Primary Evaluator	William T. Drew, Chemist (WTDrew) RAB2/HED (7509C)	Date: 8/12/2005
Approved by	Richard A. Loranger, Branch Senior Scientist RAB2/HED (7509C) R. Loranger	Date: 12/28/2005

This DER was originally prepared under contract by Dynamac Corporation (1910 Sedwick Road, Building 100, Suite B; Durham, NC 27713). It has been reviewed by HED and revised to reflect current OPP policies.

STUDY REPORTS

MRID #46346801. Diane E. Reed (2004) *Independent Laboratory Validation for the Determination of TM-444 and TMG in Grapes*. Laboratory Study ID/Pyxant Study #Arvesta-1506. Unpublished study prepared by Arvesta Corporation and Pyxant Labs Incorporated. 53 pages. {OPPTS Residue Chemistry Test Guideline 860.1340}

EXECUTIVE SUMMARY

Arvesta Corporation has proposed an LC/MS/MS method to enforce tolerances for residues of clothianidin and its metabolite TMG in/on grape and potato commodities. The method, entitled *Determination of TM-444 and TMG in Grape and Potato Raw Agricultural and Processed Commodities* (Morse Method #Meth-164), was used for the determination of clothianidin and TMG residues in samples from the crop field trials and processing studies.

The method includes instructions for analysis of clothianidin and TMG in potato and grape raw agricultural commodities (RACs) and processed fractions. The LC/MS/MS method (Morse Method #Meth-164) was adequately validated in conjunction with the field trial analyses. For this method, residues are extracted with ACN/water/guanidine-HCl (20:80:1 vol/vol/wt), filtered, and concentrated. Residues of clothianidin and TMG are then cleaned up separately using ChemElut™ liquid/liquid extraction (LLE) or ENVI-Carb™ solid phase extraction (SPE) columns, respectively. Residues are concentrated, reconstituted in 1% acetic acid, and analyzed by LC/MS/MS. The validated limit of quantitation (LOQ) for each analyte is 0.020 ppm in all grape and potato matrices, except for raisins and potato chips (in these the LOQ is 0.040 ppm). The limit of detection (LOD) for each analyte is 0.007 ppm in all grape and potato matrices, except for raisins and potato chips (with LODs of 0.013 ppm).

Method validation data for the LC/MS/MS method demonstrated adequate method recoveries of clothianidin and TMG from all grape and potato commodities. Clothianidin recoveries averaged 75 to 92%, with low standard deviations (± 4 to 14%), while TMG recoveries averaged 77 to 102%, also with low standard deviations (± 5 to 11%). The fortification levels and samples used in method validation are adequate to bracket expected residue levels.

A successful independent laboratory validation (ILV) trial was conducted using the above method with samples of grapes fortified at 0.020 and 0.040 ppm. Although the LC/MS/MS method was not validated at the proposed tolerance level for grapes (0.50 ppm), as recommended by Agency guidelines, the available ILV trial will be considered adequate since the ILV data together with the method validation data from the developing laboratory indicate that recoveries of both clothianidin and TMG are likely to be acceptable at the proposed 0.50 ppm tolerance.

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 US EPA Residue Chemistry Summary Document (DP Barcodes D309473 and D309474).

COMPLIANCE

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Clothianidin (also known by its development code numbers, TM-444, TI-435, or V-10066) is a systemic insecticide, belonging to the chloronicotinyl (and nitroguanidine) class of chemicals, which enters the transpiration stream through the roots and cotyledons of newly germinating seedlings and protects below- and above-ground plant parts from insect damage. It binds (via ingestion and contact routes) with the nicotinic acetylcholine receptor sites, interfering with transmission of stimuli and eventually inhibiting reproduction of the insect. Clothianidin is a major metabolite of thiamethoxam. It is currently registered (40CFR §180.586) for use on various crops.

Arvesta has submitted a petition (PP#4F6869) requesting the establishment of tolerances for residues of clothianidin in/on grape and potato commodities. The 50% active ingredient (ai) water-dispersible granule (WDG) formulation is proposed for foliar applications to grapes and potatoes (Clutch™ 50WDG, EPA Registration #66330-40). The 16% ai water-soluble granule (WSG) formulation is proposed for soil applications to grapes and potatoes (Belay™ 16WSG, EPA Registration #66330-52).

TABLE A.1 Nomenclature of Test Compound and its Metabolite.

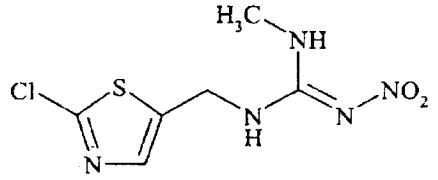
Compound	
Empirical Formula	$C_6H_8ClN_5O_2S$
Common Name	Clothianidin
Company Experimental Names	TM-444, TI-435, V-10066
IUPAC Name	*(E)*-1-(2-Chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine
CAS Name	[*C(E)*]-*N*-[(2-Chloro-5-thiazolyl)methyl]-*N'*-methyl-*N''*-nitroguanidine
CAS Number	210880-92-5 (formerly 205510-53-8)
Chemical Class	Chloronicotinyl
Known Impurities of Concern	None
End-Use Product (EUP)	Clutch™ 50WDG, EPA Registration #66330-40 Belay™ 16WSG, EPA Registration #66330-52

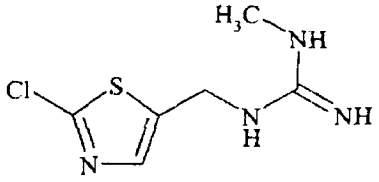
TABLE A.1 Nomenclature of Test Compound and its Metabolite.	
Metabolite	
Common Name	Metabolite TMG
Company Experimental Name	TMG
CAS Name	*[C(E)]*-*N*-[(2-Chloro-5-thiazolyl)methyl]-*N'*-methylguanidine

TABLE A.2 Physicochemical Properties (from MRID #45422301).	
Parameter	**Value**
Molecular Weight	249.7
Melting Point (°C)	176.8
pH at 23°C	6.24 [1% solution/suspension]
Density (g/cm^3) at 20°C	1.61 [PAI], 1.59 [TGAI]
Water Solubility (g/L) at 20°C	0.327
Solvent Solubility (g/L) at 25°C	n-Heptane <0.00104 Xylene 0.0128 1-Octanol 0.938 Dichloromethane 1.32 Ethyl Acetate 2.03 Methanol 6.26 Acetone 15.2
Vapor Pressure (Pa) at 25°C	1.3×10^{-10}
Dissociation Constant (pK_a) at 20°C	11.09
Octanol/Water Partition Coefficient (Log K_{OW}) at 25°C	0.7
UV/Visible Absorption Spectrum, Maximum (nm)	265.5 [acidic, neutral sol'ns]. 246.0 [basic sol'n].

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

An LC/MS/MS method entitled *Determination of TM-444 and TMG in Grape and Potato Raw Agricultural and Processed Commodities* (Morse Method #Meth-164), was used for the determination of residues of clothianidin in the crop field trials and processing studies.

B.1.1. Principle of the Method

The method includes instructions for analysis of potato, potato processed (peel, chips, and granules), grape, and grape processed (juice and raisins) samples. Residues of both clothianidin and TMG are extracted with ACN/water/guanidine-HCl (20:80:1 vol/vol/wt) and filtered through Celite. The filtrate is concentrated and diluted with water. Separate aliquots are then taken for further cleanup and subsequent determination of clothianidin and TMG. Residues of clothianidin are cleaned up using a ChemElut™ LLE column eluted with cyclohexane/ethyl acetate (1:1

vol/vol). Residues of TMG are cleaned up using a ENVI-Carb™ SPE cartridge eluted with methanol/water/acetic acid (80:20:1 vol/vol/vol). The purified residues are concentrated and re-dissolved in 1% acetic acid for separate determination by LC/MS/MS. The HPLC system consists of a C_{18} column with a mobile phase gradient of water to methanol, each containing 1% formic acid. The retention times for clothianidin and TMG are approximately 7.3 and 4.5 minutes, respectively. The monitored transitions are m/z 250 to 169 for clothianidin and m/z 205 to 132 for TMG. The validated LOQ for each analyte is 0.020 ppm for all matrices, except for potato chips and raisins (with LOQs of 0.040 ppm). The LODs for each analyte are 0.007 ppm for all matrices, except for potato chips and raisins (with LODs of 0.013 ppm).

TABLE B.1.1 Summary of Parameters for the Analytical Method Used for the Quantitation of Clothianidin Residues in Grape and Potato Matrices.

Method ID	Morse Method #Meth-164
Analytes	Clothianidin and its metabolite, TMG.
Extraction Solvent	ACN/water/guanidine-HCl (20:80:1 vol/vol/wt)
Cleanup Strategies	ChemElut™ LLE column eluted with cyclohexane/ethyl acetate (1:1 vol/vol) for clothianidin. ENVI-Carb™ SPE cartridge eluted with methanol/water/acetic acid (80:20:1 vol/vol/vol) for TMG.
Instrument/Detector	HPLC C_{18} column (15cm x 2.0mm, 0.45-µm filter thickness), with tandem mass spectrometry (MS/MS) detection. The ions monitored for quantitation and confirmation are m/z 250 to 169 for clothianidin and m/z 205 to 132 for TMG.
Standardization Method	External standard calibration.
Stability of Standard Solutions	Standard solutions are to be stored frozen (at less than -10°C).
Retention Times	Approximately 7.3 and 4.5 minutes for clothianidin and TMG, respectively.

B.1.2. Method Validation

For method validation, a total of 6 samples each of potato tubers, potato peel, potato granules, grape and grape juice were fortified separately with clothianidin and TMG at 0.020 and 0.500 ppm. For raisins and potato chips, control samples were fortified at 0.040 and 1.00 ppm with each analyte. In addition, the method was validated in conjunction with the potato and grape field trials and processing studies. Control samples (3 to 14 samples each) of potato, potato peel, potato chips, potato granules, grape, grape juice, and raisins were fortified with clothianidin and TMG at 0.020 to 10.0 ppm.

B.2. Enforcement Method

The proposed enforcement method for grape and potato commodities is the same as the data-gathering method.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

The sponsor provided method validation and concurrent recovery data from potato, potato processed fractions (peel, chips, and granules), grape, and grape processed fractions (juice and raisins). These data were reviewed in separate data evaluation records (DERs).

In the method validation trials, recoveries from plant commodities (potato, potato peel, potato chips, potato granules, grape, grape juice, and raisins) which had been fortified with clothianidin and TMG at 0.020 to 1.00 ppm were all within the generally recognized acceptable range (70 to 120%) for all matrices (see Table C.1.1), with the exception of one potato granule sample with a recovery of 61% for clothianidin. Clothianidin recoveries averaged 75 to 92%, with low standard deviations (± 4 to 14%, n = 6) and the TMG recoveries averaged 77 to 102%, also with low standard deviations (± 5 to 11%, n = 6). The fortification levels and samples used in method validation are adequate to bracket expected residue levels.

Concurrent method recoveries from plant commodities (potato, potato peel, potato chips, potato granules, grape, grape juice, and raisins) which had been fortified with clothianidin at 0.050 to 10.0 ppm were all within the generally recognized acceptable range (70 to 120%) for all matrices analyzed (see Table C.1.1). The clothianidin recoveries averaged 80 to 88%, with low standard deviations (± 1 to 7%, n = 3 to 14), while the TMG recoveries averaged 82 to 93%, also with low standard deviations (± 2 to 9%, n = 3 to 14).

TABLE C.1.1 Summary of Method Validation and Concurrent Recoveries from Grape and Potato Commodities Using the Data-Gathering Analytical Method.*

Analyte	Crop [Matrix]	Spiking Level (mg/kg)	Sample Size	Recoveries (%)	Mean Recovery ± Std Dev (%)
Method Validation Recoveries					
Clothianidin	Grape [Fruit]	0.020	3	87-90	92 ± 4
		0.500	3	92-97	
	Grape [Juice]	0.020	3	73-81	84 ± 8
		0.500	3	88-93	
	Grape [Raisins]	0.040	3	76-97	82 ± 10
		1.00	3	70-87	
	Potato [Tuber]	0.020	3	83-96	86 ± 8
		0.500	3	79-82	
	Potato [Granules]	0.020	3	61-81	75 ± 7
		0.500	3	76-78	
	Potato [Chips]	0.040	3	70-76	78 ± 7
		1.00	3	81-87	
	Potato [Wet Peel]	0.020	3	85-119	92 ± 14
		0.500	3	82-90	
TMG	Grape [Fruit]	0.020	3	92-106	102 ± 6
		0.500	3	100-106	
	Grape [Juice]	0.020	3	88-98	96 ± 6
		0.500	3	97-104	
	Grape [Raisins]	0.040	3	84-102	90 ± 8
		1.00	3	80-95	
	Potato [Tuber]	0.020	3	87-113	96 ± 11
		0.500	3	83-100	
	Potato [Granules]	0.020	3	72-76	77 ± 5
		0.500	3	79-83	

TABLE C.1.1 Summary of Method Validation and Concurrent Recoveries from Grape and Potato Commodities Using the Data-Gathering Analytical Method.*

Analyte	Crop [Matrix]	Spiking Level (mg/kg)	Sample Size	Recoveries (%)	Mean Recovery ± Std Dev (%)
	Potato [Chips]	0.040	3	90-103	93 ± 6
		1.00	3	86-96	
	Potato [Wet Peel]	0.020	3	87-106	93 ± 10
		0.500	3	82-103	
Concurrent Method Recoveries					
Clothianidin	Grape [Fruit]	0.020	5	81-85	86 ± 4
		0.100	3	78-88	
		0.200	2	84-87	
		0.500	2	92-93	
		1.00	2	86-90	
	Grape [Juice]	0.020	1	84	84 ± 0.6
		0.500	1	85	
		1.00	1	84	
	Grape [Raisins]	0.040	1	86	80 ± 5
		0.500	1	78	
		10.0	1	77	
	Potato [Tuber 1]	0.020	2	79-93	88 ± 7
		0.05	1	86	
		0.500	1	92	
	Potato [Tuber 2]	0.020	8	73-91	84 ± 5
		0.050	8	79-95	
		0.100	4	81-92	
		0.500	1	82	
TMG	Grape [Fruit]	0.020	12	84-98	93 ± 6
		1.00	2	100-104	
	Grape [Juice]	0.020	1	91	92 ± 2
		0.500	1	90	
		1.00	1	94	
	Grape [Raisins]	0.040	1	74	82 ± 8
		0.100	1	82	
		10.0	1	89	
	Potato [Tuber 1]	0.020	2	93-94	93 ± 2
		0.050	1	94	
		0.500	1	90	
	Potato [Tuber 2]	0.020	8	73-101	89 ± 9
		0.050	8	82-97	
		0.100	4	82-98	
		0.500	1	105	

* Method validation & concurrent method recovery data for grape & potato were submitted with the field trial &

processing studies, MRIDs #46346802 (Grape), 46357301 (Potato Tuber 1), & 46357302 (Potato Tuber 2).

TABLE C.1.2 Characteristics of the Data-Gathering Analytical Method Used for the Quantitation of Clothianidin Residues in/on Grape and Potato Commodities.	
Analytes	Clothianidin and TMG.
Equipment ID	Perkin-Elmer SCIEX API 3000 HPLC/MS/MS, Phenomenex Aqua C_{18} column.
Limit of Quantitation (LOQ)	0.020 ppm for each analyte in all grape and potato matrices except raisins and potato chips, which have an LOQ of 0.040 ppm.
Limit of Detection (LOD)	0.004 ppm for each analyte in all grape and potato matrices except raisins and potato chips, which have an LOD of 0.013 ppm.
Accuracy/Precision	Average method recoveries for grapes were 72 to 74% for clothianidin with standard deviations of <10% and 80 to 87% for TMG with standard deviations of <10%.
Reliability of the Method/ILV	An ILV of the proposed enforcement method was conducted to verify reliability of the method for determination of residues of clothianidin in/on plant commodities. The values obtained indicate that the method is reliable (see Section C.3, below).
Linearity	Example standard curves for clothianidin at concentrations of 0.003 to 0.06 µg/mL had correlation coefficients of >0.999. TMG at concentrations of 0.0003 to 0.006 µg/mL had correlation coefficients of >0.997.
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 proposed enforcement method for grape and potato commodities is the same as the data-gathering method.

C.3. Independent Laboratory Validation

An ILV of the proposed enforcement method was conducted by Pyxant Labs Incorporated (Colorado Springs, CO) using grape samples. Frozen, homogenized control samples of grapes (obtained by Pyxant Labs from Morse Laboratories) were each fortified with clothianidin and TMG at 0.020 ppm (the LOQ) and 0.040 ppm. The reviewers noted that the proposed tolerance level for grapes is 0.50 ppm. For each matrix, duplicate control samples were analyzed along with five fortified samples at each fortification level using the proposed enforcement method as described in Table B.1.1 (Morse Method #Meth-164). Grape samples were chosen for the ILV study as representative commodities for validation of this method.

Recoveries of clothianidin and TMG from grape samples were adequate with low variability (see Table C.3.1). Average recoveries were 72 to 74% for clothianidin and 80 to 87% for TMG, with low standard deviations (± 2 to 3%). The method was successfully validated in the first trial. The laboratory reported that a set of 12 samples required 8 hours for extraction, instrumental analysis required 8 hours, and data processing required an additional 2 hours. No critical steps were identified by the ILV laboratory.

Communications between the ILV laboratory and the study sponsor were provided in Appendix 3, and concerned selection of the appropriate matrix and fortification levels, and a request for reference standards. No other communication occurred during the testing.

TABLE C.3.1. Recovery Results Obtained from ILV of the LC/MS/MS Method (Morse Method #Meth-164) for Determination of Clothianidin and TMG Residues in Grapes.

Matrix	Spiking Level (ppm)	Number of Samples	Clothianidin		TMG	
			Recoveries (%)	Mean Recovery ± Std Dev (%)	Recoveries (%)	Mean Recovery ± Std Dev (%)
Grapes	0.020	5	71-76	72 ± 2	82-89	87 ± 3
	0.040	5	71-76	74 ± 2	77-83	80 ± 3

D. CONCLUSION

Although the LC/MS/MS method was not validated at the proposed tolerance level for grapes (0.50 ppm), as recommended by Agency guidelines, the available ILV trial will be considered adequate as the ILV data together with the method validation data (from the developing laboratory) indicate that recoveries of both clothianidin and TMG are likely to be acceptable at the proposed 0.50 ppm tolerance.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: W. T. Drew (8/12/2005), R. A. Loranger (12/28/2005)
Petition Number: 4F6869
DP Barcode: D309473 and D309474
PC Code: 044309