US EPA ARCHIVE DOCUMENT

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Processed Food and Feed - Grape (Juice and Raisins)

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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 REPORT

MRID #46346802. Sandra J. Carringer (2003) *Magnitude of the Residue of TM-444 and its Metabolite in Grape Raw Agricultural and Processed Commodities*. Study #TCI-03-076. Unpublished study prepared by Arvesta Corporation, Morse Laboratories Incorporated, and The National Food Laboratory Incorporated. 393 pages. {OPPTS Residue Chemistry Test Guideline 860.1520}

EXECUTIVE SUMMARY

In a field trial conducted during 2003 in California, clothianidin was applied to grapes in two side-by-side tests using either a water-dispersible granule (WDG) formulation or a water-soluble granule (WSG) formulation. In one test, clothianidin (Clutch™ 50WDG) was applied as two foliar broadcast applications during fruit development at the rate of 0.486 and 0.506 pounds of active ingredient per acre (lb ai/A) per application, with a re-treatment interval (RTI) of 14 days, for a total application rate of 0.992 lb ai/A (essentially 5X the proposed maximum seasonal use rate of 0.2 lb ai/A). In the other test, clothianidin (Belay™ 16WSG) was applied during fruit development as a single drip-irrigation application (to soil) at the rate of 0.992 lb ai/A (5X). Grapes were harvested at commercial maturity, 30 days after treatment (DAT) at the drip-irrigation trials, or immediately following the second application (0 DAT) at the foliar broadcast trials. A single bulk sample of grapes was collected for processing from each test, and duplicate treated samples for each matrix were collected either prior to (whole grapes) or after (juice and raisins) processing. Prior to analysis, whole fruit were stored frozen for roughly 2 months, a duration supported by the available storage stability data, while juice and raisin samples were analyzed within 1 month.

The LC/MS/MS method (Morse Method #Meth-164) used to determine residues of clothianidin and its metabolite, TMG, in grape matrices was adequately validated in conjunction with the sample analyses. For each matrix, 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 0.040 ppm for raisins and 0.020 ppm for whole fruit and juice, and the limit of detection (LOD) is 0.013 ppm for raisins and 0.007 ppm for whole fruit and juice.

Residues of both clothianidin and TMG were each less than the LOQ in grapes, juice, and raisins resulting from the drip-irrigation application at 5X; therefore, processing factors could not be calculated for these samples. However, residues were detectable in samples from the foliar broadcast applications. For the 5X foliar broadcast applications, average residues of

clothianidin were 0.621, 0.707, and 1.02 ppm in samples of whole fruit, juice, and raisins, respectively, and average residues of TMG were less than 0.004, less than or equal to 0.004, and 0.359 ppm. Residues of clothianidin concentrated by 1.1X in juice and 1.6X in raisin, and residues of TMG concentrated by 1X in juice and roughly 90X in raisins. Based on the average combined residues (expressed in parent equivalents), concentration factors for the combined residues would be 1.1X in juice and 2.3X in raisins.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

Under the conditions and parameters used in the study, the grape processing 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% 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.

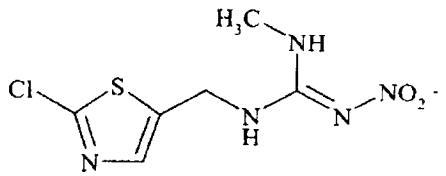
Compound	
Empirical Formula	$C_6H_8ClN_5O_2S$
Common Name	Clothianidin
Company Experimental Names	TM-444, TI-435, V-10066

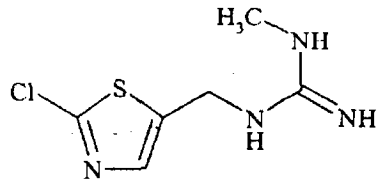
TABLE A.1 Nomenclature of Test Compound and its Metabolite.	
IUPAC Name	*(E)*-1-(2-Chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine
CAS Name	[*C(E)*]-*N*-[(2-Chloro-5-thiazolyl)methyl]-*N'*-methyl-*N''*-nitroguanidine
CAS Number	210880-92-5 (formerly 205510-53-8)
Chemical Class	Chloronicotinyl
Known Impurities of Concern	None
End-Use Product (EUP)	Clutch™ 50WDG, EPA Registration #66330-40 Belay™ 16WSG, EPA Registration #66330-52
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. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

TABLE B.1 Study Use Pattern on Grape.

Location (City, State/Year)	Application						
	EUP	Method[1]; Timing[2]	Volume (GPA)[3]	Single Rate (lb ai/A)[4]	Number	RTI (Days)	Total Rate (lb ai/A)[4]
Delano, CA/2003	Clutch™ 50WDG	Foliar broadcast; fruit development.	115-163	0.486-0.506	2	14	0.992
	Belay™ 16WSG	Drip-irrigation; fruit development.	NA	0.992	1	NA[5]	0.992

1. All applications were made using ground equipment; no tank mix adjuvants were used.
2. All applications were made during fruit development.
3. GPA = Gallons Per Acre.
4. The target single rate was 0.992 lb ai/A for the drip-irrigation treatment, and 0.496 lb ai/A for the foliar broadcast treatment. The total target rate was 0.992 lb ai/A for all treatments.
5. NA = Not Applicable.

B.2. Processing Procedures

After collection of a single bulk sample of grapes (at least 92 kg) from each of the control and two treatment plots, the sample were shipped directly to The National Food Laboratory in Dublin, California, and placed in cool storage (35 to 40°F) prior to processing. A single control and duplicate treated samples of grapes (fruit), the raw agricultural commodity (RAC) were collected and the remaining bulk grape samples were processed into raisins and canned juice (duplicate samples from each treatment) using simulated commercial procedures. All samples were then placed in frozen storage (-10 to 32°F) within 2 hours of collection. The processed samples were stored frozen for 0 to 6 days and the RAC samples were stored for 38 to 40 days. All samples were then shipped frozen to the analytical laboratory, Morse Laboratories in Sacramento, California, and placed in frozen storage (-20 ± 5°C). The RAC and raisin samples were homogenized prior to analysis.

B.3. Analytical Methodology

Grape fruit, juice, and raisin samples were analyzed for residues of clothianidin and its metabolite, TMG, using 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). A detailed description of Method #Meth-164 is presented in the data evaluation record (DER) for MRID #46346801, in conjunction with an independent laboratory validation (ILV) of that method.

Prior to extraction, raisin samples are hydrated with water for one hour. Residues of both clothianidin and TMG are then 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 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 an 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 values for clothianidin residues are 0.040 ppm for raisins and 0.020 ppm for whole fruit and juice. The LOD is 0.013 ppm for raisins and 0.007 ppm for whole fruit and juice.

Prior to analysis of field trial samples, the method was validated using triplicate control samples of whole fruit and juice fortified with clothianidin at 0.020 and 0.500 ppm and raisins fortified with clothianidin at 0.040 and 1.00 ppm. The method was also validated concurrently with the field trial samples using control samples of grapes (whole fruit) and juice fortified with clothianidin at 0.020 to 1.00 ppm and raisins fortified with clothianidin at 0.040 to 10.0 ppm.

C. RESULTS AND DISCUSSION

The LC/MS/MS method (Morse Method #Meth-164) used to determine residues of clothianidin and TMG in grapes (whole fruit), raisins, and juice is adequate for data collection. Average concurrent method recoveries for clothianidin were 80 to 86% from all grape matrices with standard deviations of ± 1 to 5% (see Table C.1). Average recoveries for metabolite TMG were 82 to 93% from all grape matrices with standard deviations of ± 2 to 8%. Apparent residues of each analyte were less than the LOD in all control samples. The validated LOQ for each analyte is 0.040 ppm for raisins and 0.020 ppm for whole fruit and juice; the LOD for each analyte is 0.013 ppm for raisins and 0.007 ppm for whole fruit and juice.

TABLE C.1 Summary of Method Validation and Concurrent Recovery Results for the LC/MS/MS Method from Grape Matrices.

Analyte	Crop [Matrix]	Spiking Level (mg/kg)	Sample Size	Clothianidin	
				Recoveries (%)	Mean Recovery ± Std Dev (%)
Method Validation Recoveries					
Clothianidin	Grape [Fruit] (RAC)	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	
	Raisins	0.040	3	76-97	82 ± 10
		1.00	3	70-87	
TMG	Grape [Fruit] (RAC)	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	
	Raisins	0.040	3	84-102	90 ± 8
		1.00	3	80-95	

TABLE C.1 Summary of Method Validation and Concurrent Recovery Results for the LC/MS/MS Method from Grape Matrices.

Analyte	Crop [Matrix]	Spiking Level (mg/kg)	Sample Size	Clothianidin	
				Recoveries (%)	Mean Recovery ± Std Dev (%)
Concurrent Method Recoveries					
Clothianidin	Grape [Fruit] (RAC)	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 ± 1
		0.500	1	85	
		1.00	1	84	
	Raisins	0.040	1	86	80 ± 5
		0.500	1	78	
		10.0	1	77	
TMG	Grape [Fruit] (RAC)	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	
	Raisins	0.040	1	74	82 ± 8
		0.100	1	82	
		10.0	1	89	

Samples of whole grapes were stored frozen from collection to analysis for up to approximately 2 months (see Table C.2), a duration supported by the available storage stability data for clothianidin and TMG on grapes, as presented in the DER for MRID #46346802. As samples of grape juice and raisins were analyzed within 1 month of sampling, storage stability data are not required for these matrices.

In a grape field trial conducted during 2003 in California, the Clutch™ 50WDG and Belay™ 16WSG formulations of clothianidin were compared via side-by-side treatments at 5X the proposed maximum seasonal application rate. In one test, clothianidin (Clutch™ 50WDG) was applied as two foliar broadcast applications during fruit development at 0.486 and 0.506 lb ai/A per application, with an RTI of 14 days, for a total rate of 0.992 lb ai/A (5X). In the second test, clothianidin (Belay™ 16WSG) was applied during fruit development as a single drip-irrigation application at 0.992 lb ai/A (5X). Grapes were harvested at commercial maturity, immediately following the second foliar application (0 DAT) or 30 DAT for the drip-irrigation application. A single bulk sample of grapes was collected for processing from each test, and duplicate treated samples for each matrix were collected either prior to (whole grapes) or after (juice and raisins) processing.

For the foliar applications of the Clutch™ 50WDG formulation at 5X, average residues of clothianidin were 0.621, 0.707, and 1.02 ppm in whole fruit, juice, and raisins, respectively

(see Table C.3), and average residues of TMG were less than 0.004, less than or equal to 0.004, and 0.359 ppm in the same matrices. For the Belay™ 16WSG treated samples, average clothianidin residues were 0.007, 0.004, and 0.010 ppm in/on whole fruit, juice, and raisins, respectively, and TMG residues were less than 0.004, less than 0.004, and 0.010 ppm.

As residues were less than the LOQ in commodities from the Belay™ 16WSG treatment, processing factors were only calculated for the samples from the foliar applications with Clutch™ 50WDG. Residues of clothianidin concentrated by 1.1X in juice and 1.6X in raisin, and residues of TMG concentrated by 1X in juice and roughly 90X in raisins. Based on the average combined residues of clothianidin and TMG (1.22 conversion factor) in whole fruit (0.626 ppm), juice (0.712 ppm), and raisins (1.458 ppm), concentration factors for the combined residues would be 1.1X in juice and 2.3X in raisins.

TABLE C.2 Summary of Freezer Storage Conditions.

Crop [Matrix]	Storage Temperature (°C)	Actual Storage Duration (Days)[1]	Limit of Demonstrated Storage Stability (Months)[2]
Grape [Fruit]	-20 ± 5	66-67	5
Grape [Juice]		9-30	
Grape [Raisins]		6-17	

1. Extracts were stored frozen for up to 13 days prior to analysis.
2. Concurrent storage stability data are available indicating that clothianidin and TMG are stable under frozen conditions in whole grapes for up to 5 months (DER for MRID #46346802).

TABLE C.3 Residue Data from Grape Processing Study with Clothianidin.

Processed Commodity	Total Rate (lb ai/A)	PHI (Days)	Residues (ppm)[1]		Processing Factor	
			Clothianidin	TMG	Clothianidin	TMG
Clutch™ 50WDG						
Fruit (RAC)	0.992	0	0.738, 0.504 [0.621]	ND[2], ND [0.004]	NA[3]	NA
Juice			0.712, 0.702 [0.707]	ND, 0.003 [0.004]	1.1	1
Raisins			0.947, *1.02, 1.03*[4] 0.992, *1.05, 1.07* [1.02]	0.352, *0.377, 0.396* 0.338, *0.362, 0.331* [0.359]	1.6	90
Belay™ 16WSG						
Fruit (RAC)	0.992	30	0.008, 0.005 [0.007]	ND, ND	NA	NA
Juice			0.004, 0.003 [0.004]	ND, ND	NC[5]	NC
Raisins			0.006, *0.010, 0.010* 0.006, *0.010, 0.020* [0.010]	0.102[6], *0.007, 0.009* 0.005, *0.006, 0.007* [0.007]	NC	NC

1. The validated LOQ values for clothianidin residues are 0.040 ppm for raisins, and 0.020 ppm for whole fruit and juice. The LOD is 0.013 ppm for raisins, and 0.007 ppm for whole fruit and juice. Average residues are reported in brackets. For calculation of average residues, ½LOD was used for samples reported as ND.
2. ND = Not Detected (less than the LOD).
3. NA = Not Applicable.
4. Residues in *italics* are the replicate analyses of a single sample.
5. NC = Not Calculated.
6. This initial value for TMG in raisins was excluded from the average because the value was not confirmed by repeated analyses.

D. CONCLUSION

The grape processing data are adequate. Residues of clothianidin concentrated by 1.1X in juice and 1.6X in raisin, and residues of TMG concentrated by 1X in juice and roughly 90X in raisins. Concentration factors for the combined residues, expressed in parent equivalents, are 1.1X in juice and 2.3X in raisins.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: W. T. Drew (8/17/2005), R. A. Loranger (12/28/2005)
Petition Number: 4F6869
DP Barcode: D309473 and D309474
PC Code: 044309

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Chemical: **Clothianidin**

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044309
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