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This DER was originally prepared under contract by Dynamac Corporation (1910 Sedwick Rd., Building 100, Suite B; Durham, NC 27713; submitted 2/20/2005). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

44107102 Crook, S. (1994) The Determination of Acetochlor and R-25788 (Dichlormid) in Maize Grain, Forage, and Fodder; Soybean Seed and Hay: Lab Project Number: RAM244/02: RAM 244/02. Unpublished study prepared by Zeneca Agrochemicals. 25 p.

EXECUTIVE SUMMARY:

A method description and validation data were provided for a GC/nitrogen-phosphorus specific detector (NPD) method for determining residues of acetochlor in plant commodities. This method (RAM 244/02) was used for the determining residues of acetochlor, *per se*, in sweet corn field trials and rotational crop field trials.

For this method, residues are extracted with methanol, filtered, and concentrated. Residues are then diluted with a sodium chloride solution and partitioned into toluene. Residues are cleaned up using solid phase amino (NH_2) and silica gel columns eluted with ethyl acetate:hexane (40:60, v/v). Residues are analyzed by GC/NPD and quantified using an external standard. The LOQ for acetochlor residues is 0.01 ppm; the LOD was not reported.

Method validation average recoveries at the method LOQ (0.10 ppm) were 92% ± 2.2% in corn grain, 106% ± 6.6% in corn forage, 107% ± 6.1% in corn fodder, 115% ± 5.3% in soybean seed and 94% ± 12% in soybean hay. Of the 20 individual samples at the LOQ, all were within the 70% – 120% acceptable recovery range with the exception of a single soybean seed sample at 123%. Further, for the 24 method validation samples fortified at 0.05 ppm to 0.20 ppm in corn and soybean matrices, recoveries were all within the acceptable 70% to 120% range for both individual samples and for fortification level averages. In addition, a total of 93 samples were fortified with acetochlor in conjunction with field trials on corn, sugar beets, dried peas, dried

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beans, sunflowers and potatoes. Corn grain (19), corn forage (24), corn stover (15), sugar beet roots (5), sugar beet tops (8), dried peas (6), dried beans (6), sunflower seeds (4), and potato tubers (6) were fortified at 0.2 – 0.10 ppm. For all samples, recoveries were within the acceptable range of 70% - 120% with the exception of a sugar beet top sample fortified at 0.02 ppm with a recovery of 122% and a dried bean sample fortified at 0.02 ppm with a recovery of 124%.

The registrant has demonstrated that the proposed data collection method can recover acetochlor *per* se from various plant matrices that have been spiked with acetochlor including: corn grain, forage, and stover, sugar beet roots and tops, soybean seeds, dried peas and beans, sunflower seeds, and potato tubers. However, this method represents a substantial change to the enforcement method extraction scheme. Since the method has not been radiovalidated, it is not possible to determine if the revised extraction scheme is able to extract field weathered residues of acetochlor from plant matrices. Therefore; HED concludes that analytical method, RAM 244/02 has not been adequately validated as a data collection method beyond its utility for recovering residues of acetochlor *per se* in laboratory spiked samples. Should the registrant desire to use this method as a data collection method to examine residues of acetochlor in field weathered samples, radiovalidation data demonstrating the method's ability to extract field weathered residues from various plant matrices would be required.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the analytical method residue data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U. S. EPA document entitled *Acetochlor: Petitions for Tolerances on Sweet Corn and Rotational Crops of Nongrass Animal Feeds (Group 18), Sugar Beets, Dried Shelled Beans and Peas (Subgroup 6C), Sunflowers, Potatoes, Cereal Grains (Group 15), and Forage, Fodder, and Straw of Cereal Grains (Group 16). Summary of Analytical Chemistry and Residue Data* (D. Davis, D230310).

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported.

A. BACKGROUND INFORMATION

Acetochlor is a chloroacetanilide herbicide used for preemergence control of weeds in corn. In the United States, acetochlor is conditionally registered for use on corn to the Acetochlor Registration Partnership (ARP), which is comprised of Monsanto and Dow AgroSciences. Acetochlor is formulated as a variety of emulsifiable concentrate (EC), emulsion in water (EW), microencapsulated (Mcap), or granular (G) formulations that can be applied to corn as a preplant, preemergence, or early post-emergence application using only ground equipment. Tolerances are established for the combined residues of acetochlor and its ethyl methyl aniline- (EMA) and hydroxyethyl methyl aniline- (HEMA) producing metabolites, expressed as acetochlor equivalents [40 CFR §180.470]. Tolerances range from 0.05 to 1.5 ppm in/on corn commodities resulting from the direct use of acetochlor and from 0.02 to 1.0 ppm in commodities from rotational crops of sorghum, soybean, or wheat.

The ARP has submitted a petition (PP#6F4791) proposing the direct use of acetochlor (EC) on sweet corn and requesting tolerances on sweet corn commodities and tolerances for inadvertent residues in rotated non-grass animal feeds. The ARP has also proposed (PP#1F6263) tolerances for inadvertent residues in rotated dried peas and beans (subgroup 6C), sugar beets, sunflowers, potatoes, cereal grains (group 15, except corn and rice), and the forage, fodder, and straw of cereal grains (group 16, except corn and rice). In conjunction with these petitions, ARP has submitted a GC/NPD method for determining residues of acetochlor *per se* in crops.

TABLE A.1. Acetochlor Nomenclature

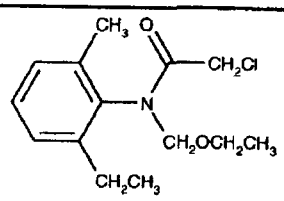
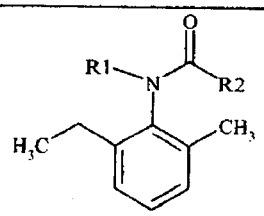
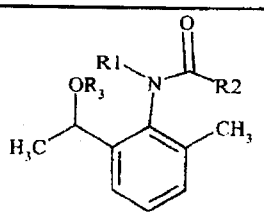
Chemical structure	
Common name	Acetochlor
Molecular Formula	$C_{14}H_{20}ClNO_2$
Molecular Weight	269.8
IUPAC name	2-chloro-N-ethoxymethyl-6'-ethylacet-o-toluidide
CAS name	2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide
CAS #	34256-82-1
PC Code	121601
End-use Product	7.5 lb/gal EC

TABLE A.2. Physicochemical Properties of Acetochlor.		
Parameter	Value	Reference
Boiling point/range	163 °C at 10 mm Hg; decomposition occurs before the boiling point at atmospheric pressure; (calculated by extrapolation of vapor pressure at lower temperature)	Acetochlor HED Chapter of the TRED 3/1/06
pH	4.41, 1% solution in acetone:water (1:1, v:v)	
Density at 20 °C	1.123 g/mL	
Water solubility at 25 °C	223 mg/L	
Solvent solubility at 25 °C	Infinitely soluble in acetone, benzene, carbon tetrachloride, ethanol, chloroform, and toluene	
Vapor pressure at 25 °C	0.045 µHg (4.5×10^{-5} mm Hg)	
Dissociation constant, pK_a	Not applicable because acetochlor is neither an acid nor a base.	
Octanol/water partition coefficient	970 or 1082	
UV/visible absorption spectrum	Not available	

Table A.3. Acetochlor Metabolite Structures	
Metabolite Type	Structure
EMA-type metabolites	
HEMA-type metabolites	

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

This GC/NPD method (RAM 244/02) was used for the determining residues of acetochlor *per se* in the sweet corn field trials and field rotational crop trials.

B.1.1. Principle of the Method

Residues of acetochlor are extracted with methanol, filtered, and concentrated. Residues are then diluted with a sodium chloride solution and partitioned into toluene. Residues are cleaned up using solid phase amino (NH_2) and silica gel columns eluted with ethyl acetate:hexane (40:60, v/v). Residues are analyzed by GC/NPD and quantified by comparison to external standards. Mass selective detection (MSD) may be used for confirmation. The LOQ for acetochlor is 0.01 ppm, and the LOD was not reported.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Residues of Acetochlor in Plant Matrices.

Method ID	RAM 244/02
Analytes	Acetochlor
Extraction solvent/technique	Extract with methanol
Cleanup strategies	Partitioning into toluene and cleanup using solid phase amino (NH_2) and silica gel columns; eluted using ethyl acetate:hexane (40:60, v/v).
Instrument/Detector	GC using fused silica capillary column, HP5890 series with BP_x5 column (25-m x 0.25-mm id, 0.25-µm film thickness) with a Nitrogen-phosphorus detector.
Standardization method	External standards
Stability of std solutions	Standard solutions are to be stored refrigerated (<5°C); standards are reportedly stable under these conditions for 4 months. The storage interval for standards used was not reported.
Retention times	Acetochlor - 24.5 minutes

B.1.2. Method Validation

For Method validation, control samples of corn grain, forage, and fodder, and soybean seed and hay were fortified with acetochlor at 0.01-0.20 ppm. Fortified samples were analyzed along with control samples using the procedures described above.

In addition, the above method was also validated in conjunction with the rotational crop field trials using control samples fortified with acetochlor at 0.02-0.10 ppm.

RAM 244/02 was not subjected to radiovalidation to confirm that the revised extraction scheme was able to extract field weathered residues from various plant matrices.

B.2. Enforcement Method

A tolerance enforcement method is available for determining residues of acetochlor and its EMA and HEMA producing metabolites in corn commodities. The method is an HPLC method using a oxidative coulometric electrochemical detector (OCED) and is listed as Method I in PAM Vol. II (180.470).

For this method, residues are solvent extracted into aqueous acetonitrile, concentrated, and base hydrolyzed to yield EMA and HEMA. The resulting residues are steam-distilled into dilute acid, adjusted to a basic pH, and partitioned into methylene chloride. HEMA is methylated using acidic methanol and residues of EMA and methylated HEMA (MEMA) are separated and determined using HPLC/OCED. Residues of EMA and HEMA are expressed in acetochlor equivalents and the validated method LOQ is 0.02 ppm for each analyte.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

Method validation average recoveries at the method LOQ (0.10 ppm) were 92% ± 2.2% in corn grain, 106% ± 6.6% in corn forage, 107% ± 6.1% in corn fodder, 115% ± 5.3% in soybean seed and 94% ± 12% in soybean hay. Of the 20 individual samples at the LOQ, all were within the 70% – 120% acceptable recovery range with the exception of a single soybean seed sample at 123%. Further, for the 24 method validation samples fortified at 0.05 ppm to 0.20 ppm in corn and soybean matrices, recoveries were all within the acceptable 70% to 120% range for both individual samples and for fortification level averages. In addition, a total of 93 samples were fortified with acetochlor in conjunction with field trials on corn, sugar beets, dried peas, dried beans, sunflowers and potatoes. Corn grain (19), corn forage (24), corn stover (15), sugar beet roots (5), sugar beet tops (8), dried peas (6), dried beans (6), sunflower seeds (4), and potato tubers (6) were fortified at 0.2 – 0.10 ppm. For all samples, recoveries were within the acceptable range of 70% - 120% with the exception of a sugar beet top sample fortified at 0.02 ppm with a recovery of 122% and a dried bean sample fortified at 0.02 ppm with a recovery of 124%. Individual sample recovery data and fortification level averages are summarized in the table below.

TABLE C.1.1. Recovery Results from Method Validation of Plant Matrices using the Data-Gathering GC/NPD Analytical Method (RAM 244/02).[1]

Matrix	Spiking Level (ppm)	Sample size	Acetochlor	
			Recoveries (%)	Mean Recovery ± Std
Method Validation				
Corn grain	0.01	4	90, 91, 93, 95	92 ± 2.2
	0.05	1	87	n/a
	0.10	1	90	n/a
	0.20	2	102, 104	n/a
Corn forage	0.01	4	98, 102, 111, 111	106 ± 6.6

TABLE C.1.1. Recovery Results from Method Validation of Plant Matrices using the Data-Gathering GC/NPD Analytical Method (RAM 244/02).[1]

Matrix	Spiking Level (ppm)	Sample size	Acetochlor	
			Recoveries (%)	Mean Recovery ± Std
	0.05	1	103	n/a
	0.10	1	96	n/a
	0.20	2	103, 112	n/a
Corn fodder	0.01	4	98, 108, 109, 112	107 ± 6.1
	0.05	2	92, 97	n/a
	0.10	2	74, 88	n/a
	0.20	2	82, 83	n/a
Soybean seed	0.01	4	111, 113, 114, 123	115 ± 5.3
	0.05	2	94, 94	n/a
	0.10	1	94	n/a
	0.20	1	83	n/a
Soybean hay	0.01	4	77, 95, 98, 107	94 ± 12
	0.05	2	77, 77	n/a
	0.10	2	83, 92	n/a
	0.20	2	82, 87	n/a
Concurrent Method Recovery				
Corn grain	0.02	7	113, 89, 115, 92, 107, 87, 92	99 ± 12
	0.05	6	101, 92, 97, 91, 87, 81	92 ± 7.1
	0.10	6	107, 108, 112, 110, 78, 91	101 ± 14
Corn forage	0.02	8	106, 88, 119, 96, 99, 93, 90, 111	101 ± 11
	0.05	9	100, 84, 106, 118, 93, 119, 80, 91, 108	100 ± 14
	0.10	7	103, 118, 102, 113, 89, 92, 91	101 ± 11
Corn stover	0.02	5	111, 115, 87, 109, 88	102 ± 13
	0.05	5	93, 109, 104, 71, 92	94 ± 12
	0.10	5	94, 117, 98, 116, 86	102 ± 14
Sugar beet roots	0.02	3	89, 108, 103	100 ± 9.8
	0.05	2	75, 93	n/a
Sugar beet tops	0.02	4	100, 113, 109, 122	111 ± 9.1
	0.05	4	95, 89, 108, 111	101 ± 10
Dried Peas	0.02	3	95, 97, 89	94 ± 4.2
	0.05	3	90, 108, 100	99 ± 9.0
Dried Beans	0.02	3	99, 111, 124	111 ± 12
	0.05	3	94, 101, 94	96 ± 4.0
Sunflower seeds	0.02	2	71, 76	n/a
	0.10	2	82, 80	n/a
Potato tubers	0.02	3	93, 70, 101	88 ± 16
	0.10	3	92, 102, 85	93 ± 8.5

[1] The concurrent method recovery data for all plant matrices are identical to those submitted with the field trial studies and field rotational crop studies (46010501.DER through 46010509.DER).
n/a = not applicable

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Acetochlor in Plant Matrices.	
Analytes	Acetochlor
Equipment ID	Hewlett-Packard Model 5890 GC; BP_x5 column (25-m x 0.25-mm id, 0.25-µm film thickness) with a NPD.
Limit of quantitation (LOQ)	0.01 ppm
Limit of detection (LOD)	Not reported
Accuracy/Precision	Average method recoveries were 88-105% for acetochlor, with relatively coefficients of variation (± 6-14%) for corn grain, forage and fodder, and soybean seed and hay.
Reliability of the Method/ [ILV]	An independent laboratory method validation [ILV] of the proposed data collection method has not been conducted to verify the reliability of the method for the determination of residues of acetochlor in plant commodities.
Linearity	An example standard curve for acetochlor at concentrations of 0.01-1.0 µg/mL was provided and indicated a linear response, however, the correlation coefficient was not provided.
Specificity	The control chromatograms generally have no peaks above the chromatographic background and the spiked sample chromatograms contain only the analyte peak of interest. Peaks were well defined and symmetrical. There appeared to be no carryover to the following chromatograms.

C.2. Enforcement Method

The HPLC/OCED enforcement method for plant commodities has been adequately validated by the Agency and is listed as Method I in PAM Vol. II (180.470).

C.3. Independent Laboratory Validation

An independent laboratory validation (ILV) of the data collection method has not been conducted. The method was used only for data collection and is not being proposed for enforcing tolerances. Therefore, an ILV trial is not required.

D. CONCLUSION

Method validation average recoveries at the method LOQ (0.10 ppm) were 92% ± 2.2% in corn grain, 106% ± 6.6% in corn forage, 107% ± 6.1% in corn fodder, 115% ± 5.3% in soybean seed and 94% ± 12% in soybean hay. Of the 20 individual samples at the LOQ, all were within the 70% – 120% acceptable recovery range with the exception of a single soybean seed sample at 123%. Further, for the 24 method validation samples fortified at 0.05 ppm to 0.20 ppm in corn and soybean matrices, recoveries were all within the acceptable 70% to 120% range for both individual samples and for fortification level averages. In addition, a total of 93 samples were fortified with acetochlor in conjunction with field trials on corn, sugar beets, dried peas, dried beans, sunflowers and potatoes. Corn grain (19), corn forage (24), corn stover (15), sugar beet roots (5), sugar beet tops (8), dried peas (6), dried beans (6), sunflower seeds (4), and potato

tubers (6) were fortified at 0.2 – 0.10 ppm. For all samples, recoveries were within the acceptable range of 70% - 120% with the exception of a sugar beet top sample fortified at 0.02 ppm with a recovery of 122% and a dried bean sample fortified at 0.02 ppm with a recovery of 124%.

The registrant has demonstrated that the proposed data collection method can recover acetochlor *per* se from various plant matrices that have been spiked with acetochlor including: corn grain, forage, and stover, sugar beet roots and tops, soybean seeds, dried peas and beans, sunflower seeds, and potato tubers. However, this method represents a substantial change to the enforcement method extraction scheme. Since the method has not been radiovalidated, it is not possible to determine if the revised extraction scheme is able to extract field weathered residues of acetochlor from plants. Therefore; HED concludes that analytical method, RAM 244/02 has not been adequately validated as a data collection method beyond its utility for recovering residues of acetochlor *per se* in laboratory spiked samples.

E. REFERENCES

DP Barcode: D292336
Subject: **ACETOCHLOR**. Revised HED Chapter of the Tolerance Reassessment Eligibility Decision (TRED) Document.
From: A. Protzel
To: F. Fort
Dated: 3/1/06
MRID(s): None

F. DOCUMENT TRACKING

RDI: D. Davis (3/13/06), T. Goodlow (3/16/06)
Petition Number(s): 6F4791
DP Barcode(s): D230310 and D275019
PC Code: 121601