional information**: Assessment of the analytical method
Study ID	Springborn Smithers Study Number: 12550.6430 Dow AgroSciences Study Number: 060246
Performing Laboratory	Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts 02571-1037
Guidelines	SANCO/3029/99 rev. 4
GLP	Yes
Method Principle	Methodology was validated to quantify the amount of clopyralid technical grade present in recovery samples prepared in AAP medium (a freshwater algal medium). The recovery samples were acidified with 10% phosphoric acid in purified reagent water and diluted with acetonitrile. The high-level recovery samples were further diluted with 20:80:0.1 acetonitrile:water:phosphoric acid and analyzed by automated injection on a high performance liquid chromatographic system equipped with ultraviolet detection (HPLC/UV). Equipment/conditions: Hewlett Packard LC/UV, wavelength 225 nm LC Column: Phenomenex Hydro-RP, 80 Å, 250 mm x 4.6 mm, 4 µm Mobile phase: 0.1% phosphoric acid in purified reagent water and 100% acetonitrile, gradient elution.
Recovery	The validity of the method was proven by determination of clopyralid in AAP medium specimens at two different fortification levels (0.100 and 100 mg/L). In addition, five control samples were analysed. The mean recoveries were in the range of 70 – 110% at each fortification level. **Table 1. Recovery of Clopyralid in AAP Medium**

Fortification Level (mg/L)	Mean Recovery %	% RSD	n
0.100	101	1.32	5
100	102	0.606	5

Repeatability	Relative standard deviations at each fortification level were below the 20% criterion, demonstrating good repeatability of the method.
Specificity	The method is specific for the determination of clopyralid by virtue of the chromatographic separation and detection system used. No residues of clopyralid above the minimum detectable limit (MDL) of 0.0117 mg a.i./L were found in control samples.
Limit of Quantitation	0.100 mg/L.
Linearity	Calibration curves were calculated using linear regression with 6 data points over the concentration of 0.02 to 0.50 mg/L with a coefficient of determination (r^2) of 0.9997.

Study Comments: 4.1.2(f)/1	The method is acceptably validated for the determination of clopyralid in AAP medium (a freshwater algal medium). The LOQ is fit for this purpose.
Agreed endpoint: 4.1.2(f)/1	HPLC/UV

Report	**CA 4.1.2(f)/2** Aufderheide, J. (2015) Study submitted under CA 8.2.6.2-2 (140515)
Title	Clopyralid Technical: Growth Inhibition Test with the Freshwater Diatom, *Navicula pelliculosa* **Additional information**: Assessment of the analytical method
Study ID	ABC Study Number: 81018 Dow AgroSciences Study Number: 140515
Performing Laboratory	ABC Laboratories, Inc. 7200 E. ABC Lane Columbia, Missouri 65202, USA
Guidelines	SANCO/3029/99 rev. 4
GLP	Yes
Method Principle	Test solutions of clopyralid technical were analyzed using a high performance liquid chromatography system with ultraviolet detection (HPLC-UV). A sample volume of approximately 10 mL was collected from the control and each test substance treatment. Samples were centrifuged for approximately ten minutes at approximately 3,400 rpm to remove the algal biomass. Five mL of the resulting supernatant from each sample was transferred to a culture tube and diluted with 5 mL of methanol. The samples were further diluted as necessary with 50:50 methanol:water to provide final sample concentrations within the analytical standard concentrations range (i.e., 0.00504 to 0.202 mg a.i./L). QC samples were prepared by fortifying dilution medium with clopyralid technical at concentrations of 1.20 and 52.8 mg a.i./L. The samples were vialed prior to analysis by HPLC-UV.

<table>
<tr><td></td><td>Equipment:
Hewlett Packard HPLC system (or equivalent) equipped with an UV detector, wavelength 280 nm, LC Column: YMC pack pro C-18, 150 mm x 4.6 mm, 3 µm, isocratic elution: 40:60, 0.1% formic acid (aq): methanol</td></tr>
<tr><td>Recovery</td><td>The validity of the method was demonstrated by determination of clopyralid in freshwater algal medium with sodium silicate at two different fortification levels (1.2 and 52.8 mg/L). The mean recoveries were in the range of 70 – 110% at each fortification level.

Table 1. Clopyralid in freshwater algal medium with sodium silicate
<table><tr><th>Fortification Level (mg/L)</th><th>Mean % Recovery</th><th>% RSD</th><th>n</th></tr><tr><td>1.20</td><td>105.3</td><td>4.49</td><td>3</td></tr><tr><td>52.8</td><td>109.7</td><td>4.68</td><td>3</td></tr></table></td></tr>
<tr><td>Repeatability</td><td>Relative standard deviations at each fortification level were below the 20% criterion, demonstrating good repeatability of the method.</td></tr>
<tr><td>Specificity</td><td>The method is specific for the individual determination of clopyralid by virtue of the chromatographic separation and the detection system used.

No residues of clopyralid above the minimum quantifiable limit (MQL) of 0.202 mg/L were found in the control samples.</td></tr>
<tr><td>Limit of Quantitation</td><td>1.20 mg/L</td></tr>
<tr><td>Linearity</td><td>Calibration curves were calculated using linear regression with 6 data points over the concentration of 0.05 to 0.20 mg/L with a correlation coefficient of 0.9999.</td></tr>
</table>

Study Comments: 4.1.2(f)/2	The method was successfully validated and met the requirements of SANCO/3029/99 rev. 4, except for the number of procedural recovery sample replicates. However, the method and the LOQ can be considered to be fit for purpose.
Agreed endpoint: 4.1.2(f)/2	HPLC/UV

Report	**CA 4.1.2(f)/3** Banman, C.S., Moore, S (2015) Method used in study CA 8.2.7-2 (140735)
Title	Clopyralid: Toxicity to the Aquatic Macrophyte, *Myriophyllum spicatum* **Additional information**: Assessment of the analytical method
Study ID	Syntech Study Number: 14SRLS14C2 Dow AgroSciences Study Number:140735
Performing Laboratory	SynTech Research Laboratory Services LLC 17745 South Metcalf Avenue Stilwell, Kansas 66085-9104
Guidelines	SANCO/3029/99 rev. 4
GLP	Yes
Method Principle	Test solutions from the study were analyzed to determine the concentrations of clopyralid. Aliquots of water samples (25 mL) were acidified with 1N HCl and clopyralid was extracted using SPE (Waters Oasis HLB). Clopyralid was eluted from the cartridge using dichloromethane. The dichloromethane was then evaporated to dryness and reconstituted in 1.0 mL of methanol/0.1% formic acid (10:90) solution, with sonication and vortexing. Samples were transferred to autosampler vials and 20 µL was injected on a Liquid Chromatography-Mass spectrometry/Mass Spectroscopy (LC-MS/MS). LC-MS/MS: electrospray ionization in negative ion mode, column Thermo Scientific Accucure Phenyl-hexyl, 50 x 4.6 mm, 2.6 µm, gradient elution with MeOH/ACN (60:40) with 0.01% formic acid and water with 0.01% formic acid.

<table>
<tr><td>Recovery</td><td>The validity of the method was demonstrated by determination of clopyralid in fortified hard water specimens at three different fortification levels (2, 20, and 4000 ppb). The mean recoveries were in the range of 70 – 110% at each fortification level.

Table 1. Recovery of Clopyralid in Hard Water
<table><tr><th>Fortification Level (ppb)</th><th>Mean % Recovery</th><th>RSD</th><th>n</th></tr><tr><td>2.0</td><td>94</td><td>4.1</td><td>5</td></tr><tr><td>20</td><td>102</td><td>0.9</td><td>5</td></tr><tr><td>4000</td><td>99</td><td>1.8</td><td>5</td></tr></table></td></tr>
<tr><td>Repeatability</td><td>Relative standard deviations at each fortification level were below the 20% criterion, demonstrating good repeatability of the method.</td></tr>
<tr><td>Specificity</td><td>The method is specific for the individual determination of clopyralid by virtue of the chromatographic separation and selective detection system used. The m/z monitored was:

Clopyralid m/z 189.88/146.00

No residues of clopyralid above the limit of quantification were found in specimens generated from the control group.</td></tr>
<tr><td>Limit of Quantitation</td><td>2.0 ppb</td></tr>
<tr><td>Linearity</td><td>The peak of the LC/MS/MS to clopyralid was linear over a range of 0.025 µg/mL to 0.50 µg/mL (1.0 ppb to 20.0 ppb sample equivalents) (6 x 2 different concentrations). The coefficient of determination of the linearity curve was ≥0.99</td></tr>
</table>

Study Comments: 4.1.2(f)/3	The method is acceptably validated for the determination of clopyralid and the LOQ is fit for this purpose.
Agreed endpoint: 4.1.2(f)/3	LC-MS/MS

(g) Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

Any methods used for physical or chemical properties which are not listed under Chapter B.5.2 are described within each individual study. Refer to study report for specific information on the method used.

No new studies were submitted.

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

The following table provides a justification for the use of a different method to that evaluated for the Active Approval.

Data Point/Method	Rationale
CA 4.2 (a)/1-6	New guidelines: Guidance document on pesticide residue analytical methods (SANCO/825/00 rev 8.1, 16/11/2010)
CA 4.2 (b)/1-4	New guidelines: Guidance document on pesticide residue analytical methods (SANCO/825/00 rev 8.1, 16/11/2010)
CA 4.2 (c)/1	New guidelines: Guidance document on pesticide residue analytical methods (SANCO/825/00 rev 8.1, 16/11/2010)
CA 4.2 (d)/1	New guidelines: Guidance document on pesticide residue analytical methods (SANCO/825/00 rev 8.1, 16/11/2010)

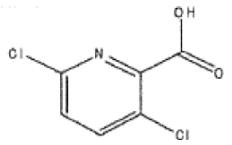
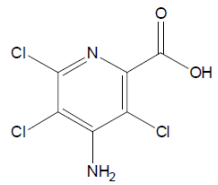
Table 5.2-1: Summary of monitoring methods

Matrix	Agreed Method	Analyte	Limit of Quantification	Proposed Method	Analyte	Limit of Quantification	Reference
Crops - Post Approval							
Dry Crops: Wheat Grain				DAS 120610	Clopyralid and Picloram[1]	0.01 mg/kg	**CA 4.2 (a)/1**
Wet Crops: Wheat Forage				DAS 120610	Clopyralid and Picloram[1]	0.01 mg/kg	**CA 4.2 (a)/1**
Acidic Crops: Orange				DAS 120610	Clopyralid and Picloram[1]	0.01 mg/kg	**CA 4.2 (a)/1**
Oily Crops: Oilseed Rape Seeds				DAS 120610	Clopyralid and Picloram[1]	0.01 mg/kg	**CA 4.2 (a)/1**
Crops – Independent Laboratory Validation							
Wet Crops: Wheat Whole Plant				DAS 120614	Clopyralid and Picloram[1]	0.01 mg/kg	**CA 4.2 (a)/2**
Oily Crops: Oilseed Rape Seeds				DAS 120614	Clopyralid and Picloram[1]	0.01 mg/kg	**CA 4.2 (a)/2**
Animal Tissues – Post Approval							
Kidney, Bovine				DAS 120483	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/3**
Liver, Bovine				DAS 120483	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/3**
Fat, Bovine				DAS 120483	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/3**
Milk, Bovine				DAS 120483	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/3**
Muscle, Bovine				DAS 120483	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/3**
Fat, Poultry				DAS 120483	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/3**
Muscle, Poultry				DAS 120483	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/3**
Liver, Poultry				DAS 120483	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/3**
Egg, Poultry				DAS 120483	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/3**
Animal Tissues – Independent Laboratory Validation							
Muscle, Bovine				DAS 120484	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/4**
Milk, Bovine				DAS 120484	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/4**

Matrix	Agreed Method	Analyte	Limit of Quantification	Proposed Method	Analyte	Limit of Quantification	Reference
Liver, Poultry				DAS 120484	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/4**
Egg, Poultry				DAS 120484	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/4**
Multi-Residue – QuEChERS - Post Approval							
Dry Crop: Rye Grain				DAS 130729	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/5**
Wet Crop: Lettuce				DAS 130729	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/5**
Acidic Crop: Lemon				DAS 130729	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/5**
Oily Crop: Oilseed Rape Seed				DAS 130729	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/5**
Muscle, Bovine				DAS 130729	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/5**
Liver, Bovine				DAS 130729	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/5**
Fat, Bovine				DAS 130729	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/5**
Milk, Bovine				DAS 130729	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/5**
Egg, Poultry				DAS 130729	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/5**
Multi-Residue – QuEChERS - Independent Laboratory Validation							
Wet Crop: Lettuce				DAS 130728	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/6**
Acidic Crop: Lemon				DAS 130728	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/6**
Fat, Bovine				DAS 130728	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/6**
Milk, Bovine				DAS 130728	Clopyralid	0.01 mg/kg	**CA 4.2 (a)/6**
Soil – Post Approval							
Soil				DAS 120612	Clopyralid and Picloram[1]	0.50 µg/kg	**CA 4.2 (b)/1**
Soil – Independent Laboratory Validation							
Soil				DAS 140079	Clopyralid and Picloram[1]	0.50 µg/kg	**CA 4.2 (b)/2**

Matrix	Agreed Method	Analyte	Limit of Quantification	Proposed Method	Analyte	Limit of Quantification	Reference
Water – Post Approval							
Water: Drinking, Ground, Surface				DAS 120611	Clopyralid and Picloram[1]	0.050 µg/L	**CA 4.2 (b)/3**
Water – Independent Laboratory Validation							
Water: Drinking, Ground, Surface				DAS 120613	Clopyralid and Picloram[1]	0.050 µg/L	**CA 4.2 (b)/4**
Air							
Air				DAS 120601	Clopyralid	4.5 µg/m^3	**CA 4.2 (c)/1**
Body Fluids							
Human Urine				DAS 130727	Clopyralid	0.05 mg/L	**CA 4.2 (d)/1**
Human Blood				DAS 130727	Clopyralid	0.05 mg/L	**CA 4.2 (d)/1**

[1] Picloram is included in the method validation summary since both clopyralid and picloram were validated in the same method due to their similarity in structure. For clarity, the results for picloram have been marked with grey colour.

Common Name of Compound	Structural Formula and Chemical Name
Clopyralid Molecular Formula: $C_6H_3Cl_2NO_2$ Formula Weight: 192.00 Nominal Mass: 192 CAS Number 1702-17-6	 3,6-Dichloropicolinic Acid
Picloram Molecular Formula: $C_6H_3Cl_3N_2O_2$ Formula Weight: 241.46 Nominal Mass: 241 CAS Number 1918-02-1	 4-Amino-3,5,6-trichloropyridine-2-carboxylic acid

(a) Methods for the determination of all components included in the monitoring residue definition as submitted in accordance with the provision of Point 6.7.1 in order to enable Member States to determine compliance with established maximum residue levels (MRLs); they shall cover residues in or on food and feed of plant and animal origin

CA 4.2(a)/1 Method for the Determination of Clopyralid in Crop Matrices

Report	**CA 4.2 (a)/1**, Vogl, E. (2012)
Title	Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS
DAS Report Number	120610
Guidelines	SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1 EPA Guideline; OPPTS 860.1340 and PR Notice 2011-3 PMRA Residue Chemistry Guidelines as Regulatory Directive Dir 98-02
GLP	Yes

Matrix, LOQ	Wet Crops –Wheat Forage	0.010 mg/kg
	Dry Crops – Wheat Grain	0.010 mg/kg
	Acidic Crops – Orange	0.010 mg/kg
	Oily Crops – Canola Seed	0.010 mg/kg
Scope	This method is applicable for the quantitative determination of residues of clopyralid and picloram in crop matrices representative of the four European crop groupings (wet crops, dry crops, oily crops, and acidic crops). The method was validated over the concentration range of 0.010-1.0 mg/kg (µg/g) with a verification of the limit of detection at 0.003 mg/kg.	
Principle	Residues of clopyralid are extracted from crop samples with 100:1 methanol:10N sodium hydroxide by blending for approximately of 1 minute and shaking for 1 hour on a reciprocal shaker. The extracts are allowed to set ambient overnight. An aliquot of the extract is submitted to a nitrogen stream to remove the methanol and then brought back to volume with 1N sodium hydroxide. The cleanup for crops is affected by partitioning the basic extract with dichloromethane (DCM). An aliquot of the extract is acidified with HCl and submitted to a polymeric reversed-phase solid phase extraction column (Waters, HLB SPE) cleanup and elution with DCM. After removal of the DCM using nitrogen blow down, the sample is reconstituted in 10:90, methanol:0.1% formic acid. The final extract is filtered through a 0.2-µm PTFE syringe filter and then analyzed by liquid chromatography (Accucore Phenyl-hexyl column, 4.6x50 mm, 2.6 µm; Mobile Phase: A) water containing 0.01% formic acid, B) 60:40 methanol:acetonitrile containing 0.01% formic acid, gradient elution) coupled with negative-ion electrospray ionization tandem mass spectrometry (ESI LC/MS/MS).	

Linearity	Linear regression analysis with 1/x weighting was used to describe the detector response as a function of the calibration standard concentrations. For the least squares regression equations describing the detector response as a function of the standard calibration curve concentrations, the correlation coefficients (r) were greater than or equal to 0.990 for all of the calibration curve determinations during the method validation. The results indicate linearity of the detector response as a function of the standard concentration.
Validation	The method validation study was conducted to determine the recovery levels and the precision of the method for the determination of residues of clopyralid and picloram in crop matrices. The performance of the analytical method was determined with each set of samples by fortifying aliquots of the appropriate control matrix with a mixed clopyralid and picloram solution and analyzing the set following the procedures described within this report. Samples were fortified at the limit of detection (LOD) of 0.003 mg/kg the limit of quantitation (LOQ) of 0.010 mg/kg, and at 1.0 mg/kg. Samples fortified at the LOD were analyzed only to demonstrate observable peaks at the LOD level; the results were not included for average percent recovery calculations. An unfortified control matrix and reagent blank were also included in each set. For the quantitation results, the individual recoveries for all samples fell within the range of 70 to 110%, with the exception of one 1 mg/kg clopyralid fortification for oranges at 68%. The average recoveries at each fortification level in each crop matrix group also fell within the range of 70 to 110%. The average recoveries for all fortification levels in each crop matrix group fell within the range of 70 to 110%. Relative standard deviations at each fortification level were all less than 15%.

Selectivity/ Confirmation	The method is selective for the determination of clopyralid and picloram by virtue of the chromatographic separation and MS/MS detection system used. Using published guidelines, when detection is by tandem mass spectrometry methods, confirmation of the presence of the analyte should require the observation of a precursor ion plus one structurally significant product ion observed at the same retention time. MS/MS ion transitions monitored are: Clopyralid *m/z* Q1/Q3 190/146 (quantitation) *m/z* Q1/Q3 192/148 (confirmation) Picloram *m/z* Q1/Q3 241/197 (quantitation) *m/z* Q1/Q3 239/195 (confirmation) By monitoring multiple MS/MS ion transitions for each analyte, the confirmation ratios were calculated for each analyte in each sample set and compared to the average for the calibration standards. The confirmation ratios for each analyte in all sample matrices were within ±20% of the average found for the standards (with the exceptions of one slightly higher than 20% for the determination of picloram in canola seed fortified at 0.010 mg/kg and one slightly higher than 20% for the determination of picloram in orange fortified at 0.003 mg/kg), indicating that the method is selective for the determination of clopyralid and picloram in crops.
Solution and Sample Extract Stability	Results indicate that clopyralid and picloram fortification solutions prepared in methanol and clopyralid and picloram calibration standard solutions prepared in a 0.1% formic acid:methanol (90:10) solution are stable for at least 13 days when stored under refrigerated conditions. Results indicate that sample extracts containing clopyralid and picloram are stable for at least 12 days when stored under refrigerated conditions.
Matrix Effects	For clopyralid quantitation samples, the matrix effects ranged from -6.3% to 4.0% across all matrices. For clopyralid confirmatory samples, matrix effects ranged from -4.6% to 3.1% across all matrices. For picloram quantitation samples, the matrix effects ranged from -4.2% to 4.5% across all matrices. For picloram confirmatory samples, matrix effects ranged from -6.5% to 2.5% across all matrices. Therefore, no significant matrix effects were observed in any of the crops studied.

Extraction Efficiency	For clopyralid, no new extraction efficiency studies were conducted in conjunction with the validation of the new residue analytical method for the determination of residues of clopyralid. The same alkaline extraction solution and extraction procedure used in previous methods and nature of residue studies for the determination of residues of clopyralid in agricultural commodities were used in this method. For picloram, a new extraction efficiency study (DAS Study ID110573, Shackelford, D.D., 2012) was conducted in conjunction with the validation of the new residue analytical method for the determination of residues of picloram. The same alkaline extraction solution and extraction procedure used in the extraction efficiency study for the determination of residues of picloram in agricultural commodities was used in this method

Recovery of Clopyralid in Crops (m/z 190/146) - Quantitation

Matrix Group	Matrix	Fortification Level (mg/kg)	Recovery (%)		RSD (%)	n
			mean	range		
Oily	Canola Seed	0.01	82	74-90	7.6	5
		1.00	79	78-81	1.8	5
Wet	Wheat Forage	0.01	85	77-91	8.2	5
		1.00	81	74-88	8.1	5
Acidic	Orange	0.01	88	84-97	5.7	5
		1.00	80	68-85	8.5	5
Dry	Wheat Grain	0.01	81	79-82	1.5	5
		1.00	83	74-91	8.0	5

Recovery of Clopyralid in Crops (m/z 192/148) - Confirmation

Matrix Group	Matrix	Fortification Level (mg/kg)	Recovery (%)		RSD (%)	n
			mean	range		
Oily	Canola Seed	0.01	81	75-91	8.1	5
		1.00	80	76-83	3.1	5
Wet	Wheat Forage	0.01	87	78-97	8.1	5
		1.00	81	77-85	4.5	5
Acidic	Orange	0.01	85	80-95	6.7	5
		1.00	80	69-86	7.9	5
Dry	Wheat Grain	0.01	80	77-84	3.3	5
		1.00	82	73-92	10.0	5

Recovery of Picloram in Crops (m/z 241/197) - Quantitation

Matrix Group	Matrix	Fortification Level (mg/kg)	Recovery (%)		RSD (%)	n
			mean	range		
Oily	Canola Seed	0.01	80	71-94	11.7	5
		1.00	83	79-86	3.5	5
Wet	Wheat Forage	0.01	83	81-87	3.2	5
		1.00	84	80-87	3.4	5
Acidic	Orange	0.01	80	83-98	7.9	5
		1.00	87	74-92	8.6	5
Dry	Wheat Grain	0.01	85	81-93	5.5	5
		1.00	89	75-99	11.2	5

Recovery of Picloram in Crops (m/z 239/195) - Confirmation

Matrix Group	Matrix	Fortification Level (mg/kg)	Recovery (%)		RSD (%)	n
			mean	range		
Oily	Canola Seed	0.01	78	65-90	11.7	5
		1.00	82	77-87	5.3	5
Wet	Wheat Forage	0.01	89	86-94	3.4	5
		1.00	88	79-94	7.3	5
Acidic	Orange	0.01	88	81-94	7.1	5
		1.00	84	67-90	11.4	5
Dry	Wheat Grain	0.01	89	84-94	4.6	5
		1.00	88	77-100	10.5	5

Study Comments: 4.2(a)/1	Specificity: Highly specific method Linearity: Linear over the concentration range of 0.5 to 50 ng/mL (0.0025-0.25 mg/kg) for clopyralid (5 different concentrations) Correlation coefficient $r \geq 0.990$ Precision: RSD =1.5–10 % Accuracy: Mean recovery 79 – 88 % According to SANCO/825/00 rev. 8.1, recovery and precision data must be reported for the fortification levels LOQ and 10 x LOQ. Here the fortification level 10 x LOQ is missing, and the level 100 x LOQ has been used instead. Otherwise the method is acceptably validated and suitable for the determination of clopyralid in wheat forage (wet crops), wheat grain (dry crops), orange (acidic crops) and canola seed (oily crops).
Agreed endpoint: 4.2(a)/1	LC-MS/MS, LOQ for clopyralid was established at 0.01 mg/kg for wheat forage (wet crops), wheat grain (dry crops), orange (acidic crops) and canola seed (oily crops).

CA 4.2(a)/2 Independent Laboratory Validation of the Enforcement Method for the Determination of Clopyralid in Crop Matrices

Report	**CA 4.2 (a)/2**, Austin, R. (2012)
Title	Independent Laboratory Validation of Dow AgroSciences Method 120610, "Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS"
DAS Report Number	120614
Guidelines	EC Regulation No. 1107/2009 (21-Oct-09) repealing Directive 91/414/EEC SANCO/825/00 rev. 8.1, (16-Nov-10) EPA Guideline; OPPTS 860.1340, PR Notice 96-1 and PR Notice 2011-3
GLP	Yes

Matrix, LOQ	Wet Crop – Wheat Whole Plant 0.01 mg/kg Oily Crop – Oilseed Rape Seed 0.01 mg/kg
Scope	The objective of this study was to assess and to independently validate the Dow AgroSciences Method 120610, "Method Validation Study for the Determination of Residues of Clopyralid and picloram in Agricultural Commodities by LC-MS/MS". The methodology was successfully independently validated over the concentration range of 0.01 - 0.1 mg/kg with a verification of the limit of quantification of 0.010 mg/kg. Plant matrices were represented by wheat (whole plant) (commodity with high water content) and oilseed rape (seeds) (commodity with high oil content).
Principle	Residues of clopyralid and picloram are extracted from crop samples with methanol/10 N sodium hydroxide (100:1) by blending for approximately 1 minute and shaking for 1 hour on a reciprocal shaker. The extracts are allowed to stand at room temperature overnight. An aliquot of the extract is submitted to a nitrogen stream to remove the methanol and then brought back to volume with 1 N sodium hydroxide. The clean-up for crops is performed by partitioning the basic extract with dichloromethane. An aliquot of the extract is acidified with hydrochloric acid and submitted to a solid phase extraction column (Waters HLB) clean-up and elution with dichloromethane. After removal of the dichloromethane using nitrogen blow down, the sample is reconstituted in methanol/0.1% formic acid (10:90). The final extract is filtered through a 0.2-µm PTFE syringe filter and then analyzed by liquid chromatography (Accucore Phenyl-hexyl column, 4.6x50 mm, 2.6 µm; Mobile Phase: A) water containing 0.01% formic acid, B) 60:40 methanol:acetonitrile containing 0.01% formic acid, gradient elution) coupled with negative-ion electrospray ionization tandem mass spectrometry (ESI LC-MS/MS).

Linearity	Linear regression with 1/x weighting was used to describe the detector response as a function of the standard calibration curve concentrations, and the correlation coefficients (r) were always greater than or equal to 0.995 for all of the calibration curve determinations during the method validation study.
Validation	The method independent validation study was conducted to determine the recovery levels and the precision of the method for the determination of residues of clopyralid and picloram in crop matrices. The performance of the analytical method was determined with each set of samples by fortifying aliquots of the appropriate control matrix with a mixed clopyralid and picloram solution and analyzing the set following the procedures described within this report. Samples were fortified at the limit of detection (LOD) of 0.003 mg/kg (µg/g), the limit of quantitation (LOQ) of 0.01 mg/kg and at 0.1 mg/kg. Samples fortified at the LOD were analyzed only to demonstrate observable peaks at the LOD level. Average recoveries at each fortification level were all within the acceptance range of 70-120 %. The relative standard deviation (RSD) did not exceed 20 % at any fortification level for either of the analytes. These results comply with the standard acceptance criteria of SANCO Guideline 825/00 rev 8.1 which specifies that the mean recovery for both fortification levels should be in the range of 70 - 120 % with a relative standard deviation of ≤ 20 %.
Selectivity/ Confirmation	The method is selective for the determination of clopyralid and picloram by virtue of the chromatographic separation and MS/MS detection system used. Using published guidelines, when detection is by tandem mass spectrometry methods, confirmation of the presence of the analyte should require the observation of a precursor ion plus one structurally significant product ion observed at the same retention time. MS/MS ion transitions monitored are: Clopyralid *m/z* Q1/Q3 190/146 (quantitation) *m/z* Q1/Q3 192/148 (confirmation) Picloram *m/z* Q1/Q3 241/197 (quantitation) *m/z* Q1/Q3 239/195 (confirmation) Interferences were < 30 % of the LOQ.
Matrix Effects	The effect of plant matrices on the LC-MS/MS signal was assessed by preparing standards in the presence of matrix and comparing the peak areas of the analytes prepared in matrix against solvent based standards at an equivalent concentration. Matrix effects were less than 10% for clopyralid and picloram in wheat (whole plant) and oilseed rape (seeds). Matrix effects in the crops tested were deemed acceptable since matrix effects less than 20% were observed.

Recovery of Clopyralid in Crops (m/z 190/146) - Quantitation

Matrix Group	Matrix	Fortification Level (mg/kg)	Recovery (%)		RSD (%)	n
			mean	range		
Wet/Oily Crop	Wheat, Whole Plant	0.01	83	79-89	4.8	5
		0.10	70	60-75	8.6	5
	Oilseed Rape, Seeds	0.01	77	73-79	3.3	5
		0.10	75	74-78	2.0	5

Recovery of Clopyralid in Crops (m/z 192/148) - Confirmation

Matrix Group	Matrix	Fortification Level (mg/kg)	Recovery (%)		RSD (%)	n
			mean	range		
Wet/Oily Crop	Wheat, Whole Plant	0.01	79	75-86	5.6	5
		0.10	70	60–76	8.8	5
	Oilseed Rape, Seeds	0.01	80	75–83	4.4	5
		0.10	74	72-77	2.6	5

Recovery of Picloram in Crops (m/z 241/197) - Quantitation

Matrix Group	Matrix	Fortification Level (mg/kg)	Recovery (%)		RSD (%)	n
			mean	range		
Wet/Oily Crop	Wheat, Whole Plant	0.01	83	77-92	6.7	5
		0.10	85	78-92	7.4	5
	Oilseed Rape, Seeds	0.01	73	72-75	1.8	5
		0.10	76	73-80	3.6	5

Recovery of Picloram in Crops (m/z 239/195) - Confirmation

Matrix Group	Matrix	Fortification Level (mg/kg)	Recovery (%)		RSD (%)	n
			mean	Range		
Wet/Oily Crop	Wheat, Whole Plant	0.01	83	79-87	4.5	5
		0.10	83	77–89	6.0	5
	Oilseed Rape, Seeds	0.01	84	81–90	4.4	5
		0.10	76	73-80	3.9	5

Study Comments: 4.2(a)/2	Specificity: No interferences > 30% LOQ, highly specific method Linearity: Linear over the concentration range of 0.5 to 50 ng/mL for clopyralid (5 different concentrations) Correlation coefficient r $\geq$ 0.995 Precision: RSD = 2.0–8.8 % Accuracy: Mean recovery 70 – 83 % The ILV is acceptably validated and suitable for the determination of clopyralid in wheat whole plant (wet crops) and oilseed rape seed (oily crops).
Agreed endpoint: 4.2(a)/2	LC-MS/MS, LOQ for clopyralid was established at 0.01 mg/kg for wheat whole plant (wet crops) and oilseed rape seed (oily crops).

CA 4.2(a)/3 Method for the Determination of Clopyralid in Animal Matrices

Report	**CA 4.2 (a)/3**, [redacted] (2012)
Title	Method Validation Study for the Determination of Residues of Clopyralid in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection
Das Report Number	120483
Guidelines	European Commission Guidance Document on Residue Analytical Methods, Section 3 of SANCO/3029/99 rev. 4, Section 2 of SANCO/825/00 rev. 8.1, EPA Guideline; OPPTS 860.1340, PMRA Residue Chemistry Guidelines as Regulatory Directive Dir 98-02
GLP	Yes

Matrix, LOQ	Bovine Muscle, Fat, Kidney, Liver and Milk 0.010 mg/kg Poultry Eggs, Fat, Muscle and Liver 0.010 mg/kg
Scope	This method is applicable for the quantitative determination of residues of clopyralid in animal matrices. The method was validated over the concentration range of 0.003-1.0 mg/kg with a validated limit of quantitation of 0.010 mg/kg.
Principle	Residues of clopyralid are extracted from animal tissue samples with 2.5N NaOH with heating at approximately 105 °C for a minimum of 2 hours. Optional cleanup for poultry liver is affected by partioning the basic extract with dichloromethane (DCM). An aliquot of the extract is acidified with HCl and submitted to a polymeric reversed-phase solid phase extraction column (Waters, HLB SPE) cleanup and elution with DCM. After removal of the DCM using nitrogen blow down, the sample is reconstituted in 10:90, acetonitrile:0.1% formic acid. The final extract is filtered through a 0.2-µm PTFE syringe filter and then analyzed by liquid chromatography (Accucore Phenyl-hexyl column, 4.6x50 mm, 2.6 µm; Mobile Phase: A) water containing 0.01% formic acid, B) 60:40 methanol:acetonitrile containing 0.01% formic acid, gradient elution) coupled with negative-ion electrospray ionization tandem mass spectrometry (ESI LC/MS/MS).

Linearity	Linear regression analysis with 1/x weighting was used to describe the detector response as a function of the calibration standard concentrations. For the least squares regression equations describing the detector response as a function of the standard calibration curve concentrations, the correlation coefficients (r) were greater than or equal to 0.995 for all of the calibration curve determinations during the method validation. The results indicate linearity of the detector response as a function of the standard concentration.
Validation	The method validation study was conducted to determine the recovery levels and the precision of the method for the determination of residues of clopyralid in animal matrices. The performance of the analytical method was determined with each set of samples by fortifying aliquots of the appropriate control matrix with a clopyralid solution and analyzing the set following the procedures described within this report. Samples were fortified at the limit of detection (LOD) of 0.003 mg/kg the limit of quantitation (LOQ) of 0.010 mg/kg, and at 1.0 mg/kg. Samples fortified at the LOD were analyzed only to demonstrate observable peaks at the LOD level; the results were not included for average percent recovery calculations. An unfortified control matrix and reagent blank were also included in each set. For the quantitation results, the individual recoveries for all samples fell within the range of 70 to 110% and the average recoveries at each fortification level in each animal tissue matrix group also fell within the range of 70 to 110%. The average recoveries for all fortification levels in each animal tissue matrix group fell within the range of 70 to 110%. Relative standard deviations at each fortification level were all less than 20%. These results comply with the standard acceptance criteria of SANCO Guideline 825/00 rev 8.1.

Selectivity/ Confirmation	The method is selective for the determination of clopyralid by virtue of the chromatographic separation and MS/MS detection system used. Using published guidelines, when detection is by tandem mass spectrometry methods, confirmation of the presence of the analyte should require the observation of a precursor ion plus one structurally significant product ion observed at the same retention time. MS/MS transitions monitored are: Clopyralid *m/z* Q1/Q3 190/146 (quantitation) *m/z* Q1/Q3 192/148 (confirmation) By monitoring multiple MS/MS ion transitions for each analyte, the confirmation ratios were calculated for each analyte in each sample set and compared to the average for the calibration standards. The confirmation ratios for each analyte in all sample matrices were within ±20% of the average found for the standards (with one exception higher than 20% for the determination of clopyralid in poultry eggs fortified at 0.010 mg/kg), indicating that the method is selective for the determination of clopyralid in animal tissue.
Solution and Sample Extract Stability	The stability of the fortification solutions and the calibration standards was demonstrated in this study. The results indicate that clopyralid solutions prepared in methanol and clopyralid calibration standards and spiking solutions prepared in a formic acid:acetonitrile (90:10) solution are stable for at least 53 days when stored under refrigerated conditions. Results indicate that final sample extracts containing clopyralid are stable for at least 11 days when stored under refrigerated conditions.
Matrix Effects	For clopyralid quantitation samples, the matrix effects ranged from -3.1% to 2.7% across all matrices. For clopyralid confirmation samples, matrix effects ranged from -1.6% to 3.6% across all matrices. No significant matrix effects were observed in the animal matrices studied.
Extraction Efficiency	No new extraction efficiency studies were conducted in conjunction with the validation of this residue analytical method. According to the notifier, the extraction efficiency for clopyralid using heated alkaline extraction was previously determined and deemed acceptable using in-grown poultry and bovine tissues (Poultry Tissue DAS Report ID: GH-C 752, [redacted] 1974 and Bovine Tissue DAS Report ID: GH-C 811, [redacted] 1975).

Recovery of Clopyralid in Animal Tissues (m/z 190/146) – Quantitation

Matrix	Fortification Level (mg/kg)	Recovery (%)		RSD (%)	n
		mean	range		
Bovine Muscle	0.01	87	74-95	9.2	5
	1.00	91	89-94	2.0	5
Bovine Fat	0.01	80	73-87	7.6	5
	1.00	82	80-85	2.7	5
Bovine Liver	0.01	81	79-82	1.6	5
	1.00	87	85-89	2.0	5
Bovine Kidney	0.01	89	85-93	3.8	5
	1.00	90	87-92	2.1	5
Bovine Milk	0.01	82	78-89	5.4	5
	1.00	82	80-84	2.1	5
Poultry Muscle	0.01	84	78-88	4.8	5
	1.00	87	85-89	2.1	5
Poultry Fat	0.01	90	80-94	6.6	5
	1.00	89	87-91	2.1	5
Poultry Liver	0.01	77	75-80	2.9	5
	1.00	87	83-89	2.6	5
Poultry Eggs	0.01	86	79-91	4.9	5
	1.00	88	80-99	9.1	5

Recovery of Clopyralid in Animal Tissues (m/z 192/148) – Confirmation

Matrix	Fortification Level (mg/kg)	Recovery (%)		RSD (%)	n
		mean	range		
Bovine Muscle	0.01	85	74-100	13.1	5
	1.00	90	89-94	2.7	5
Bovine Fat	0.01	92	79-103	10.9	5
	1.00	82	80-85	2.8	5
Bovine Liver	0.01	87	80-96	7.8	5
	1.00	87	84-88	1.7	5
Bovine Kidney	0.01	88	83-95	5.3	5
	1.00	88	86-91	2.2	5
Bovine Milk	0.01	80	76-92	8.5	5
	1.00	82	79-84	2.3	5
Poultry Muscle	0.01	93	80-100	7.0	5
	1.00	89	86-92	2.6	5
Poultry Fat	0.01	88	81-94	5.6	5
	1.00	89	88-92	1.7	5
Poultry Liver	0.01	69	65-80	8.6	5
	1.00	87	81-90	4.0	5
Poultry Eggs	0.01	106	94-136	16.3	5
	1.00	88	79-99	9.8	5

Study Comments: 4.2(a)/3	Specificity: Highly specific method Linearity: Linear over the concentration range of 0.4 to 50 ng/mL (0.0027-0.33 mg/kg) for clopyralid (5 different concentrations) Correlation coefficient $r \geq 0.995$ Precision: RSD = 1.6–9.2 % (quantifying transition) RSD = 1.7–16.3 % (confirmatory transition) Accuracy: Mean recovery 77 – 91 % (quantifying transition) Mean recovery 69 – 106 % (confirmatory transition) According to SANCO/825/00 rev. 8.1, recovery and precision data must be reported for the fortification levels LOQ and 10 x LOQ. Here the fortification level 10 x LOQ is missing, and the level 100 x LOQ has been used instead. Otherwise the method is acceptably validated and suitable for the determination of clopyralid in bovine muscle, fat, kidney, liver and milk as well as poultry eggs, fat, muscle and liver.
Agreed endpoint: 4.2(a)/3	LC-MS/MS, LOQ for clopyralid was established at 0.01 mg/kg for bovine muscle, fat, kidney, liver and milk as well as poultry eggs, fat, muscle and liver.

CA 4.2(a)/4 Independent Laboratory Validation of the Method for the Determination of Residues of Clopyralid in Animal Matrices

Report	**CA 4.2 (a)/4**, [redacted] (2012)
Title	Independent Laboratory Validation of an Analytical Method for the Determination of Clopyralid in Animal Matrices
DAS Report Number	120484
Guidelines	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21. October 2009, European Commission Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4, EPA Guideline; OPPTS860.1340, PR Notice 96-1 and PR Notice 2011-3
GLP	Yes

Matrix, LOQ	Bovine Milk and Muscle	0.01 mg/kg
	Poultry Liver and Egg	0.01 mg/kg
Scope	This study was conducted to provide independent laboratory validation data for the determination of residues of clopyralid in bovine muscle, milk, chicken liver and eggs, following the analytical method, Dow AgroSciences LLC, Study Number 120483, "Method Validation Study for the Determination of Residues of Clopyralid in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection" (**CA 4.2 (a)/3**). The validated limit of quantification was 0.01 mg/kg, for bovine muscle, milk, chicken liver and eggs samples.	
Principle	Specimens were assayed according to the analytical method Dow AgroSciences Study Number 120483, "Method Validation Study for the Determination of Residues of Clopyralid in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection". The method was internally referenced at [redacted] under the number AGR/MOA/CLOP-1. The animal sample matrices were extracted with 2.5 N NaOH at 105°C for 2 hours. When sample was back to room temperature, the extract was centrifuged. A partition with dichloromethane was carried out (for poultry liver only). An aliquot of extract was acidified with 1 N HCl before purification with a Waters Oasis HLB (0.2g/6mL) SPE cartridge. The eluate was then evaporated to dryness and dissolved in acetonitrile/0.1% formic acid solution (10/90, v/v) prior to quantification by ESI LC-MS/MS (Accucore Phenyl-hexyl column, 4.6x50 mm, 2.6 µm; Mobile Phase: A) ultra-pure water containing 0.01% formic acid, B) 60:40 methanol:acetonitrile containing 0.01% formic acid, gradient elution).	

Linearity	Single determinations of the calibration standards at 7 different concentration levels in matrix extracts were made over the concentration range 0.4 ng/mL to 50 ng/mL. The linearity of response of the analytical instrumentation to clopyralid over this concentration range was acceptable, with a coefficient of determination (r^2) of better than 0.995 for all analytical determinations. This calibration range covers from 30% of the LOQ to at least 20% above the highest measured gross concentration level found in the samples.
Validation	For the independent validation of the method, for bovine muscle, milk, chicken liver and eggs, after fortification with the analytes, the following specimens were analysed by LC-MS/MS: ▪ 5 specimens fortified at the LOQ level of 0.01 mg/kg ▪ 5 specimens fortified at 10 LOQ level of 0.1 mg/kg ▪ 2 unfortified, untreated control specimens ▪ 1 specimen fortified at the LOD level of 0.003 mg/kg ▪ 1 reagent blank, taken through sample clean-up with the samples All of the individual recovery values as well as the average recoveries at each fortification level in all of the animal samples were within the acceptable range of 70-120% for clopyralid. The relative standard deviation (RSD) never exceeded ± 20% at any fortification level. There were no interferences present greater than 30% of the LOQ seen in the chromatograms of the untreated control samples for the quantification or confirmatory transitions in any of the blank and unfortified specimens.
Selectivity/ Confirmation	The LC-MS/MS method is highly selective for the determination of residues of clopyralid in bovine muscle, milk, chicken liver and eggs by virtue of the chromatographic separation and selective detection system used. To demonstrate further confirmation, a second MS/MS ion transition was monitored for clopyralid. Calculations of %Recovery and %RSD were carried out on the confirmation ions data and passed the same acceptance criteria as the quantitation ion data. MS/MS ion transitions monitored: Clopyralid *m/z* Q1/Q3 190/146 (quantitation) *m/z* Q1/Q3 192/148 (confirmation)
Matrix Effects	The matrix effects were assessed on both transitions by comparing a standard prepared in the presence of matrix with a non-matrix standard at the same concentration (equivalent to the LOQ). Matrix effects ranged from 28.3% to -26.5% for the quantitation transition and from 28.7% to -19.8% for the confirmation transition.

Recovery of Clopyralid (m/z 190/146) in Animal Tissue – Quantitation

Matrix	Fortification Level (µg/L)	Number of Samples	Recovery Range (%)	Mean (%)	RSD %
Bovine Muscle	0.01	5	88-93	90	2
	0.10	5	80-90	87	5
Bovine Milk	0.01	5	90- 96	92	3
	0.10	5	88- 94	93	3
Poultry Liver	0.01	5	89-94	92	2
	0.10	5	90-99	97	4
Poultry Egg	0.01	5	79-86	82	3
	0.10	5	81-93	88	5

Recovery of Clopyralid (m/z 192/148) in Animal Tissue – Confirmation

Matrix	Fortification Level (µg/L)	Number of Samples	Recovery Range (%)	Mean (%)	RSD %
Bovine Muscle	0.01	5	87-91	89	2
	0.10	5	80-91	88	5
Bovine Milk	0.01	5	90-95	93	2
	0.10	5	88-95	92	3
Poultry Liver	0.01	5	88-96	92	4
	0.10	5	91-101	97	4
Poultry Egg	0.01	5	81-90	85	4
	0.10	5	81-91	87	5

Study Comments: 4.2(a)/4	Specificity: No interferences > 30% LOQ, highly specific method Linearity: Linear over the concentration range of 0.4 to 50 ng/mL for clopyralid (7 different concentrations) Coefficient of determination $r^2 \geq 0.995$ Precision: RSD = 2–5 % (quantifying transition) RSD = 2–5 % (confirmatory transition) Accuracy: Mean recovery 82 – 97 % (quantifying transition) Mean recovery 85 – 97 % (confirmatory transition) The ILV is acceptably validated and suitable for the determination of clopyralid in bovine muscle and milk as well as poultry eggs and liver.
Agreed endpoint: 4.2(a)/4	LC-MS/MS, LOQ for clopyralid was established at 0.01 mg/kg for bovine muscle and milk as well as poultry eggs and liver.

CA 4.2(a)/5 Multiresidue Method Testing for the Determination of Clopyralid in Crop and Animal Matrices

Report	**CA 4.2 (a)/5**, [redacted] (2013)
Title	Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin
DAS Report Number	130729
Guidelines	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21-Oct-2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, SANCO/825/00 rev. 8.1, OECD ENV/JM/MONO(2007)17 Guidance document on pesticide residue analytical methods, 13 Aug 2007
GLP	Yes

Matrix , LOQ	Dry crop – Rye Grain	0.010 mg/kg
	Wet crop - Lettuce	0.010 mg/kg
	Oily crop - Oilseed Rape Seed	0.010 mg/kg
	Acidic crop - Lemon	0.010 mg/kg
	Bovine Milk	0.010 mg/kg
	Poultry Eggs	0.010 mg/kg
	Bovine Meat	0.010 mg/kg
	Bovine Fat	0.010 mg/kg
	Bovine Liver	0.010 mg/kg
Scope	The study objective was to validate an analytical multi-residue method for the determination of clopyralid following the QuEChERS sample preparation technique followed by analysis using liquid chromatography with tandem mass spectrometric detection. The study was performed in four different matrices of plant origin as represented by rye (grain) which is a dry commodity (high protein/high starch content), lettuce (head) which is a commodity with high water content, lemon (whole fruit) which is a commodity with high acid content and oilseed rape (seeds) which is a commodity with high oil content and in five matrices of animal origin (represented by bovine whole milk, poultry eggs, bovine meat, bovine fat and bovine liver) in accordance with the EC guidance document SANCO/825/00 rev. 8.1. The intended limit of quantitation (LOQ) was 0.01 mg/kg for clopyralid in all nine matrices of plant and animal origin.	

Principle	Specimen material is extracted with acetonitrile followed by the addition of a mixture of citrate salts. The specimens of bovine fat are melted completely by heating at approximately 60 °C in a water bath after adding acetonitrile and before adding the citrate salts. The samples are shaken. For samples of lemon (whole fruit) only, 600 µL 5 M sodium hydroxide is additionally added. The extracts are centrifuged. For oilseed rape (seeds) and bovine fat only, an additional clean-up of the acetonitrile phase is done by freezing out. Clean-up with PSA (primary/secondary amine) was omitted since clopyralid is a carbonic acid and the risk would be that it interacts with the PSA sorbent. For determination of clopyralid in all matrices of plant and animal origin, an aliquot of the supernatant is evaporated to dryness and reconstructed in acetonitrile / water plus 0.1 % formic acid (1/9, v/v). All sample extracts fortified at 100x LOQ level were diluted 1:10 with solvent acetonitrile / water plus 0.1 % formic acid (1/9, v/v) prior to analysis to keep them in the range of the calibration curve. The final sample extract as it was prepared for analysis is analyzed for residues of clopyralid by positive-ion electrospray ionization tandem mass spectrometry LC-MS/MS (Accucore Phenyl-hexyl column, 4.6x50 mm, 2.6 µm; Mobile Phase: A) methanol containing 0.1% formic acid, B) water containing 0.1% formic acid, gradient elution).
Linearity	The linearity of the detector response was confirmed for solvent-based standard solutions and for matrix-matched standards of oilseed rape (seeds). All coefficients of determination (R^2) in all of the sample sets were ≥ 0.996 for the least squares equation which describes the detector response as a function of standard curve concentration. The solvent-based calibration standards were used for the determination of recoveries in all matrix types with the exception of the determination of residues of clopyralid in oilseed rape (seeds) for which matrix-matched standards were used. The calibration curves always covered the range of no greater than ≤ 30 % of the LOQ to at least 20 % above the highest analysed sample extract concentration level as described in SANCO Guideline 825/00 rev. 8.1.

Validation	The accuracy of the method was determined by comparing theoretical and found residue levels in the recovery experiments. Determination of the relative standard deviation of the recoveries allowed for assessing the method's precision with regard to the repeatability. For clopyralid in matrices of lettuce (head), lemon (whole fruit), bovine whole milk and bovine fat, the mean recovery values at the fortification level of 0.01 mg/kg (LOQ) for both mass transitions are between 70 % and 93 % with relative standard deviations ≤ 8.3 %. At the fortification level of 1.0 mg/kg (100x LOQ), all mean recovery values, for these matrices and for both mass transitions, are between 73 % and 87 % with relative standard deviations ≤ 6.9 %. These results comply with the standard acceptance criteria of SANCO Guideline 825/00 rev 8.1 which specifies that the mean recovery for the lower fortification level should be in the range of 70 - 120 % with a relative standard deviation of < 20 % and for the 100x LOQ level (1.0 mg/kg) in the range of 70 - 110 % with a relative standard deviation of < 15 %. For clopyralid in matrices of rye (grain), oilseed rape (seeds), poultry eggs, bovine meat and bovine liver, the mean recovery values at the fortification level of 0.01 mg/kg (LOQ) for both mass transitions are between 34 % and 69 % with relative standard deviations ≤ 20 %. At the fortification level of 1.0 mg/kg (100x LOQ), all mean recovery values, for all matrices and for both mass transitions, are between 38 % and 64 % with relative standard deviations ≤ 7.7 %. **While the relative standard deviations did not exceed 20 %, the use of the multi-residue method following the QuEChERS sample preparation technique for the determination of clopyralid in matrices of rye (grain), oilseed rape (seeds), poultry eggs, bovine meat and bovine liver does not yield acceptable results as outlined in SANCO Guideline 825/00 rev 8.1 which specifies that the mean recovery for the lower fortification level should be in the range of 70 - 120 % and for the 100x LOQ level (1.0 mg/kg) in the range of 70 - 110 %.**

Selectivity/ Confirmation	The presence of the analyte is confirmed by comparing the liquid chromatography retention times of the analyte in the calibration standards with those found in the samples when monitoring two characteristic MS/MS transitions. Clopyralid *m/z* Q1/Q3 192/110 (quantitation) *m/z* Q1/Q3 194/112 (confirmation) The concentration of clopyralid in the final recovery sample extracts was determined by high performance liquid chromatography with MS/MS detection. The presence of the analyte was confirmed by monitoring and evaluating two ion mass transitions. No significant interferences in the unfortified control specimen matrices were observed at the expected retention time of clopyralid for either ion mass transition. The two ion mass transitions could be used interchangeably for quantitation and confirmation. The mass ion transition *m/z* 192→110 is proposed for quantitation because of the higher sensitivity.
Solution and Sample Extract Stability	Stock solutions of clopyralid in methanol can be considered to be stable for at least 58 days of refrigerated storage. Calibration solutions of clopyralid prepared in acetonitrile/0.1 % formic acid (1/9, v/v) can be considered to be stable for at least 30 days of storage at 5 ± 4 °C in the dark. The final sample extracts, those that were prepared for analysis and which had been fortified at the level of the LOQ, along with one control sample for each matrix, were stored in a refrigerator at 5 ± 4 °C for at least 14 days following their initial injection for analysis. After this storage period, the final sample extracts were re-analysed in order to confirm their stability, quantifying against freshly prepared standard solutions. Only the obtained mean recoveries for lettuce (head) and bovine fat were within the required range of 70 - 120 % and within ± 20 % of their initial values. The obtained mean recoveries of rye (grain), lemon (whole fruit), oilseed rape (seeds), bovine whole milk, poultry eggs, bovine meat and bovine liver were below 70 %. Nevertheless all sample extracts were considered to be stable because all values were within ± 20 % of their initial values.

Matrix Effects	Matrix effects (signal suppression/enhancement) on LC-MS/MS detection were investigated by comparing peak areas of solvent-based standard solutions to peak areas of matrix-matched standard solutions prepared for each matrix type at two concentration levels (e.g. LOQ with 4.0 ng/mL and 10x LOQ with 40 ng/mL). With the exception of oilseed rape (seeds), the matrix effects for all matrices at both concentrations levels were within ± 19 % and thus considered to be insignificant. The matrix effects of oilseed rape (seeds) were up to -53 % (signal suppression) and thus considered to be significant. For quantitation of clopyralid in undiluted sample extracts of oilseed rape (seeds) matrix-matched standards were used.

Recovery of Clopyralid (m/z 192/110) - Quantitation

Matrix Group	Matrix	Fortification Level (mg/kg)	Recovery (%)		RSD (%)	n
			mean	range		
Dry Crop	Rye Grain	0.01	52[a]	45-64	14	5
		1.00	61[a]	58-64	3.6	5
Wet Crop	Lettuce	0.01	83	74-89	8.3	5
		1.00	87	82-95	5.8	5
Acidic Crop	Lemon	0.01	72	69-75	3.1	5
		1.00	80	77-83	2.8	5
Oily Crop	Oilseed Rape Seeds	0.01	43[a]	41-45	3.5	5
		1.00	39[a]	37-43	7.7	5
Animal	Bovine Whole Milk	0.01	70	68-72	2.3	5
		1.00	74	70-78	5.3	5
	Poultry Eggs	0.01	62[a]	48-74	18	5
		1.00	55[a]	52-57	4.3	5
	Bovine Muscle	0.01	59[a]	56-64	5.5	5
		1.00	64[a]	58-68	6.1	5
	Bovine Fat	0.01	92	86-97	4.5	5
		1.00	87	83-90	3.6	5
	Bovine Liver	0.01	37[a]	34-40	6.9	5
		1.00	41[a]	39-44	4.7	5

[a] Does not meet the acceptance criteria of SANCO Guideline 825/00 rev. 8.1

Recovery of Clopyralid (m/z 194/112) - Confirmation

Matrix Group	Matrix	Fortification Level (mg/kg)	Recovery (%)		RSD (%)	n
			mean	Range		
Dry Crop	Rye Grain	0.01	44[a]	38-51	11	5
		1.00	61[a]	59-63	2.9	5
Wet Crop	Lettuce	0.01	79	75-82	3.4	5
		1.00	87	78-93	6.9	5
Acidic Crop	Lemon	0.01	84	79-87	3.6	5
		1.00	76	73-80	3.5	5
Oily Crop	Oilseed Rape Seeds	0.01	54[a]	47-60	11	5
		1.00	38[a]	35-40	5.5	5
Animal	Bovine Whole Milk	0.01	71	69-73	2.3	5
		1.00	73	67-78	6.6	5
	Poultry Eggs	0.01	69[a]	54-86	20	5
		1.00	55[a]	53-57	2.7	5
	Bovine Muscle	0.01	49[a]	41-56	12	5
		1.00	64[a]	60-70	5.8	5
	Bovine Fat	0.01	93	85-99	5.6	5
		1.00	86	84-87	1.8	5
	Bovine Liver	0.01	34[a]	27-43	17	5
		1.00	40[a]	36-41	5.2	5

[a] Does not meet the acceptance criteria of SANCO Guideline 825/00 rev. 8.1

Study Comments: 4.2(a)/5	Specificity: Highly specific method Linearity: Linear over the concentration range of 1.2 to at least 60 ng/mL for clopyralid (≥ 6 different concentrations) Coefficient of determination $r^2 \geq 0.996$ Precision: RSD = 2.3–18 % (quantifying transition) RSD = 1.8–20 % (confirmatory transition) Accuracy: Mean recovery 70 – 92 % (lettuce, lemon, bovine milk and fat; quantifying transition) Mean recovery 71 – 93 % (lettuce, lemon, bovine milk and fat; confirmatory transition) Mean recovery 37 – 64 % (rye, oilseed rape, poultry eggs, bovine muscle and liver; quantifying transition) Mean recovery 34 – 69 % (rye, oilseed rape, poultry eggs, bovine muscle and liver; confirmatory transition)

	According to SANCO/825/00 rev. 8.1, recovery and precision data must be reported for the fortification levels LOQ and 10 x LOQ. Here the fortification level 10 x LOQ is missing, and the level 100 x LOQ has been used instead. Otherwise the method is acceptably validated and suitable for the determination of clopyralid in lettuce, lemon as well as bovine milk and fat. However, the method is not acceptably validated for the determination of clopyralid in rye, oilseed rape, poultry eggs, bovine muscle and liver as the obtained mean recoveries are too low.
Agreed endpoint: 4.2(a)/5	LC-MS/MS, LOQ for clopyralid was established at 0.01 mg/kg for lettuce, lemon as well as bovine milk and fat.

CA 4.2(a)/6 Independent Laboratory Validation of the Multiresidue Method for the Determination of Clopyralid in Crop and Animal Matrices

Report	**CA 4.2 (a)/6**, [redacted] (2014)
Title	Independent Laboratory Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin
DAS Report Number	130728
Guidelines	EU Commission Regulation No. 283/2013 in accordance with EC Regulation No. 1107/2009, SANCO/825/00 rev. 8.1, (16-Nov-10)
GLP	Yes

Matrix, LOQ	Wet Crop - Lettuce	0.01 mg/kg
	Acidic Crop - Lemon	0.01 mg/kg
	Bovine Milk	0.01 mg/kg
	Bovine Fat	0.01 mg/kg
Scope	The objective of this study was to assess and to independently validate a multi-residue method that was developed following the multi-residue QuEChERS sample preparation technique. The method was developed on behalf of Dow AgroSciences LLC by [redacted] as Study Number S13-02878 "Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin" (**CA 4.2 (a)/5**) in accordance with the guidance document SANCO/825/00 rev. 8.1 of the European Commission. The intended limit of quantitation (LOQ) was 0.01 mg/kg. The study was performed in two different matrices of plant origin as represented by lettuce which is a commodity with high water content and lemon which is a commodity with high acid content and in two matrices of animal origin as represented by bovine whole milk and bovine fat. Only these four matrices were evaluated during the independent laboratory validation study because only these matrices complied with the standard acceptance criteria of SANCO Guideline 825/00 rev 8.1 in the method validation study.	

Principle	The multi-residue QuEChERS sample preparation technique as found in the method uses acetonitrile/water to extract residues of clopyralid from plant and animal samples. After addition of $MgSO_4$, NaCl and buffering citrate salts, the samples are shaken and centrifuged. Bovine fat extracts are stored for > 4 hours in a freezer in order to precipitate the majority of fat from the sample. For all samples, an aliquot of the acetonitrile phase is evaporated to dryness before reconstitution in acetonitrile/water plus 0.1% formic acid (1:9). For bovine milk samples, the final extracts are centrifuged before analysis. Residues of clopyralid are determined in the final sample extracts by positive-ion electrospray ionization tandem mass spectrometry LC-MS/MS analysis (Accucore Phenyl-hexyl column, 4.6x50 mm, 2.6 µm; Mobile Phase: A) water containing 0.1% formic acid, B) methanol containing 0.1% formic acid, gradient elution).
Linearity	Linear regression with 1/x weighting was used to describe the detector response as a function of the standard calibration curve concentrations, and the correlation coefficients (r) were always greater than or equal to 0.9978 for all of the calibration curve determinations during the ILV study. Calibration curves should be generated using standards prepared in unfortified control matrix extracts (matrix matched standards) for all sample materials included in the independent validation study according to SANCO/825/00 rev. 8.1 guidelines. Only if experiments clearly demonstrate that matrix effects are not significant (i.e. < 20 %), may calibration with standards in solvent be used. Matrix-matched standards were used for the quantitation of undiluted sample extracts of lettuce, lemon and bovine milk because matrix effects were significant for these matrices in the independent validation study. Matrix effects were >20% for lemons. Even though matrix effects were <20% for lettuce and bovine milk, due to the method having low recoveries, matrix matched standards were used. Calibration solutions prepared in neat solvent were used for quantitation of undiluted sample extracts of bovine fat (because this matrix showed an insignificant matrix effect of 0%) and for quantitation of diluted sample extracts for all matrices in the independent validation study.

Validation	The method independent laboratory validation study was conducted to provide data to support the method validation study for the determination of residues of clopyralid. For the independent laboratory validation study, crops from two European crop matrix groups (representative samples were lettuce and lemons) and two animal matrices (bovine milk and bovine fat) were evaluated using the QuEChERS multi-residue sample preparation technique. Only these four matrices were used because only they complied with the standard acceptance criteria of SANCO Guideline 825/00 rev 8.1 in the method validation study. The stated limit of quantitation (LOQ) was 0.01 mg/kg for all matrices. Untreated control samples were fortified at 0.01 mg/kg and at 1.0 mg/kg with clopyralid and analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). The ILV successfully passed on the first trial for clopyralid in lemons and bovine fat at all fortification levels. The ILV for clopyralid in lettuce successfully passed on the first trial at the LOQ fortification level and passed on the second trial at the 100×LOQ fortification level. For lettuce, lemon and bovine fat, the mean recovery values at fortification levels of 0.01 mg/kg (LOQ) and 1.0 mg/kg (100×LOQ) comply with the standard acceptance criteria of SANCO Guideline 825/00 rev 8.1. **However, the mean recoveries of bovine whole milk at the LOQ fortification level were below the EU acceptance range of 70-120 %, and the mean recoveries at the 100×LOQ fortification level were below the EU acceptance range of 70-110 % in all three trials attempted. T**he relative standard deviations (RSDs) did not exceed the level of ±20 % at the LOQ fortification level or ±15 % at the 100×LOQ fortification level.
Selectivity/ Confirmation	Interferences were < 30 % of the LOQ in all unfortified control matrices. Residues were confirmed by monitoring two structurally characteristic MS/MS transitions. MS/MS transitions monitored are: Clopyralid *m/z* Q1/Q3 192/110 (quantitation) *m/z* Q1/Q3 194/112 (confirmation)

Matrix Effects	The effect of plant and animal matrices on the LC-MS/MS signal was assessed by preparing standards in the presence of matrix and comparing the peak areas of the analyte as prepared in matrix against calibration solutions prepared in neat solvent at an equivalent concentration. Matrix-matched standards were used for the quantitation of undiluted sample extracts of lettuce, lemon and bovine milk. Matrix effects were >20% for lemons. Even though matrix effects were <20% for lettuce and bovine milk, due to the method having low recoveries, matrix matched standards were used. Calibration solutions prepared in neat solvent were used for quantitation of undiluted sample extracts of bovine fat and for quantitation of diluted sample extracts for all matrices.

Recovery of Clopyralid (m/z 192/110) - Quantitation

Matrix Group	Matrix	Fortification Level (mg/kg)	Recovery (%)		RSD (%)	n
			mean	Range		
Wet Crop	Lettuce	0.010	72	69-78	5.0	5
		1.00	70	62–78	9.2	5
Acidic Crop	Lemon	0.010	82	68–91	10.5	5
		1.00	72	62–82	11.0	5
Animal	Bovine Fat	0.010	80	78–85	3.7	5
		1.00	76	72–79	4.1	5
	Bovine Milk	0.010	57	54–59	3.8	5
		1.00	61	60-62	1.6	5

Recovery of Clopyralid (m/z 194/112) - Confirmation

Matrix Group	Matrix	Fortification Level (mg/kg)	Recovery (%)		RSD (%)	n
			mean	Range		
Wet Crop	Lettuce	0.010	72	68-78	5.5	5
		1.00	71	65–79	8.2	5
Acidic Crop	Lemon	0.010	82	70–91	9.7	5
		1.00	73	63–82	10.5	5
Animal	Bovine Fat	0.010	79	77–84	3.5	5
		1.00	76	72–79	4.1	5
	Bovine Milk	0.010	57	53–59	4.0	5
		1.00	61	60-62	1.5	5

Study Comments: 4.2(a)/6	Specificity: No interferences > 30% LOQ, highly specific method Linearity: Linear over the concentration range of 1.2 to 80 ng/mL for clopyralid (7 different concentrations) Correlation coefficient r $\geq$ 0.9978 Precision: RSD = 1.6–11.0 % (quantifying transition) RSD = 1.5–10.5 % (confirmatory transition) Accuracy: Mean recovery 70 – 82 % (lettuce, lemon, bovine fat; quantifying transition) Mean recovery 71 – 82 % (lettuce, lemon, bovine fat; confirmatory transition) Mean recovery 57 – 61 % (bovine milk; both transitions) According to SANCO/825/00 rev. 8.1, recovery and precision data must be reported for the fortification levels LOQ and 10 x LOQ. Here the fortification level 10 x LOQ is missing, and the level 100 x LOQ has been used instead. Otherwise the ILV is acceptably validated and suitable for the determination of clopyralid in lettuce, lemon as well as bovine fat. However, the method is not acceptably validated for the determination of clopyralid in bovine milk as the obtained mean recoveries are too low.
Agreed endpoint: 4.2(a)/6	LC-MS/MS, LOQ for clopyralid was established at 0.01 mg/kg for lettuce, lemon and bovine fat.

(b) Methods for the determination of all components included for monitoring purposes in the residue definitions for soil and water as submitted in accordance with the provisions of Point 7.4.2

CA 4.2(b)/1 Method for the Determination of Clopyralid in Soil

Report	**CA 4.2 (b)/1**, Vincent, T. P. (2013)
Title	Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS
DAS Report Number	120612
Guidelines	European Commission Guidance Document on Residue Analytical Methods, SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1, EPA Guideline OCSPP 850.6100 PMRA Residue Chemistry Guidelines as Regulatory Directive Dir 98-02
GLP	Yes

Matrix, LOQ	Loamy Sand	0.50 µg/kg
	Sandy Clay Loam	0.50 µg/kg
	Loam	0.50 µg/kg
	Silt Loam	0.50 µg/kg

Scope	The objective of this study is to provide residue method validation data for the determination of clopyralid and picloram in various soil types utilizing SPE sample purification. This method is applicable for the quantitative determination of residues of clopyralid and picloram in soil matrices (loamy sand, sandy clay loam, loam, and silt loam soil, per USDA Soil Class, equivalent to loamy sand, sandy clay, clay loam, and clay loam, respectively, per International Soil Class) (see Table below). The method was validated over the concentration range from the limit of quantitation (0.50 µg/kg) to 2000x the limit of quantitation (1000 µg/kg) with a limit of detection verification of 0.15 µg/kg.

PARAMETER	RESULTS			
ABC Designation	484	485	498	508
Geographic Location	Tift County , Georgia, USA	Willacy County, Texas, USA	Tehama County, California, USA	Boone County, Missouri, USA
Common Name	Tift	Raymondville	Tehama	Boone
Textural Class (USDA)	Loamy Sand	Sandy Clay Loam	Loam	Silt Loam
% Sand	87	53	39	23
% Silt	8	18	40	58
% Clay	5	29	21	19
Textural Class (International)	Loamy Sand	Sandy Clay	Clay Loam	Clay Loam
% Sand	91	63	57	37
% Silt	4	8	22	44
% Clay	5	29	21	19
Bulk Density (g/cc)	1.39	1.16	1.10	0.97
CEC (meq/100 g)	4.7	20.8	15.4	10.2
% Moisture at 0 bar (%)	32.4	67.1	57.5	70.7
% Moisture at 1/10 bar (%)	5.0	32.6	32.5	41.7
% Moisture at 1/3 bar (%)	3.8	20.9	21.3	34.3
% Moisture at 15 bar (%)	2.3	11.4	8.5	13.8
% Organic Matter (Walkley Black)	1.1	0.90	2.9	4.0
% Organic Carbon[b]	0.64	0.52	1.7	2.4
pH[a]	5.6	7.9	6.3	5.5
Nitrogen, Total (%)	0.05	0.07	0.18	0.21
Soluble Salts (mmhos/cm)	0.20	0.52	0.49	0.10
Calcium (ppm)	374	2880	1560	851
Magnesium (ppm)	49	326	491	143
Sodium (ppm)	13	93	54	18
Potassium (ppm)	102	441	236	64
Hydrogen (ppm)	21	22	26	46
Olsen Phosphorus (ppm)	23	12	48	9

Note: Soil characterization analyses were performed at Agvise Laboratories, Northwood, North Dakota.
[a] pH in 1:1 soil:water ratio
[b] Organic Carbon = Organic Matter / 1.72

Principle	Residues of clopyralid and picloram are extracted from soil samples by adding 25 mL of acetone:1N hydrochloric acid (90:10) then shaking and centrifuging, followed by 10 mL of additional acetone:1N hydrochloric acid (90:10) and further shaking and centrifuging. The acetone is then evaporated using nitrogen and brought to 8 mL final volume with 1N sodium hydroxide before vortexing and sonication. Approximately 8 mL of dichloromethane is added, with sonication, vortexing, and centrifuging to mix well, and the upper 6 mL extract layer is transferred to a clean glass tube and 6 mL of 1N hydrochloric acid is added. The sample is then passed through a pre-conditioned Waters HLB solid phase extraction (SPE) column. The sample bottle is then rinsed with 1N hydrochloric acid which is used to rinse the SPE column. The sample bottle is then rinsed with acetonitrile/1N formic acid (15:85) solution which is then used to rinse the SPE column, followed by drying under full vacuum. The SPE column is eluted with dichloromethane, which is evaporated to dryness using a gentle steam of nitrogen. The sample residue is reconstituted with a methanol/0.1% formic acid (10:90) solution filtered through a 0.2-µm PTFE syringe filter and then analyzed by liquid chromatography (Accucore Phenyl-hexyl column, 4.6x50 mm, 2.6 µm; Mobile Phase: A) water containing 0.01% formic acid, B) 60:40 methanol:acetonitrile containing 0.01% formic acid, gradient elution) coupled with negative-ion electrospray ionization tandem mass spectrometry (ESI LC-MS-MS).
Linearity	Linear regression analysis with 1/x weighting was used to describe the detector response as a function of the calibration standard concentrations. For the least squares regression equations describing the detector response as a function of the clopyralid standard calibration curve concentrations, the correlation coefficients (r) were greater than 0.9980 (r^2 greater than 0.9960) for both quantification and confirmatory results. For the least squares regression equations describing the detector response as a function of the picloram standard calibration curve concentrations, the correlation coefficients (r) were greater than 0.9969 (r^2 greater than 0.9938) for both quantification and confirmatory results.. The results indicate linearity of the detector response as a function of the standard concentration.

Validation	The method validation study was conducted to determine the recovery levels and the precision of the method for the determination of residues of clopyralid and picloram in soil matrices. The performance of the analytical method was determined with each set of samples by fortifying aliquots of the appropriate control matrix with a mixed clopyralid and picloram solution and analyzing the set following the procedures described within this report. Samples were fortified at the limit of detection (LOD) of 0.15 µg/kg, the limit of quantitation (LOQ) of 0.50 µg/kg, and at 1000 µg/kg. Samples fortified at the LOD were analyzed only to demonstrate observable peaks at the LOD level; the results were not included for average percent recovery calculations. An unfortified control matrix and reagent blank were also included in each set. For the clopyralid quantitation results, the individual recoveries for all of the samples fell within the range of 70 to 120%. The average clopyralid recoveries at each fortification level in each soil matrix group fell within the range of 70 to 120%. Relative standard deviations at each fortification level of clopyralid were all less than 20% except for the confirmatory transition of silt loam (20.4% at 0.50 µg/kg;). For the fortification level 1000 µg/kg (=1 mg/kg), the mean recoveries are below 110% and the RSDs below 15%. For the picloram quantitation results; 1) the individual recoveries for all of the sandy clay loam samples fell within the range of 70 to 120%; 2) the individual recoveries for all loam samples fell within the range of 70 to 120%; 3) the individual recoveries for all silt sand samples fell within the range of 70 to 120%, with the exception of a 1000 µg/kg fortified sample that yielded a 65% recovery; and 4) the individual recoveries for the loamy sand samples fortified at 1000 µg/kg fell within the range of 70 to 120%, however, the loamy sand samples fortified at 0.50 µg/kg fell within the range of 64 to 75%. These 0.50 µg/kg fortifications were accepted as best possible results based on repeated attempts indicating an elevated level of bonding in this soil type and the tightness of the data (RSD <7%). The average picloram recoveries at each fortification level in each soil matrix group fell within the range of 70 to 120%, with the exception of the loamy sand at 0.50 µg/kg (69%). The average picloram recoveries for all fortification levels in each soil matrix group fell within the range of 70 to 120%. Relative standard deviations at each fortification level were all less than 20%.

Selectivity/ Confirmation	The method is selective for the determination of clopyralid and picloram by virtue of the chromatographic separation and MS/MS detection system used. Using published guidelines, when detection is by tandem mass spectrometry methods, confirmation of the presence of the analyte should require the observation of a precursor ion plus one structurally significant product ion observed at the same retention time. MS/MS transitions monitored are: Clopyralid *m/z* Q1/Q3 190/146 (quantitation) *m/z* Q1/Q3 192/148 (confirmation) Picloram *m/z* Q1/Q3 241/197 (quantitation) *m/z* Q1/Q3 239/195 (confirmation) By monitoring multiple MS/MS ion transitions for each analyte, the confirmation ratios were calculated for each analyte for each sample within a set and compared to the average ratio for the calibration standards within that set. The confirmation ratios for most analytes in sample matrices were within ±20% of the average found for the standards, indicating that the method is selective for the determination of clopyralid and picloram in soils.
Solution and Sample Extract Stability	As part of this method validation study, the stability of the fortification solutions and the calibration standards was evaluated. The results indicate that clopyralid and picloram fortification solutions prepared in methanol are stable for at least 75 days and clopyralid and picloram calibration standard solutions prepared in a methanol/0.1% formic acid (10:90) solution are stable for at least 24 days when stored under refrigerated conditions. As part of this method validation study, the stability of sample extracts from representative soils was evaluated over a period of 7 days. The results ranged from -2.6% to 1.6% for the clopyralid quantitation transition, -2.1% to 2.2% for the clopyralid confirmatory transition, -2.5% to 1.0% for the picloram quantitation transition, and –3.4% to 2.1% for the picloram confirmatory transition. The results indicate that sample extracts containing clopyralid and picloram are stable for at least 7 days when stored under refrigerated conditions.

Matrix Effects	The determination of matrix effects were evaluated by comparing the response of the analyte at a concentration of 10 ng/mL in neat solvent to the response of the analyte at a concentration of 10 ng/mL that was fortified in control matrix (post extraction and cleanup following the analysis procedure) for each of the matrices. For clopyralid quantitation samples, the matrix effects ranged from -5.1% to -0.3% across all matrices. For clopyralid confirmatory samples, matrix effects ranged from -2.4% to 1.2% across all matrices. For picloram quantitation samples, the matrix effects ranged from -5.1% to 0.8% across all matrices. For picloram confirmatory samples, matrix effects ranged from -3.8% to 0.2% across all matrices. The results indicate there were no significant matrix effects for any of the soil types.
Extraction Efficiency	No new extraction efficiency studies were conducted in conjunction with the validation of this residue analytical method. In a previous study (DAS Study ID GRM 95.01, Harnick, B.J., and Olberding, E.L., 1995), extraction efficiency of clopyralid was determined by incubating soil for 10 days at 25 °C with 14C-labeled clopyralid. The resulting aged soil samples were extracted using 90% acetone/10% 1.0 N hydrochloric acid extraction solution. The extraction procedure was found to extract 92-98% of the remaining clopyralid from the soil matrix.

Recovery of Clopyralid (m/z 190/146) - Quantitation

Matrix Group	Matrix	Fortification Level (µg/kg)	Recovery (%)		RSD (%)	n
			mean	range		
Soil	Loamy Sand	0.50	90	78-113	15.5	5
		1000	74	72-75	2.0	5
	Sandy Clay Loam	0.50	87	81-96	6.6	5
		1000	76	75-77	1.1	5
	Loam	0.50	89	81-95	7.4	5
		1000	87	82-93	5.1	5
	Silt Loam	0.50	90	75-109	15.3	5
		1000	78	68-92	11.6	5

Recovery of Clopyralid (m/z 192/148) - Confirmation

Matrix Group	Matrix	Fortification Level (µg/kg)	Recovery (%)		RSD (%)	n
			mean	range		
Soil	Loamy Sand	0.50	80	68-103	17.2	5
		1000	74	72-76	1.9	5
	Sandy Clay Loam	0.50	87	80-98	8.1	5
		1000	75	74-77	1.3	5
	Loam	0.50	86	74-98	12.4	5
		1000	87	81-94	5.7	5
	Silt Loam	0.50	88	72-117	20.4	5
		1000	78	68-91	10.7	5

Recovery of Picloram (m/z 241/197) - Quantitation

Matrix Group	Matrix	Fortification Level (µg/kg)	Recovery (%)		RSD (%)	n
			mean	range		
Soil	Loamy Sand	0.50	69	64-75	6.7	5
		1000	75	73-77	2.2	5
	Sandy Clay Loam	0.50	87	77-93	7.4	5
		1000	85	79-89	4.3	5
	Loam	0.50	91	73-118	18.3	5
		1000	94	84-104	8.4	5
	Silt Loam	0.50	85	75-103	13.3	5
		1000	80	65-97	14.8	5

Recovery of Picloram (m/z 239/195) - Confirmation

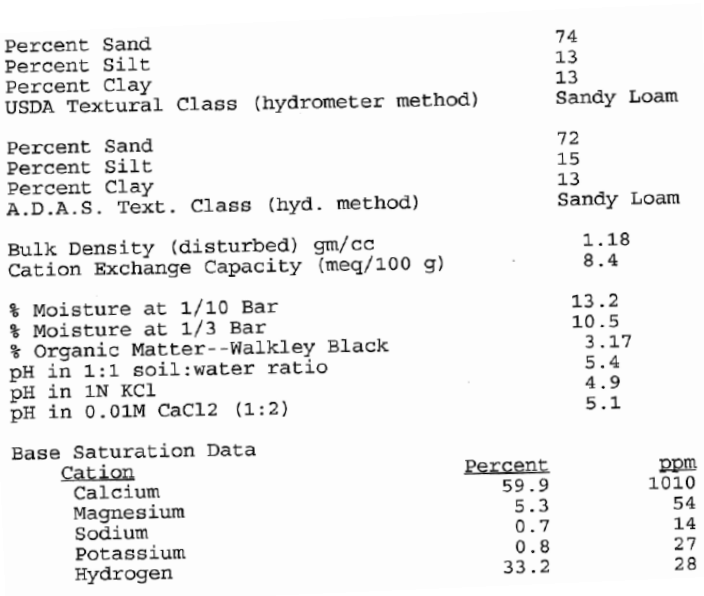
Matrix Group	Matrix	Fortification Level (µg/kg)	Recovery (%)		RSD (%)	n
			mean	range		
Soil	Loamy Sand	0.50	65	61-69	4.8	5
		1000	75	73-78	3.0	5
	Sandy Clay Loam	0.50	80	74-86	5.7	5
		1000	85	80-89	4.2	5
	Loam	0.50	85	67-116	23.9	5
		1000	94	85-104	7.9	5
	Silt Loam	0.50	85	66-99	17.8	5
		1000	81	66-98	14.8	5

Study Comments: 4.2(b)/1	Specificity: Highly specific method Linearity: Linear over the concentration range of 0.4 to 50 ng/mL (0.11-13-µg/kg) for clopyralid (5x2 different concentrations) Correlation coefficient $r \geq 0.9980$ Precision: RSD = 1.1–15.5 % (quantifying transition) RSD = 1.3–20.4 % (confirmatory transition) Accuracy: Mean recovery 74 – 90 % (quantifying transition) Mean recovery 74 – 88 % (confirmatory transition) According to SANCO/825/00 rev. 8.1, recovery and precision data must be reported for the fortification levels LOQ and 10 x LOQ. Here the fortification level 10 x LOQ is missing, and the level 2000 x LOQ has been used instead. Otherwise the method is acceptably validated and suitable for the determination of clopyralid in soil matrices (loamy sand, sandy clay loam, loam, and silt loam soil).
Agreed endpoint: 4.2(b)/1	LC-MS/MS, LOQ for clopyralid was established at 0.5 µg/kg for soil matrices (loamy sand, sandy clay loam, loam, and silt loam soil).

CA 4.2(b)/2 Independent Laboratory Validation of the Method for the Determination of Clopyralid in Soil

Report	**CA 4.2 (b)/2**, Austin, R., Turner, R. (2014)
Title	Independent Laboratory Validation of a Dow AgroSciences Method for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS
DAS Report Number	140079
Guidelines	EU Commission Regulation No. 283/2013 in accordance with EC Regulation No. 1107/2009, SANCO/825/00 rev. 8.1, (16-Nov-10), EPA Guideline OCSPP 850.6100
GLP	Yes

Matrix, LOQ	Sandy Loam 0.50 µg/kg
Scope	The objective of this study was to assess and to independently validate Dow AgroSciences Method 120612 for the determination of clopyralid and picloram in soil. The method was developed on behalf of Dow AgroSciences LLC at ABC Laboratories, Inc. as study number 68931, "Method Validation Study for the Determination of Residues of Clopyralid and picloram in Soil by LC-MS/MS" (**CA 4.2 (b)/1**). The independent laboratory validation demonstrated that the method can be considered applicable for use in the determination of residues of clopyralid and picloram in soil matrices. The methodology was successfully independently validated over the concentration range of 0.50 – 1000.0 µg/kg with an independently validated lower limit of quantification of 0.50 µg/kg. The soil matrix was represented by a fully characterized Lufa Speyer 2.2 soil sample (see Table below).



Percent Sand	74	
Percent Silt	13	
Percent Clay	13	
USDA Textural Class (hydrometer method)	Sandy Loam	
Percent Sand	72	
Percent Silt	15	
Percent Clay	13	
A.D.A.S. Text. Class (hyd. method)	Sandy Loam	
Bulk Density (disturbed) gm/cc	1.18	
Cation Exchange Capacity (meq/100 g)	8.4	
% Moisture at 1/10 Bar	13.2	
% Moisture at 1/3 Bar	10.5	
% Organic Matter--Walkley Black	3.17	
pH in 1:1 soil:water ratio	5.4	
pH in 1N KCl	4.9	
pH in 0.01M CaCl2 (1:2)	5.1	
Base Saturation Data		
Cation	Percent	ppm
Calcium	59.9	1010
Magnesium	5.3	54
Sodium	0.7	14
Potassium	0.8	27
Hydrogen	33.2	28

Principle	Residues of clopyralid and picloram are extracted from soil samples by adding 25 mL of acetone/1N hydrochloric acid (90:10) solution followed by shaking and centrifugation. The solvent is decanted before an additional 10 mL of acetone/1N hydrochloric acid (90:10) solution is added to the sample followed by further shaking and centrifugation. The two solvent extracts are combined, and the acetone is evaporated using nitrogen before being brought to a final volume of 8 mL using a 1N sodium hydroxide solution. The sample is vortex mixed and sonicated. Approximately 8 mL of dichloromethane is added and the sample is mixed well using vortex mixing and sonication. The sample is centrifuged before a 6 mL aliquot of the upper extract layer is transferred to a new glass tube and 6 mL of 1N hydrochloric acid is added to the upper extract layer. The acidified upper extract layer is then passed through a pre-conditioned Waters HLB SPE cartridge, and the sample tube is rinsed with 1N HCl which is then transferred to and passed through the SPE cartridge. This is followed by rinsing the sample tube with acetonitrile/1N formic acid (15:85) and passing this rinse through the SPE cartridge also. The cartridge is dried under full vacuum for 30 minutes before elution of the analytes with dichloromethane. The sample is evaporated to dryness using nitrogen and reconstituted in 1.0 mL of methanol/0.1% formic acid (10:90) solution before being filtered through a 0.2 µm PTFE syringe filter. The final sample is analysed for clopyralid and picloram by liquid chromatography (Accucore Phenyl-hexyl column, 4.6x50 mm, 2.6 µm; Mobile Phase: A) water containing 0.01% formic acid, B) 60:40 methanol:acetonitrile containing 0.01% formic acid, gradient elution) coupled with negative-ion electrospray tandem mass spectrometry (LC-MS/MS).
Linearity	Linear regression analysis with 1/x weighting and the least squares regression was used to describe the detector response as a function of the calibration standard concentrations. The correlation coefficients (r) were greater than or equal to 0.9993 for all of the linear calibration curve determinations during the independent laboratory validation which indicates linearity of the detector response as a function of the standard concentration.

Validation	The independent laboratory validation study was conducted to determine the recovery levels and the precision of the method for the determination of clopyralid and picloram in soil matrices. The performance of the analytical method was determined with each set of samples by fortifying aliquots of appropriate control matrix with clopyralid and picloram and analyzing the set following the procedures described in this report. Samples were fortified at the limit of detection (LOD) of 0.15 µg/kg, the limit of quantitation (LOQ) of 0.5 µg/kg, and at the higher fortification level of 1000 µg/kg (2000 x LOQ). Samples fortified at the LOD were analyzed only to demonstrate that observable peaks at the LOD level could be distinguished from untreated control samples; the results were not included for average percent recovery calculations. Two unfortified control matrices and a reagent blank were also included in each set. The individual recoveries for both analytes fell within the range of 70 to 120% for both the quantitative and confirmatory transitions. The average recoveries at each fortification level for both analytes fell within the range of 70 to 120% for both the quantitative and confirmatory transitions. Relative standard deviations at each fortification level for both analytes were less than 20% for the quantitative and confirmatory transitions.
Selectivity/ Confirmation	The method is selective for the determination of clopyralid and picloram by virtue of the chromatographic separation and MS/MS detection. The presence of the analytes is confirmed by comparing the liquid chromatography retention time of the analytes in the calibration standards with that found in the samples, while monitoring two structurally characteristic MS/MS transitions. The following ion transitions were monitored: Clopyralid *m/z* Q1/Q3 190/146 (quantitative) *m/z* Q1/Q3 192/148 (confirmatory) Picloram *m/z* Q1/Q3 241/197 (quantitative) *m/z* Q1/Q3 239/195 (confirmatory) When detection is performed by tandem mass spectrometry methods, confirmation of the presence of the analyte requires the observation of a precursor ion plus one structurally significant product ion observed at the same retention time. The blank values (non-fortified control samples) were determined from the matrix used in the fortification experiments. It was demonstrated that any interferences in these specimens were less than 30% of the analyte contribution found at the LOQ.

Matrix Effects	Matrix effects were evaluated by comparing the response of the analytes (clopyralid and picloram) fortified into the final control extract following extraction and purification to the response of the analytes fortified in neat solvent. Matrix effects were evaluated for each analyte at a concentration of 10.0 ng/mL. Matrix effects for the quantitative and confirmatory transitions for each analyte were calculated. For the quantitative transitions of clopyralid and picloram, matrix effects were 1% and 3% respectively. For the confirmatory transitions, matrix effects were 0% and 4% respectively. The results demonstrate that matrix effects are within ±20%; therefore, the effects are not significant according to SANCO guidelines.

Recovery of Clopyralid (m/z 190/146) - Quantitation

Matrix	Fortification Level (µg/kg)	Recovery (%)		RSD (%)	n
		mean	range		
Soil	0.50	85	73-91	8.8	5
	1000	89	81-93	5.3	5

Recovery of Clopyralid (m/z 192/148) - Confirmation

Matrix	Fortification Level (µg/kg)	Recovery (%)		RSD (%)	n
		mean	range		
Soil	0.50	86	81-90	4.3	5
	1000	87	80-92	5.5	5

Recovery of Picloram (m/z 241/197) - Quantitation

Matrix	Fortification Level (µg/kg)	Recovery (%)		RSD (%)	n
		mean	range		
Soil	0.50	94	87-97	4.4	5
	1000	90	83-93	4.6	5

Recovery of Picloram (m/z 239/195) - Confirmation

Matrix	Fortification Level (µg/kg)	Recovery (%)		RSD (%)	n
		mean	range		
Soil	0.50	82	74-93	8.9	5
	1000	91	83-95	5.2	5

Study Comments: 4.2(b)/2	Specificity: No interferences > 30% LOQ, highly specific method Linearity: Linear over the concentration range of 0.4 to 50 ng/mL (0.09-13 µg/kg) for clopyralid (5 different concentrations) Correlation coefficient r ≥ 0.9993 Precision: RSD = 4.3–8.8 % Accuracy: Mean recovery 85 – 89 % According to SANCO/825/00 rev. 8.1, recovery and precision data must be reported for the fortification levels LOQ and 10 x LOQ. Here the fortification level 10 x LOQ is missing, and the level 2000 x LOQ has been used instead. Otherwise the ILV is acceptably validated and suitable for the determination of clopyralid in soil matrice (sandy loam).
Agreed endpoint: 4.2(b)/2	LC-MS/MS, LOQ for clopyralid was established at 0.5 µg/kg for soil matrice (sandy loam).

CA 4.2(b)/3 Method for the Determination of Clopyralid in Water

Report	**CA 4.2 (b)/3**, Shaffer, S. (2012)
Title	Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water, and Surface Water by LC-MS/MS
DAS Report Number	120611
Guidelines	European Commission Guidance Document on Residue Analytical Methods, SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1, EPA Guideline OCSPP 850.6100 PMRA Residue Chemistry Guidelines as Regulatory Directive Dir 98-02
GLP	Yes

Matrix, LOQ	Drinking Water 0.050 µg/L Ground Water 0.050 µg/L Surface Water 0.050 µg/L
Scope	This method is applicable for the quantitative determination of residues of clopyralid and picloram in water matrices (ground water, drinking water, and surface water; see characterisation table below). The method was validated over the concentration range of 0.050-10 µg/L with a verification of the limit of detection of 0.015 µg/L.

Specimen (Date of Collection/ Characterization)	Conductivity (µS)	Alkalinity (mg/L)[a]	Total Hardness (mg/L)[a]	DO	pH	Dissolved Organic Carbon (ppm)	Total Organic Carbon (ppm)
Ground Water (06 Aug 12/09 Aug 12/17 Aug 12)	341	150	142	8.47	8.37	5.22	3.84
Drinking Water (06 Aug 12/09 Aug 12/17 Aug 12))	669	306	288	8.39	7.62	5.49	4.37
Surface Water (06 Aug 12/09 Aug 12/17 Aug 12))	111.1	30	30	8.33	9.30	14.32	13.23

[a]Calculated value of endpoint as $CaCO_3$.

Principle	Residues of clopyralid and picloram are extracted from water samples by passing 100 mL of water through a pre-conditioned Waters HLB solid phase extraction (SPE) column after adjusting the pH to below 2 with 1N HCl. The sample bottle is then rinsed with 1N HCl which is used to rinse the SPE column. The sample bottle is then rinsed with acetonitrile/1N formic acid (15:85) solution which is then used to rinse the SPE column, followed by drying under full vacuum. The SPE column is eluted with dichloromethane, which is evaporated to dryness using a gentle steam of nitrogen. The sample residue is reconstituted with a methanol/0.1% formic acid (10:90) solution filtered through a 0.2-µm PTFE syringe filter and then analyzed by liquid chromatography (Accucore Phenyl-hexyl column, 4.6x50 mm, 2.6 µm; Mobile Phase: A) water containing 0.01% formic acid, B) 60:40 methanol:acetonitrile containing 0.01% formic acid, gradient elution) coupled with negative-ion electrospray ionization tandem mass spectrometry (ESI LC-MS-MS).
Linearity	Linear regression analysis with 1/x weighting was used to describe the detector response as a function of the calibration standard concentrations. For the least squares regression equations describing the detector response as a function of the standard calibration curve concentrations, the correlation coefficients (r) were greater than 0.9995 for all of the calibration curve determinations during the method validation. The results indicate linearity of the detector response as a function of the standard concentration.

Validation	The method validation study was conducted to determine the recovery levels and the precision of the method for the determination of residues of clopyralid and picloram in water matrices. The performance of the analytical method was determined with each set of samples by fortifying aliquots of the appropriate control matrix with a mixed clopyralid and picloram solution and analyzing the set following the procedures described within this report. Samples were fortified at the limit of detection (LOD) of 0.015 µg/L, the limit of quantitation (LOQ) of 0.050 µg/L, and at 10.0 µg/L. Samples fortified at the LOD were analyzed only to demonstrate observable peaks at the LOD level; the results were not included for average percent recovery calculations. An unfortified control matrix and reagent blank were also included in each set. For the quantitation results, the individual recoveries for all samples fell within the range of 70 to 110%. The average recoveries at each fortification level in each water matrix group also fell within the range of 70 to 110%. The average recoveries for all fortification levels in each water matrix group fell within the range of 70 to 110%. Relative standard deviations at each fortification level were all less than 20%.
Selectivity/ Confirmation	The method is selective for the determination of clopyralid and picloram by virtue of the chromatographic separation and MS/MS detection system used. Using published guidelines, when detection is by tandem mass spectrometry methods, confirmation of the presence of the analyte should require the observation of a precursor ion plus one structurally significant product ion observed at the same retention time. MS/MS transitions monitored are: Clopyralid *m/z* Q1/Q3 190/146 (quantitative) *m/z* Q1/Q3 192/148 (confirmatory) Picloram *m/z* Q1/Q3 241/197 (quantitative) *m/z* Q1/Q3 239/195 (confirmatory) By monitoring multiple MS/MS ion transitions for each analyte, the confirmation ratios were calculated for each analyte for each sample within a set and compared to the average ratio for the calibration standards within that set. The confirmation ratios for each analyte in all sample matrices were within ±20% of the average found for the standards, indicating that the method is selective for the determination of clopyralid and picloram in waters.

Solution and Sample Extract Stability	Results indicate that clopyralid and picloram fortification solutions prepared in methanol are stable for at least 23 days and clopyralid and picloram calibration standard solutions prepared in a methanol:0.1% formic acid (10:90) solution are stable for at least 24 days when stored under refrigerated conditions. The sample extract stability results ranged from -9.4% to 1.5% for the clopyralid quantitation transition, -4.2% to 3.3% for the clopyralid confirmatory transition, -13.9% to 2.3% for the picloram quantitation transition, and –2.8% to 4.0% for the picloram confirmatory transition. The results indicate that sample extracts containing clopyralid and picloram are stable for at least 15 days when stored under refrigerated conditions.
Matrix Effects	The determination of matrix effects were evaluated by comparing the response of the analyte at a concentration of 10 ng/mL in neat solvent to the response of the analyte at a concentration of 10 ng/mL that was fortified in control matrix (post extraction and cleanup following the analysis procedure) for each of the matrices. For clopyralid quantitation samples, the matrix effects ranged from -2.7% to 3.6% across all matrices. For clopyralid confirmatory samples, matrix effects ranged from -8.1% to 5.6% across all matrices. For picloram quantitation samples, the matrix effects ranged from -3.6% to 2.6% across all matrices. For picloram confirmatory samples, matrix effects ranged from -2.3% to 0.6% across all matrices. The results indicate there were no significant matrix effects for any of the water types.

Recovery of Clopyralid (m/z 190/146) - Quantitation

Matrix	Fortification Level (µg/L)	Recovery (%)		RSD (%)	n
		mean	Range		
Ground Water	0.050	90	88-93	4.0	5
	10	86	80-91	5.2	5
Drinking Water	0.050	92	81-100	7.6	5
	10	85	74-93	9.3	5
Surface Water	0.050	89	86-97	5.3	5
	10	86	81-89	3.8	5

Recovery of Clopyralid (m/z 192/148) - Confirmation

Matrix	Fortification Level (µg/L)	Recovery (%)		RSD (%)	n
		mean	Range		
Ground Water	0.050	92	82-95	5.7	5
	10	85	79-90	5.9	5
Drinking Water	0.050	94	85-100	6.5	5
	10	84	76-89	7.2	5
Surface Water	0.050	86	82-90	3.4	5
	10	87	79-97	8.5	5

Recovery of Picloram (m/z 241/197) - Quantitation

Matrix	Fortification Level (µg/L)	Recovery (%)		RSD (%)	n
		mean	Range		
Ground Water	0.050	100	94-105	3.9	5
	10	99	96-101	2.0	5
Drinking Water	0.050	101	96-105	3.6	5
	10	98	96-101	2.2	5
Surface Water	0.050	93	85-102	7.0	5
	10	92	81-100	7.9	5

Recovery of Picloram (m/z 239/195) – Confirmation

Matrix	Fortification Level (µg/L)	Recovery (%)		RSD (%)	n
		mean	Range		
Ground Water	0.050	98	95-100	2.1	6
	10	98	94-102	3.3	6
Drinking Water	0.050	99	96-100	1.7	6
	10	98	95-101	2.5	6
Surface Water	0.050	91	86-100	6.2	6
	10	91	80-97	7.2	6

Study Comments: 4.2(b)/3	Specificity: Highly specific method Linearity: Linear over the concentration range of 1 to 50 ng/mL for clopyralid (5 different concentrations) Correlation coefficient $r \geq 0.9995$ Precision: RSD = 3.8–9.3 % (quantifying transition) RSD = 3.4–8.5 % (confirmatory transition) Accuracy: Mean recovery 85 – 92 % (quantifying transition) Mean recovery 84 – 94 % (confirmatory transition) According to SANCO/825/00 rev. 8.1, recovery and precision data must be reported for the fortification levels LOQ and 10 x LOQ. Here the fortification level 10 x LOQ is missing, and the level 200 x LOQ has been used instead. Otherwise the method is acceptably validated and suitable for the determination of clopyralid in water matrices (ground water, drinking water, and surface water).
Agreed endpoint: 4.2(b)/3	LC-MS/MS, LOQ for clopyralid was established at 0.05 µg/L for water matrices (ground water, drinking water, and surface water).

CA 4.2(b)/4 Independent Laboratory Validation of the Method for the Determination of Clopyralid in Water

Report	**CA 4.2 (b)/4**, Austin, R., Turner, R. (2013)
Title	Independent Laboratory Validation of Dow AgroSciences Method 120611, "Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water, and Surface Water by LC-MS/MS"
DAS Report Number	120613
Guidelines	EC Regulation No. 1107/2009 (21-Oct-09) repealing Directive 91/414/EEC SANCO/825/00 rev. 8.1, (16-Nov-10) EPA Guideline; OCSPP 850.6100, PR Notice 96-1 and PR Notice 2011-3
GLP	Yes

Matrix, LOQ	Drinking Water	0.050 µg/L
	Ground Water	0.050 µg/L
	Surface Water	0.050 µg/L

Scope

The objective of this study was to assess and to independently validate the method described in the Dow AgroSciences Method 120611, "Method Validation Study for the Determination of Residues of Clopyralid and picloram in Drinking Water, Ground Water, and Surface Water by LC-MS/MS" (**CA 4.2 (b)/3**). The methodology was successfully independently validated over the concentration range of 0.05 - 0.5 µg/L with a verification of the limit of quantification of 0.050 µg/L. Three different water matrices were used for validation. These were drinking water, ground water and surface water samples. The drinking water specimen was obtained from a 'drinking water' tap at Battelle UK Ltd, Ongar. The ground water specimen was bottled still spring water. The surface water specimen was collected from a pond at Boarded Barns Farm, Ongar, Essex, CM5 0HJ.

Drinking water:	pH	8.2
	Calcium	128 ppm
	Magnesium	4.4 ppm
	Hardness	339 mg equivalent CaCO3/L
	Conductivity	0.67 mmhos/cm
	Total Suspended Solids	18 ppm
	Total Organic Carbon	1.3 ppm
	Dissolved Organic Carbon	1.0 ppm
Ground water:	pH	8.2
	Calcium	42 ppm
	Magnesium	10 ppm
	Hardness	148 mg equivalent CaCO3/L
	Conductivity	0.29 mmhos/cm
	Total Suspended Solids	18 ppm
	Total Organic Carbon	0.7 ppm
	Dissolved Organic Carbon	0.1 ppm

	Surface water: pH 8.1 Calcium 130 ppm Magnesium 8.7 ppm Hardness 360 mg equivalent CaCO3/L Conductivity 0.73 mmhos/cm Total Suspended Solids 8 ppm Total Organic Carbon 5.6 ppm Dissolved Organic Carbon 5.3 ppm
Principle	Residues of clopyralid and picloram are extracted from water matrices by acidifying with 1 N hydrochloric acid (5 mL) followed by a solid-phase extraction (SPE) clean up. The sample is transferred onto a conditioned 0.2 g Waters HLB column at an approximate rate of 2 mL/min. The sample bottle is rinsed with 1 N hydrochloric acid (1 mL) followed by 15:85 acetonitrile/1 N formic acid (5 mL) and the column washed with the rinse before drying under full vacuum for at least 30 minutes. The column is eluted with 14 mL of dichloromethane (DCM). The extract is evaporated to dryness using nitrogen and reconstituted in methanol/0.1% formic acid in water (10:90). The final extract is filtered through a 0.2-µm PTFE syringe filter and then analyzed by liquid chromatography (Accucore Phenyl-hexyl column, 4.6x50 mm, 2.6 µm; Mobile Phase: A) water containing 0.01% formic acid, B) 60:40 methanol:acetonitrile containing 0.01% formic acid, gradient elution) coupled with negative-ion electrospray ionization tandem mass spectrometry (ESI LC-MS/MS).

Linearity	Linear regression with 1/x weighting was used to describe the detector response as a function of the standard calibration curve concentrations, and the correlation coefficients (r) were always greater than or equal to 0.996 for all of the calibration curve determinations during the independent laboratory validation study. Solvent standards were used to calculate the results for all water types.
Validation	The independent laboratory method validation study was conducted to determine the recovery levels and the precision of the method for the determination of residues of clopyralid and picloram in drinking water, ground water, and surface water. The performance of the analytical method was determined with each set of samples by fortifying aliquots of the appropriate control matrix with a mixed clopyralid and picloram solution and analyzing the set following the procedures described within this report. Samples were fortified at the limit of detection (LOD) of 0.015 µg/L, the limit of quantitation (LOQ) of 0.05 µg/L and at 0.5 µg/L. Samples fortified at the LOD were analyzed only to demonstrate observable peaks at the LOD level. Two untreated samples and a reagent blank were also analyzed. The first ILV trials for all three water types were successful. Average recoveries at each fortification level were all within the acceptance range of 70-120 %. The relative standard deviation (RSD) did not exceed 20 % at any fortification level.
Selectivity/ Confirmation	No interferences were present. Residues were confirmed by monitoring two structurally characteristic MS/MS transitions. MS/MS transitions monitored are: Clopyralid *m/z* Q1/Q3 190/146 (quantitative) *m/z* Q1/Q3 192/148 (confirmatory) Picloram *m/z* Q1/Q3 241/197 (quantitative) *m/z* Q1/Q3 239/195 (confirmatory)
Matrix Effects	The effect of matrices on the LC-MS/MS signal was assessed by preparing standards in the presence of matrix and comparing the peak areas of the analytes prepared in matrix against solvent based standards at an equivalent concentration. Matrix effects were less than or equal to 10% for clopyralid and picloram in all matrices.

Recovery of Clopyralid (m/z 190/146) in Water - Quantitation

Matrix	Fortification Level (µg/L)	Mean Recovery (%)	Recovery Range (%)	RSD %	Number of Samples
Drinking Water	0.050	99	98-101	1.1	5
	0.50	99	96-102	2.4	5
Ground Water	0.050	89	86–90	2.0	5
	0.50	98	93-100	3.1	5
Surface Water	0.050	102	100-106	2.2	5
	0.50	98	95-102	3.2	5

Recovery of Clopyralid (m/z 192/148) in Water – Confirmation

Matrix	Fortification Level (µg/L)	Mean Recovery (%)	Recovery Range (%)	RSD %	Number of Samples
Drinking Water	0.050	101	97-105	3.0	5
	0.50	99	97-101	1.5	5
Ground Water	0.050	89	87–91	2.0	5
	0.50	94	92-98	2.4	5
Surface Water	0.050	104	102-106	1.6	5
	0.50	98	94-104	4.0	5

Recovery of Picloram (m/z 241/197) in Water - Quantitation

Matrix	Fortification Level (µg/L)	Mean Recovery (%)	Recovery Range (%)	RSD %	Number of Samples
Drinking Water	0.050	100	99-101	0.8	5
	0.50	99	96-102	2.2	5
Ground Water	0.050	89	84–95	5.3	5
	0.50	96	93-100	3.2	5
Surface Water	0.050	97	92-100	4.0	5
	0.50	97	95-100	1.8	5

Recovery of Picloram (m/z 239/195) in Water – Confirmation

Matrix	Fortification Level (µg/L)	Mean Recovery (%)	Recovery Range (%)	RSD %	Number of Samples
Drinking Water	0.050	97	93-101	4.2	5
	0.50	99	98-99	0.6	5
Ground Water	0.050	94	90–100	4.2	5
	0.50	97	92-99	2.9	5
Surface Water	0.050	95	93-99	2.4	5
	0.50	92	88-93	2.3	5

Study Comments: 4.2(b)/4	Specificity: No interferences, highly specific method Linearity: Linear over the concentration range of 1 to 50 ng/mL for clopyralid (5 different concentrations) Correlation coefficient $r \geq 0.996$ Precision: RSD = 1.1–3.2 % (quantifying transition) RSD = 1.5–4.0 % (confirmatory transition) Accuracy: Mean recovery 89 – 102 % (quantifying transition) Mean recovery 89 – 104 % (confirmatory transition) The ILV is acceptably validated and suitable for the determination of clopyralid in water matrices (ground water, drinking water, and surface water).
Agreed endpoint: 4.2(b)/4	LC-MS/MS, LOQ for clopyralid was established at 0.05 µg/L for water matrices (ground water, drinking water, and surface water).

(c) Methods for the analysis in air of the active substance and relevant breakdown products formed during or after application, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible

CA 4.2(c)/1 The Development and Validation of a Method for the Analysis of Chlorpyrifos and Chlorpyrifos-oxon in Air

Report	**CA 4.2 (c)/1**, Bacher, R. (2012)
Title	The Development and Validation of a Method for the Analysis of Clopyralid in Air
DAS Report Number	120601
Guidelines	EC Regulation No. 1107/2009 (21-Oct-09) repealing Directive 91/414/EEC. European Commission Guidance Documents on Residue Analytical Methods, SANCO/825/00 rev. 8.1 (16/11/10) and SANCO/3029/99 rev. 4 (11/07/2000)
GLP	Yes

Matrix, LOQ	Ambient Air 4.5 µg/m^3 Warm, Humid Air 4.5 µg/m^3
Scope	This study was conducted to develop and validate an analytical method for the determination of clopyralid in ambient as well as warm and humid air with a limit of quantitation (LOQ) of approximately 4.5 µg/m^3. Ambient Air: Air Temperature 21 °C, Relative Humidity 58 % Warm, Humid Air: Air Temperature 36 °C, Relative Humidity 98 %
Principle	Air sampling used adsorption tubes filled with two portions of XAD adsorption material. Particles and aerosols were trapped by filtration or impact onto the adsorbent material. After sampling of air (6 hours), the front and the back adsorbent portions of the adsorption material were separated and both sections were extracted separately three times, each time with 3 mL of acetonitrile. The three extracts from the front portion were combined, and the volumes were adjusted to 10 mL with acetonitrile. Extracts obtained from recoveries fortified at 100xLOQ (front portion) were further diluted by a factor of 50 using acetonitrile/water (2/8). Combined extracts of the blank control, LOQ, and 100xLOQ from the back portion of the tubes used to check for breakthrough were diluted by a factor of 5 using acetonitrile/water (2/8). Final determination of clopyralid was performed by LC-MS/MS (YMC Triart C_{18} column, 150 x 3.0 mm, 3.0 µm particle size, Securityguard: Phenomenex, C_{18}, 4 x 3 mm; mobile phase: A – water with 0.1% formic acid, B – methanol with 0.1% formic acid, gradient elution), using the transition 192 *m/z* => 146 *m/z* as the primary transition ion of the analyte for quantification and the transition 192 *m/z* => 110 *m/z* as the secondary transition ion for confirmation of the presence of the analyte.

Linearity	For the linear regression analysis with 1/x weighting, the correlation coefficients (r) were equal to or greater than 0.99 for all of the calibration curve determinations made during the method validation. The results indicate linearity of the detector response as a function of the standard concentration.
Validation	A method validation study was conducted to determine the recovery and the precision of the method for the determination of clopyralid in ambient and warm, humid air. The validated method achieves a limit of quantification (LOQ) of approx. 4.5 µg/m^3. The average recoveries at each fortification level were between 70 and 120 %. The relative standard deviation (RSD) per fortification level was <20 %, and interferences were negligible (< 1 % of fortified amount).
Selectivity/ Confirmation	Selectivity of the LC-MS/MS method is proven by monitoring two MRMs with two characteristic daughter ions (146 *m/z* and 110 *m/z*) originating from the parent $[M+H]^+$ ion (192 *m/z*) in matrix blanks and fortified samples
Storage Stability	As evidenced by average recoveries in the range of 70 % to 120 % and relative standard deviations RSD of ≤20 %, storage stability of fortified tubes was demonstrated for a period of 7 days under all three storage conditions (i.e. at room temperature at approximately 21 °C, in a refrigerator at approximately 6 °C and in a freezer at < -18 °C, always in the dark). Stability of extracts stored at refrigerator temperature for 4 days was demonstrated with acceptable recoveries in the range of 70 -120 %.

Recovery of Clopyralid (m/z 192/146) - Quantitation

Matrix Group	Matrix	Fortification Level (µg/m^3)	Recovery (%)		RSD (%)	n
			mean	range		
Air	Ambient air	4.10	100	84-111	10	5
		415	93	87-110	10	5
	Humid air	4.16	96	89–105	8	5
		412	100	93-111	7	5

Recovery of Clopyralid (m/z 192/110) - Confirmation

Matrix Group	Matrix	Fortification Level ($\mu g/m^3$)	Recovery (%)		RSD (%)	n
			mean	range		
Air	Ambient air	4.10	99	83-111	11	5
		415	92	86-107	9	5
	Humid air	4.16	95	85-107	10	5
		412	99	91-112	8	5

Study Comments: 4.2(c)/1	Specificity: No interferences, highly specific method Linearity: Linear over the concentration range of 2.5 to 500 ng/mL for clopyralid (6 different concentrations) Correlation coefficient $r \geq 0.99$ Precision: RSD = 7–11 % Accuracy: Mean recovery 92 – 100 % According to SANCO/825/00 rev. 8.1, recovery and precision data must be reported for the fortification levels LOQ and 10 x LOQ. Here the fortification level 10 x LOQ is missing, and the level 100 x LOQ has been used instead. Otherwise the method is acceptably validated and suitable for the determination of clopyralid in air (ambient and humid air).
Agreed endpoint: 4.2(c)/1	LC-MS/MS, LOQ for clopyralid was established at 4.5 $\mu g/m^3$ for air (ambient and humid air).

(d) Methods for the analysis in body fluids and tissues for active substances and relevant metabolites

CA 4.2(d)/1 Method for the Determination of Clopyralid in Body Fluids

Report	CA 4.2 (d)/1, [redacted] (2014)
Title	Development and Validation of an Analytical Method for the Determination of Clopyralid in Body Fluid(s)
DAS Report Number	130727
Guidelines	EC Regulation No. 1107/2009 (21-Oct-09) repealing Directive 91/414/EEC European Commission Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1, 16-Nov-10. OECD Guidance document ENV/JM/MONO (2007)17.
GLP	Yes

Matrix, LOQ	Human Blood	0.05 mg/L
	Human Urine	0.05 mg/L
Scope	This method is applicable for the quantitative determination of residues of clopyralid in human blood and urine with a limit of quantitation of 0.05 mg/L.	
Principle	For the method validation study, untreated control samples of human blood and urine were fortified at 0.05 mg/L with clopyralid. Urine was acidified and cleaned up over Isolut HM-N cartridges, and eluted with dichloromethane. An aliquot of the dichloromethane phase was evaporated to dryness, and the residue was reconstituted in acetonitrile / water (2/8, v/v). Blood was extracted with acetone; an aliquot was evaporated to dryness and dissolved in acetonitrile/water (2/8, v/v). The extracts were analyzed by liquid chromatography with positive-ion electrospray ionization (ESI) tandem mass spectrometry (LC-MS/MS) (YMC, J`Sphere ODS-H 80, C_{18} column, 150 x 3.0 mm, 4.0 µm particle size, Pre-column Phenomenex C_{18}, 4 x 3 mm; mobile phase: A – water with 0.1% formic acid, B – methanol with 0.1% formic acid, gradient elution).	
Linearity	The correlation coefficients “r” met or exceeded 0.99 for all the validation sets.	
Validation	Average recoveries for the LOQ fortification level were all within the EU acceptance range of 70-120 % in both urine and blood samples. The relative standard deviation (RSD) did not exceed the level of ± 20 % at any fortification level, and interferences were all < 0.01 mg/L, respectively < 20 % of the LOQ.	

Selectivity/ Confirmation	The presence of the analyte is confirmed by comparing the liquid chromatography retention time of the analyte in the calibration standards with those found in the samples when monitoring two structurally characteristic MS/MS transitions. MS/MS transitions monitored are: Clopyralid *m/z* Q1/Q3 190/146 (quantitation) *m/z* Q1/Q3 192/148 (confirmation)
Solution and Sample Extract Stability	Stability of the stock solution is proven by comparing two solutions. The first solution is prepared by dilutions of the original stored and the second solution is prepared by dilution of the freshly prepared stock solution. The stock solution which is prepared in acetonitrile is found to be stable for at least 5 weeks. Stability of fortification solutions is proven by comparing a diluted solution prepared from a fortification solution used for fortification of recovery samples during the study with an equivalent concentration prepared by dilution of the freshly prepared stock solution. The fortification solutions prepared in acetonitrile are stable for at least 5 weeks. Stability of calibration solutions is proven by comparing one of the original stored calibration solutions with an equivalent concentration prepared by dilution of a freshly prepared stock solution made from the newly weighed standard. The calibration solutions prepared in acetonitrile/water (2/8, v/v) are stable for at least 5 weeks. All final sample extracts were analysed by LC-MS/MS directly after preparation. Selected final sample extracts as prepared for analysis were stored refrigerated for re-injection at a later date following the initial injection. Re-injection was performed after 8 days to fully demonstrate stability. As the recoveries were all within the acceptable range of 70-120 %, the stability of the sample extracts was considered sufficiently proven for at least 8 days.
Matrix Effects	Matrix-matched standards were prepared by fortifying final extracts of untreated control samples with calibration solutions prepared in neat solvent. Matrix-matched standard solutions were analyzed by LC-MS/MS together with a calibration solution in neat solvent prepared at the same analyte concentration. A significant matrix effect was observed in urine samples but not for blood samples fortified with clopyralid. However, matrix-matched standard solutions were used to quantify the analyte in both matrices.

Recovery of Clopyralid (m/z 190/146) - Quantitation

Matrix	Fortification Level (mg/L)	Recovery (%)		RSD (%)	n
		mean	range		
Human Urine	0.050	99	97-102	2	5
Human Blood	0.050	81	79-82	2	5

Recovery of Clopyralid (m/z 192/148) - Confirmation

Matrix	Fortification Level (mg/L)	Recovery (%)		RSD (%)	n
		mean	range		
Human Urine	0.050	90	84-95	5	5
Human Blood	0.050	82	80-83	2	5

Study Comments: 4.2(d)/1	Specificity: No interferences > 30% LOQ, highly specific method Linearity: Linear over the concentration range of 0.01 to 10 ng/mL for clopyralid (6 different concentrations) Correlation coefficient r ≥ 0.99 Precision: RSD = 2–5 % Accuracy: Mean recovery 81 – 99 % According to SANCO/825/00 rev. 8.1, recovery and precision data must be reported for the fortification levels LOQ and 10 x LOQ. Here the fortification level 10 x LOQ is missing. Thus the method is not acceptably validated for body fluids (blood and urine), In addition, no method has been given for body tissues.
Agreed endpoint: 4.2(d)/1	LC-MS/MS.

B.5.3. REFERENCES RELIED ON

Data were presented in the dossier submitted in April 2002 for the Active Approval and were deemed acceptable following evaluation and peer review at EU level. These data are still valid for decision making and are listed below in *italics*.

Other references listed are new tests, studies or information required to support the active substance renewal submission.

Data owner: DAS = Dow AgroSciences

Data Point	***Author(s)***	***Year***	***Title*** ***Source (where different from company)*** ***Company Report No.*** ***Date*** ***GLP or GEP status*** ***Published or not***	***Vertebrate study Y/N***	***Data protection claimed Y/N***	***Justification if data protection is claimed***	***Owner***	***Previous evaluation***
CA 4.1.1 (a)/1	*Liang, Y.Y.*	*1995*	*Analytical Method for the Determination of Clopyralid in Technical Grade Clopyralid Produced by the Penta Process* *The Dow Chemical Company, Pittsburg, CA* *DAS Report No. DECO-GP-AR 94-47020A* *GLP/GEP (Y/N): Yes* *Published (Y/N): No*	*No*	*No*	*N/A*	*DAS*	*In DAR 2003*
CA 4.1.2 (e)/1	[redacted]	*2002a*	*Determination of residues of clopyralid in animal tissues by gas chromatography with negative-ion chemical ionization mass spectrometry.* [redacted] *Report No.: GH-C 5440 (GRM 02.14),* *Date:19.04.2002,* *GLP, Non Published* *DAS No. O96*	*No*	*No*	*N/A*	*DAS*	*In DAR 2003*

Data Point	***Author(s)***	***Year***	***Title*** ***Source (where different from company)*** ***Company Report No.*** ***Date*** ***GLP or GEP status*** ***Published or not***	***Vertebrate study Y/N***	***Data protection claimed Y/N***	***Justification if data protection is claimed***	***Owner***	***Previous evaluation***
CA 4.1.2 (e)/2	*Clements, B,; Harrington, R.*	*1997*	*Determination of Residues of MCPA. Clopyralid and Fluroxypyr in Grass and Cereal Grain and Straw* *Dow AgroSciences, LLC, Letcombe, UK* *Dow AgroSciences Study Number: ERC 97.10* *15.10.1997* *GLP, Non Published*	*No*	*No*	*N/A*	*DAS*	*The study has been evaluated at EU level by Ireland (RMS) in the framework of the Fluroxypyr EU Annex I Renewal (RAR, Oct 2009)*
CA 4.1.2 (e)/3	*Hastings, M.J.*	*2002b*	*Determination of residues of clopyralid on agricultural crops by gas chromatography with negative-ion chemical ionization mass spectrometry.* *Dow AgroSciences LLC,* *Indianapolis, Indiana, USA* *Report No.: GH-C 5439,* *Date:19.04.2002,* *GLP, Non Published* *DAS No. O97*	*No*	*No*	*N/A*	*DAS*	*In DAR 2003*

Data Point	***Author(s)***	***Year***	***Title*** ***Source (where different from company)*** ***Company Report No.*** ***Date*** ***GLP or GEP status*** ***Published or not***	***Vertebrate study Y/N***	***Data protection claimed Y/N***	***Justification if data protection is claimed***	***Owner***	***Previous evaluation***
CA 4.1.2 (f)/1 *(submitted under CA 8.2.6.2/1)*	*Hoberg, J. R.*	*2006*	*Clopyralid Technical Grade - Growth Inhibition Test with Freshwater Blue Green Alga (Anabaena flos-aquae)* *Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts, United States 02571* *DAS Report No. 060246* *25.9.2006* *GLP/GEP (Y/N): Yes* *Published (Y/N): No*	*No*	*Yes*	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	*DAS*	*Submitted for the purpose of renewal*

Data Point	***Author(s)***	***Year***	***Title*** ***Source (where different from company)*** ***Company Report No.*** ***Date*** ***GLP or GEP status*** ***Published or not***	***Vertebrate study Y/N***	***Data protection claimed Y/N***	***Justification if data protection is claimed***	***Owner***	***Previous evaluation***
CA 4.1.2 (f)/2 *(submitted under CA 8.2.6.2/2)*	*Aufder-heide, J.*	*2015*	*Clopyralid Technical: Growth Inhibition Test with the Freshwater Diatom, Navicula pelliculosa* *ABC Laboratories, Inc. 7200 E. ABC Lane Columbia, Missouri 65202 USA* *DAS Report No. 140515* *15.1.2015* *GLP/GEP (Y/N): Yes* *Published (Y/N): No*	*No*	*Yes*	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	*DAS*	*Submitted for the purpose of renewal*

Data Point	***Author(s)***	***Year***	***Title*** ***Source (where different from company)*** ***Company Report No.*** ***Date*** ***GLP or GEP status*** ***Published or not***	***Vertebrate study Y/N***	***Data protection claimed Y/N***	***Justification if data protection is claimed***	***Owner***	***Previous evaluation***
CA 4.1.2 (f)/3 *(submitted under CA 8.2.7-2)*	*Banman, C. S., Moore, S.*	*2015*	*Clopyralid: Toxicity to the Aquatic Macrophyte, Myriophyllum spicatum* *SynTech Research Laboratory Services LLC 17745 South Metcalf Avenue Stilwell, Kansas 66085-9104, USA* *17.3.2015* *DAS Report No. 140735* *GLP/GEP (Y/N): Yes* *Published (Y/N): No*	*No*	*Yes*	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	*DAS*	*Submitted for the purpose of renewal*

Data Point	***Author(s)***	***Year***	***Title*** ***Source (where different from company)*** ***Company Report No.*** ***Date*** ***GLP or GEP status*** ***Published or not***	***Vertebrate study Y/N***	***Data protection claimed Y/N***	***Justification if data protection is claimed***	***Owner***	***Previous evaluation***
CA 4.2 (a)/1	*Vogl, E.*	*2012*	*Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS* *ABC Laboratories, Inc., Columbia, Missouri, USA* *DAS Report No. 120610* *21.9.2012* *GLP/GEP (Y/N): Yes* *Published (Y/N): No*	*No*	*Yes*	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	*DAS*	*Submitted for the purpose of renewal*

Data Point	***Author(s)***	***Year***	***Title*** ***Source (where different from company)*** ***Company Report No.*** ***Date*** ***GLP or GEP status*** ***Published or not***	***Vertebrate study Y/N***	***Data protection claimed Y/N***	***Justification if data protection is claimed***	***Owner***	***Previous evaluation***
CA 4.2 (a)/2	*Austin, R.*	*2012*	*Independent Laboratory Validation of Dow AgroSciences Method 120610, "Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS"* *Battelle UK Ltd, Ongar, Essex, United Kingdom* *DAS Report No. 120614* *12.10.2012* *GLP/GEP (Y/N): Yes* *Published (Y/N): No*	*No*	*Yes*	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	*DAS*	*Submitted for the purpose of renewal*

Data Point	***Author(s)***	***Year***	***Title*** ***Source (where different from company)*** ***Company Report No.*** ***Date*** ***GLP or GEP status*** ***Published or not***	***Vertebrate study Y/N***	***Data protection claimed Y/N***	***Justification if data protection is claimed***	***Owner***	***Previous evaluation***
CA 4.2 (a)/3	[redacted]	*2012*	*Method Validation Study for the Determination of Residues of Clopyralid in Bovine and Poultry Matrices by Liquid Chromatography with Tandem Mass Spectrometry Detection* [redacted] *DAS Report No. 120483* *17.9.2012* *GLP/GEP (Y/N): Yes* *Published (Y/N): No*	*Yes*	*Yes*	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	*DAS*	*Submitted for the purpose of renewal*

Data Point	***Author(s)***	***Year***	***Title*** ***Source (where different from company)*** ***Company Report No.*** ***Date*** ***GLP or GEP status*** ***Published or not***	***Vertebrate study Y/N***	***Data protection claimed Y/N***	***Justification if data protection is claimed***	***Owner***	***Previous evaluation***
CA 4.2 (a)/4	[redacted]	*2012*	*Independent Laboratory Validation of an Analytical Method for the Determination of Clopyralid in Animal Matrices* [redacted] *DAS Report No. 120484* *11.9.2012* *GLP/GEP (Y/N): Yes* *Published (Y/N): No*	*Yes*	*Yes*	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	*DAS*	*Submitted for the purpose of renewal*

Data Point	***Author(s)***	***Year***	***Title*** ***Source (where different from company)*** ***Company Report No.*** ***Date*** ***GLP or GEP status*** ***Published or not***	***Vertebrate study Y/N***	***Data protection claimed Y/N***	***Justification if data protection is claimed***	***Owner***	***Previous evaluation***
CA 4.2 (a)/5	[redacted]	*2013*	*Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin* [redacted] *7.10.2013* *DAS Report No. 130729* *GLP/GEP (Y/N): Yes* *Published (Y/N): No*	*No*	*Yes*	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	*DAS*	*Submitted for the purpose of renewal*

Data Point	***Author(s)***	***Year***	***Title*** ***Source (where different from company)*** ***Company Report No.*** ***Date*** ***GLP or GEP status*** ***Published or not***	***Vertebrate study Y/N***	***Data protection claimed Y/N***	***Justification if data protection is claimed***	***Owner***	***Previous evaluation***
CA 4.2 (a)/6	[redacted]	*2014*	*Independent Laboratory Validation of a Multi-residue Method Following the QuEChERS Sample Preparation Technique for the Determination of Clopyralid in Matrices of Plant and Animal Origin* [redacted] *DAS Report No. 130728* *25.2.2014* *GLP/GEP (Y/N): Yes* *Published (Y/N): No*	*No*	*Yes*	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	*DAS*	*Submitted for the purpose of renewal*

Data Point	***Author(s)***	***Year***	***Title*** ***Source (where different from company)*** ***Company Report No.*** ***Date*** ***GLP or GEP status*** ***Published or not***	***Vertebrate study Y/N***	***Data protection claimed Y/N***	***Justification if data protection is claimed***	***Owner***	***Previous evaluation***
CA 4.2 (b)/1	*Vincent, T. P.*	*2013*	*Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS* *ABC Laboratories, Inc., Columbia, Missouri, USA* *DAS Report No. 120612* *20.2.2013* *GLP/GEP (Y/N): Yes* *Published (Y/N): No*	*No*	*Yes*	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	*DAS*	*Submitted for the purpose of renewal*

Data Point	***Author(s)***	***Year***	***Title*** ***Source (where different from company)*** ***Company Report No.*** ***Date*** ***GLP or GEP status*** ***Published or not***	***Vertebrate study Y/N***	***Data protection claimed Y/N***	***Justification if data protection is claimed***	***Owner***	***Previous evaluation***
CA 4.2 (b)/2	*Austin, R., Turner, R.*	*2014*	*Independent Laboratory Validation of a Dow AgroSciences Method for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS* *Battelle UK Ltd, Chelmsford, Essex, United Kingdom* *DAS Report No. 140079* *19.5.2014* *GLP/GEP (Y/N): Yes* *Published (Y/N): No*	*No*	*Yes*	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	*DAS*	*Submitted for the purpose of renewal*

Data Point	***Author(s)***	***Year***	***Title*** ***Source (where different from company)*** ***Company Report No.*** ***Date*** ***GLP or GEP status*** ***Published or not***	***Vertebrate study Y/N***	***Data protection claimed Y/N***	***Justification if data protection is claimed***	***Owner***	***Previous evaluation***
CA 4.2 (b)/3	*Shaffer, S.*	*2012*	*Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water, and Surface Water by LC-MS/MS* *ABC Laboratories, Inc., Columbia, Missouri, USA* *DAS Report No. 120611* *4.12.2012* *GLP/GEP (Y/N): Yes* *Published (Y/N): No*	*No*	*Yes*	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	*DAS*	*Submitted for the purpose of renewal*

Data Point	***Author(s)***	***Year***	***Title*** ***Source (where different from company)*** ***Company Report No.*** ***Date*** ***GLP or GEP status*** ***Published or not***	***Vertebrate study Y/N***	***Data protection claimed Y/N***	***Justification if data protection is claimed***	***Owner***	***Previous evaluation***
CA 4.2 (b)/4	*Austin, R., Turner, R.*	*2013*	*Independent Laboratory Validation of Dow AgroSciences Method 120611, "Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water, and Surface Water by LC-MS/MS"* *Battelle UK Ltd, Ongar, Essex, United Kingdom* *DAS Report No. 120613* *5.4.2013* *GLP/GEP (Y/N): Yes* *Published (Y/N): No*	*No*	*Yes*	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	*DAS*	*Submitted for the purpose of renewal*

Data Point	***Author(s)***	***Year***	***Title*** ***Source (where different from company)*** ***Company Report No.*** ***Date*** ***GLP or GEP status*** ***Published or not***	***Vertebrate study Y/N***	***Data protection claimed Y/N***	***Justification if data protection is claimed***	***Owner***	***Previous evaluation***
CA 4.2 (c)/1	*Bacher, R.*	*2012*	*The Development and Validation of a Method for the Analysis of Clopyralid in Air* *PTRL Europe GmbH, D-89081 Ulm, Germany* *DAS Report No. 120601* *4.10.2012* *GLP/GEP (Y/N): Yes* *Published (Y/N): No*	*No*	*Yes*	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	*DAS*	*Submitted for the purpose of renewal*

Data Point	***Author(s)***	***Year***	***Title*** ***Source (where different from company)*** ***Company Report No.*** ***Date*** ***GLP or GEP status*** ***Published or not***	***Vertebrate study Y/N***	***Data protection claimed Y/N***	***Justification if data protection is claimed***	***Owner***	***Previous evaluation***
CA 4.2 (d)/1	[redacted]	*2014*	*Development and Validation of an Analytical Method for the Determination of Clopyralid in Body Fluid(s)* [redacted] *DAS Report No. 130727* *9.7.2014* *GLP/GEP (Y/N): Yes* *Published (Y/N): No*	*No*	*Yes*	Active substance data submitted with an application under Article 15 of the Regulation (renewal) and with the application for renewal of authorisation of the corresponding product. Applies also to data submitted with applications for authorisation for products containing AIR2 substances (Regulation (EU) No 1141/2010).	*DAS*	*Submitted for the purpose of renewal*