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Peer Reviewer	[Peer Reviewer name, title, and affiliation]	Date:
Approved by	[Approver name, title, and affiliation]	Date:

This DER was originally prepared under contract by Dynamac Corporation (2275 Research Boulevard, Suite 300; Rockville, MD 20850; submitted 05/08/2006). The DER has been reviewed by the Health Effects Division (HED) and revised to reflect current Office of Pesticide Programs (OPP) policies.

STUDY REPORTS:

46352003 Charles, E.; Doran, A.M. (2004) Independent Laboratory Validation of Analytical Method DuPont-13412 for the Determination of Thifensulfuron Methyl, Ethametsulfuron Methyl, Rimsulfuron, Tribenuron Methyl and Chlorimuron Ethyl in Olives and Soybean Seed Using SPE Purification and LC/MS/MS Detection: Project Number: DuPont-13398. Study No. 303871 Unpublished study prepared by E.I. du Pont de Nemours and Company. 65 pages.

46421901 Carringer, S.J. (2004) Magnitude of Residues of Thifensulfuron Methyl and Tribenuron Methyl in Rice, Corn, Sorghum, and Soybeans Following Pre-Plant Burn-Down Applications of DPX-M6316 75 GT XP Herbicide and DPX-L5300 75 XP Herbicide at Maximum Label Rates – U.S.A., 2003: Lab Project Number: ML03-1101-DUP. Study No. TCI-03-080. Unpublished study prepared by The Carringers, Inc. 552 pages.

EXECUTIVE SUMMARY:

E.I. du Pont de Nemours and Company have submitted an independent laboratory validation (ILV) study for an LC/MS/MS method, DuPont Method 13412, for the determination of residues of tribenuron methyl in/on oily crop matrices. This method was also used for data collection in samples of corn grain, sorghum grain, and soybean seed from the crop field trial study submitted in conjunction with DP Barcode D314429 (refer to the 860.1500 DER for MRID 46421901).

The ILV submission did not include a copy of the method. The only version of the method, entitled "Analytical Method for the Determination of Nicosulfuron, Thifensulfuron Methyl, Ethametsulfuron Methyl, Rimsulfuron, Tribenuron Methyl, and Chlorimuron Ethyl in Oily Crop Matrices Using SPE Purification and LC/MS/MS Detection," available to the study reviewer for review was a draft version, dated 12/2/03, that was included with the crop field trial submission. Based on the references included in the method, the version of the method that was subjected to ILV was dated 1/28/04.

We note that the submission contains ILV data for thifensulfuron methyl, ethametsulfuron methyl, rimsulfuron, and chlorimuron ethyl which are not reviewed herein. Data for thifensulfuron methyl were reviewed separately (46352003.del.doc).

Using DuPont Method 13412, residues in/on homogenized samples of corn grain, sorghum grain, and soybean seed are extracted with an acetonitrile/potassium phosphate buffer solution. An aliquot of the extract is partitioned with hexane, and the aqueous phase is concentrated for cleanup through an ENV solid-phase extraction (SPE) column. Residues are eluted from the column with an ammonium hydroxide/methanol solution, and the eluate is concentrated and mixed with acetonitrile and ammonium acetate solution. Samples are analyzed by LC/MS/MS using electrospray ionization in the positive ion mode. The validated limit of quantitation (LOQ) reported in the method was 0.01 ppm; an LOQ of 0.05 ppm was used for corn grain, sorghum grain, and soybean seed samples from the crop field trials submitted in conjunction with DP Barcode D314429.

Method validation data for DuPont Method 13412 demonstrated adequate method recoveries of tribenuron methyl from corn grain, olive, and soybean seed. Following fortification of samples with each analyte at 0.010 and 0.10 ppm, recoveries of tribenuron methyl averaged 81 ± 2.3%, 86 ± 4.4%, and 78 ± 2.5% from corn grain, olive, and soybean seed, respectively. Recoveries of tribenuron methyl averaged 98 ± 2.6% and 96 ± 3.1% from corn grain and soybean seed, respectively, fortified with tribenuron methyl at 0.05 and 0.5 ppm.

The fortification levels used in method validation are adequate to bracket expected residue levels; however, no validation data were provided for sorghum grain. Concurrent method recovery data were included with the sorghum crop field trial study submitted in conjunction with DP Barcode D314429 (refer to the 860.1500 DER for MRID 46421901); adequate recoveries of tribenuron methyl were obtained from sorghum grain fortified at 0.05 ppm. The method validation and concurrent method recovery data are sufficiently representative of the expected residue levels for the commodities included in the petition associated with DP Barcode D314429.

Analyte identification is to be confirmed by comparing the ion ratio for the two MS/MS ion transitions acquired during analysis with the average ion ratio obtained for the calibration standards.

A successful ILV trial was conducted using samples of olive and soybean seed fortified with tribenuron methyl at 0.01 and 0.10 ppm.

No radiovalidation data were submitted for the method. In metabolism studies conducted using oil seeds (canola and cotton; refer to the DERs for MRIDs 45089802 and 45089803, currently under review), total radioactive residues were too low in canola seed and cotton seed to allow residue characterization (0.02 ppm in canola seed and <0.01 ppm in cotton seed). Therefore, it is unlikely that residues of tribenuron methyl would be present in these commodities at levels that

would permit adequate determination of extraction efficiency. No radiovalidation data are required for this method at this time.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the analytical method test data are classified as scientifically acceptable. If the petitioner wishes DuPont Method 13412 to be used for enforcement, the final version of the method must be submitted; the version should include the modifications made by the ILV laboratory in the course of validation.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D314429.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Tribenuron methyl is a sulfonylurea herbicide (Group 1) registered for food/feed uses on barley, oats, and wheat, and grass grown for seed and for nonfood/feed use on cotton grown in TX only. A summary of the status of residue chemistry data requirements for tribenuron methyl was issued 6/24/04 (DP Barcode D304059, R. Griffin).

TABLE A.1. Tribenuron Methyl Nomenclature.

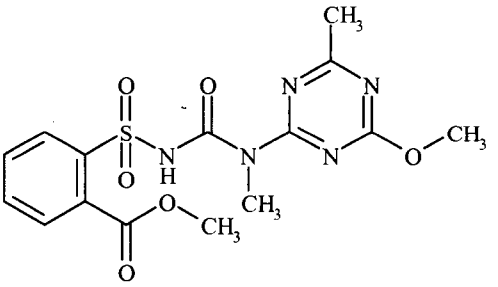
Chemical structure	
Common name	Tribenuron methyl
Company experimental name	DPX-L5300
IUPAC name	methyl-2-[4-methoxy-6-methyl-1,3,5-triazin-2-yl(methyl)carbamoyl-sulfamoyl]benzoate
CAS name	methyl-2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)methylamino]carbonyl]amino]sulfonyl]benzoate
CAS registry number	101200-48-0
End-use product (EP)	75% DF formulation (DuPont™ Express® XP Herbicide; EPA Reg. No. 352-509)

TABLE A.2. Physicochemical Properties of Tribenuron Methyl.		
Parameter	Value	Reference
Melting point	141 °C	DP Barcode D304059, 6/24/04, R. Griffin
pH	4.27 (slurry in water)	DP Barcode D304059, 6/24/04, R. Griffin
Density	1.54 g/mL	DP Barcode D304059, 6/24/04, R. Griffin
Water solubility	At 25 °C: pH 4.0 28 mg/L pH 5.0 50 mg/L pH 6.0 280 mg/L	DP Barcode D304059, 6/24/04, R. Griffin
Solvent solubility	At 25 °C: acetone - 43.8 g/L acetonitrile - 54.2 g/L carbon tetrachloride - 3.12 g/L ethyl acetate - 17.5 g/L hexane - 0.028 g/L methanol - 3.39 g/L	DP Barcode D304059, 6/24/04, R. Griffin
Vapor pressure	25 °C (Knudsen) - 3.8×10^{-10} 25 °C (gas saturation) - 2.7×10^{-7} 70 °C (gas saturation) - $<8.3 \times 10^{-7}$	DP Barcode D304059, 6/24/04, R. Griffin
Dissociation constant, pK_a	5.0	DP Barcode D304059, 6/24/04, R. Griffin
Octanol/water partition coefficient, $Log(K_{OW})$	pH 7 - 0.3 pH 5 (calculated) - 15 pH 9 (calculated) - 0.003	DP Barcode D304059, 6/24/04, R. Griffin
UV/visible absorption spectrum	Not available	

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

Samples of soybean seed from the storage stability study and samples of corn grain, sorghum grain, and soybean seed from the crop field trial study submitted in conjunction with DP Barcode D314429 were analyzed for residues of tribenuron methyl using LC/MS/MS DuPont Method 13412 entitled "Analytical Method for the Determination of Nicosulfuron, Thifensulfuron Methyl, Ethametsulfuron Methyl, Rimsulfuron, Tribenuron Methyl, and Chlorimuron Ethyl in Oily Crop Matrices Using SPE Purification and LC/MS/MS Detection" (draft dated 12/2/03).

B.1.1. Principle of the Method:

Samples of corn grain, sorghum grain, and soybean seed are extracted with an acetonitrile/potassium phosphate buffer solution. An aliquot of the extract is partitioned with hexane and the aqueous phase is concentrated for cleanup through an ENV SPE column. Residues are eluted from the column with an ammonium hydroxide/methanol solution and the eluate is concentrated and mixed with acetonitrile and ammonium acetate solution. Samples are analyzed using LC/MS/MS with electrospray ionization in the positive ion mode.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Tribenuron Methyl Residues in Oily Crop Matrices.	
Method ID	DuPont Method 13412 (draft version, dated 12/2/03)

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Tribenuron Methyl Residues in Oily Crop Matrices.	
Analyte	Tribenuron methyl [as well as nicosulfuron, thifensulfuron methyl, ethametsulfuron methyl, rimsulfuron, and chlorimuron ethyl]
Extraction solvent/technique	Homogenized samples are extracted two times with acetonitrile:pH 7 K_2HPO_4 (75:25, v:v); the extracts are isolated by centrifugation, combined, and diluted to volume with acetonitrile.
Cleanup strategies	An aliquot of the extract is partitioned with hexane and the hexane phase is discarded. An aliquot of the remaining aqueous phase is concentrated to near aqueous and then diluted with deionized water. The extract is then quantitatively transferred to a preconditioned ENV SPE column using 10 mM ammonium acetate rinses. Residues are eluted from the SPE column using 25 mM ammonium hydroxide:methanol (1:99, v:v). The eluate is mixed with 5 mM ammonium acetate, concentrated to remove the methanol, mixed with acetonitrile, and then diluted to volume with 50 mM ammonium acetate.
Instrument/Detector	HPLC employing tandem mass spectrometric (MS/MS) detection, using a phenyl-hexyl column and a gradient mobile phase of water and methanol, each containing 0.05% formic acid. The total ion chromatogram from two molecular ion transitions is used for quantitation.
Standardization method	Calibration curve of external standards, with a correlation coefficient of >0.99 using linear regression analysis.
Stability of std solutions	Stock standard solutions (100 µg/ml) in acetonitrile were reported to be stable for 6 months when stored at -10°C. Intermediate and fortification standards (10 and 1.0 µg/ml, and 100 ng/ml) were reported to be stable for 1 month when stored refrigerated. The method specifies that fresh chromatographic standard solutions be prepared from the intermediate standard on the day of analysis; these solution are reportedly stable for 2 days when stored refrigerated.
Retention times	Tribenuron methyl: ~25 minutes

B.2. Enforcement Method

The petitioner did not state whether the submitted method is intended for enforcement purposes. An LC/MS method exists for the enforcement of tolerances for residues of tribenuron methyl in/on canola, cotton, and flax commodities.

Note to EPA Reviewer: We could find nothing the petition materials identifying which method the petitioner was proposing for enforcement purposes.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

Method validation data for corn grain, olive, and soybean seed were included with the draft method. These data were generated by DuPont during method development using samples of untreated corn grain and soybean seed from crop field trials and samples of commercially purchased olives. In addition, method validation data for corn grain and soybean seed were included with the crop field trial results reported in MRID 46421901; these data were generated by Morse Laboratories, the laboratory that conducted the crop field trial analyses. Method validation data are presented in Table C.1.1; adequate recoveries of tribenuron methyl were obtained. Following fortification of samples at 0.010 and 0.10 ppm, recoveries of tribenuron methyl averaged 81 ± 2.3%, 86 ± 4.4%, and 78 ± 2.5% from corn grain, olive, and soybean seed respectively. Recoveries of tribenuron methyl averaged 98 ± 2.6% and 96 ± 3.1% from corn grain and soybean seed, respectively, fortified at 0.05 and 0.5 ppm.

The fortification levels used in method validation are adequate to bracket expected residue levels; however, no validation data were provided for sorghum grain. Concurrent method recovery data were included with the sorghum crop field trial study submitted in conjunction with DP Barcode D314429 (refer to the 860.1500 DER for MRID 46421901); adequate recoveries of tribenuron methyl were obtained from sorghum grain fortified at 0.05 ppm. The method validation and concurrent method recovery data are sufficiently representative of the expected residue levels for the commodities included in the petition associated with DP Barcode D314429.

Analyte identification is to be confirmed by comparing the ion ratio for the two MS/MS ion transitions acquired during analysis with the average ion ratio obtained for the calibration standards.

No radiovalidation data were submitted for the method. In metabolism studies conducted using oil seeds (canola and cotton; refer to the DERs for MRIDs 45089802 and 45089803, currently under review), total radioactive residues were too low in canola seed and cotton seed to allow residue characterization (0.02 ppm in canola seed and <0.01 ppm in cotton seed). Therefore, it is unlikely that residues of tribenuron methyl would be present in these commodities at levels that would permit adequate determination of extraction efficiency. No radiovalidation data are required for this method at this time.

We note that the draft method contains erroneous instructions for preparing the acetonitrile/phosphate buffer extraction solution. The solution is stated several times in the method description to be a 75/25 solution of acetonitrile and 20 mM potassium phosphate solution. However, the instructions in Section 4.2.2 specify that 250 mL of acetonitrile is to be diluted to 1 L with 20 mM potassium phosphate solution, which would result in a 25/75 solution of acetonitrile and potassium phosphate solution.

Several sections of the draft method state "To be completed" or "To be added;" these sections included Extraction Efficiency (Section 5.1.4), Second Lab Tryout (Sections 5.4.4), and Conclusions (Section 6.0).

The LOQ reported in the method is 0.01 ppm. In the analysis of crop field trial samples, Morse Laboratories reported the LOQ to be 0.05 ppm with a limit of detection of 0.02 ppm (estimated at one-third the LOQ).

TABLE C.1.1. Recovery Results from Method Validation of Corn Grain, Olive. and Soybean Seed using the Data-Gathering Analytical Method. [1]

Matrix	Spiking Level (ppm)	Recoveries Obtained (%)	Mean Recovery ± SD [CV] (%)
Method validation conducted by DuPont			
Corn grain	0.010	81, 81, 82, 83, 85	86 ± 2.3 [2.8]
	0.10	81, 82, 84, 84, 85	
Olive	0.010	85, 87, 90, 91, 95	86 ± 4.4 [5.1]
	0.10	81, 82, 84, 84, 85	

TABLE C.1.1. Recovery Results from Method Validation of Corn Grain, Olive. and Soybean Seed using the Data-Gathering Analytical Method. [1]

Matrix	Spiking Level (ppm)	Recoveries Obtained (%)	Mean Recovery ± SD [CV] (%)
Soybean seed	0.010	77, 78, 80, 80, 82	78 ± 2.5 [3.1]
	0.10	75, 76, 77, 77, 82	
Method validation conducted by Morse Laboratories			
Corn grain	0.05	100, 101	98 ± 2.6 [2.7]
	0.50	95, 98	
Soybean seed	0.05	97, 98	96 ± 3.1 [3.3]
	0.50	91, 96	

[1] Standards were prepared in acetonitrile

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Tribenuron Methyl Residues in Oily Crop Matrices.

Method ID	DuPont Method 13412 (draft version, dated 12/2/03)
Analyte	Tribenuron methyl [as well as nicosulfuron, thifensulfuron methyl, ethametsulfuron methyl, rimsulfuron, and chlorimuron ethyl]
Equipment ID	HP1100 HPLC with a MicroMass Quattro II triple quadrupole mass spectrometer with an electrospray interface; Phenomenex Luna Phenyl-Hexyl column (4.6 mm x 150 mm; 3 µm particle size diameter).
Limit of quantitation (LOQ)	0.01 ppm (target)
Limit of detection (LOD)	Estimated at 0.001 ppm; the method states that the LOD should be determined by each laboratory using the method.
Accuracy/Precision	Percent recoveries and coefficients of variance (CVs) indicate acceptable accuracy/precision for residues of tribenuron methyl in corn grain, olive, and soybean seed at the LOQ, 5x LOQ, 10x LOQ, and 50x LOQ. The overall recovery range was 75-101% with an average recovery of 85% (CV = 9%). See Table C.1.1 above.
Reliability of the Method [ILV]	An independent laboratory method validation [ILV], was conducted to verify the reliability of DuPont Method 13412 for the determination of tribenuron methyl residues in olives and soybean seed. The values obtained are indicative that the LC/MS/MS method is reliable. See Section C.3.
Linearity	The method/detector response was linear (coefficient of determination, r^2= 0.9991) within the range of 0.2-10.0 ng/mL.
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 petitioner did not state whether the submitted method is intended for enforcement purposes.

C.3. Independent Laboratory Validation

An independent laboratory validation (ILV) study was conducted for DuPont Method 13412 using olive and soybean seed samples. The ILV was conducted at Inveresk (Tranent, Scotland). Samples of homogenized untreated olives, from crop field trials, and untreated soybean seeds, purchased commercially, were fortified with tribenuron methyl at ~0.01 ppm (LOQ) and ~0.05 ppm. Fortified and unfortified (control) samples were analyzed using DuPont LC/MS/MS

Method 13412 as described in Table B.1.1. The ILV laboratory stated that olives and soybean seed were chosen for the study because these matrices are representative of the matrices to be analyzed using DuPont Method 13412.

The first trial of the ILV analysis failed for olive (actual data were not presented) and the method was modified slightly (to change the centrifuging procedures). The second trial of the ILV also failed for olives. Following a minor modification to the cleanup step, the third trial was successful for olives.

The modifications in the method made for olives were incorporated into the method before the first trial for soybean seeds. The first attempt for soybean seeds was not successful (59.1-77.0% recovery). The method was again modified by keeping the extracts under constant refrigeration during analysis but the second trial for soybeans failed due to poor recovery (actual data were not presented). Additional modifications were made to the SPE cleanup step and the third trial was successful for soybean seeds.

Recoveries of tribenuron methyl from the ILV study are reported in Table C.3.1. Residues of tribenuron methyl were less than the LOQ (<0.01 ppm) in two unfortified samples each of olive and soybean seed.

The laboratory reported that a set of 12 samples could be prepared for LC/MS/MS analysis by a single person within an 8-hour day. The LC/MS/MS analysis could be run unattended overnight. Data processing was done the following day. Overall, complete analysis of a single set of samples takes <24 hours.

The ILV report noted that several minor modifications were made to extraction/cleanup procedures to improve the recovery of tribenuron methyl. A log of communication between the ILV laboratory and the sponsor representative was included. The ILV laboratory did not identify any critical steps or recommend any modifications to the method.

TABLE C.3.1. Recovery Results Obtained by an Independent Laboratory Validation of the Enforcement Method for the Determination of Tribenuron Methyl in Olive and Soybean Seed.

Matrix	Spiking Level (ppm)	Recoveries Obtained (%)	Mean Recovery ± SD (CV) (%)
Olive	0.01	72.1, 72.9, 74.8, 75.8, 79.6	73.0 ± 3.0 [4.1]
	0.05	69.1, 70.3, 71.3, 71.8, 72.4	
Soybean Seed	0.01	79.8, 83.3, 84.4, 87.4, 91.8	89.4 ± 10.0 [11.2]
	0.05	80.1, 86.6, 92.8, 94.2, 114	

D. CONCLUSION

Adequate concurrent method recovery and method validation data have been submitted for DuPont LC/MS/MS Method 13412 for the determination of residues of tribenuron methyl in oily crop matrices. Based on the method validation and concurrent method validation, the LC/MS/MS method is adequate for data collection. No radiovalidation data have been submitted for the method; however, RAB2 has determined that radiovalidation data are not required.

The petitioner did not state whether the LC/MS method is to be used for enforcement purposes. Adequate independent laboratory validation data have been submitted for this method, using olive and soybean seed. If the petitioner wishes DuPont Method 13412 to be used for enforcement, the final version of the method must be submitted; the version should include the modifications made by the ILV laboratory in the course of validation.

E. REFERENCES

DP Barcode: D304059
Subject: Tribenuron methyl. Residue Chemistry Considerations.
From: R. Griffin
To: K. Rothwell and J. Tompkins
Dated: 6/24/04
MRIDs: None

F. DOCUMENT TRACKING

RDI: Name1 (Date); Name2 (Date); Name3 (Date); etc.
Petition Number: 4F6890
DP Barcode: D314429
PC Code: 128887

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Chemical Name: Tribenuron

PC Code: 128887
HED File Code:
Memo Date:
File ID: 00000000
Accession #: 000-00-0123

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