OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

(1)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: 06/06/2005

Subject: Amicarbazone (MKH 3586). Petition for the Establishment of Permanent Tolerances for Use on Field Corn and Imported Sugarcane. Summary of Analytical Chemistry and Residue Data. PP#0F6131.

DP Numbers:	D288216, D309766	Decision Numbers:	303867, 303868
PC Code:	114004	MRID Numbers:	45121630-45121633, 45121702-45121711, and 45121713; 46145301-46145310
40 CFR: None		Chemical Class:	Herbicide

From: Manying Xue, Chemist
Registration Action Branch 3
Health Effects Division (7509C)

Through: Leung Cheng, Senior Chemist
Registration Action Branch 3
Health Effects Division (7509C)

Chemistry Science Advisory Council
Health Effects Division (7509C)

To: James Tompkins, PM 25
Herbicide Branch
Registration Division (7505C)

This document was prepared under contract by Dynamac Corporation (1910 Sedwick Rd., Building 100, Suite B; Durham, NC 27713; submitted 1/07/2005). The document has been reviewed by the HED and revised to reflect current OPP policies.

JUN 2 1 2005

Executive Summary

Arvesta Corporation has proposed, in PP#0F6131, the establishment of permanent tolerances for residues of the herbicide amicarbazone [4-amino-4,5-dihydro-N-(1,1-dimethylethyl)-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide] and its metabolites DA amicarbazone [N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide] and iPr-2-OH DA amicarbazone [N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide] in/on the following raw agricultural commodities:

Corn, grain	0.05 ppm
Corn, forage	0.8 ppm
Corn, stover	0.5 ppm
Meat (cattle, sheep, goats, horses, hogs)	0.01 ppm
Meat byproducts (cattle, sheep, goats, horses, hogs)	0.2 ppm
Milk	0.01 ppm
Alfalfa, forage	0.05 ppm
Alfalfa, hay	0.1 ppm
Cotton, undelinted seed	0.04 ppm
Cotton, gin by-product	0.3 ppm
Cottonseed, meal	0.01 ppm
Cottonseed, refined oil	0.01 ppm
Cottonseed, hulls	0.01 ppm
Sugarcane	0.15 ppm
Sugarcane, molasses	0.8 ppm
Soybean, forage	2.5 ppm
Soybean, hay	7.0 ppm
Soybean, seed	0.7 ppm
Soybean, meal	0.25 ppm
Soybean, hulls	0.2 ppm
Soybean, oil	0.01 ppm
Wheat, forage	0.6 ppm
Wheat, hay	1.0 ppm
Wheat, grain	0.09 ppm
Wheat, straw	0.4 ppm
Wheat, bran	0.08 ppm
Wheat, shorts	0.06 ppm
Wheat, flour	0.05 ppm
Wheat, middlings	0.05 ppm
Wheat, germs	0.05 ppm

Amicarbazone (MKH 3586) is a selective herbicide that acts through inhibition of photosynthesis. In the U.S., the petitioner has proposed the use of amicarbazone for preplant, preemergence, or postemergence application to field corn for control of annual broadleaf weeds. The petitioner has also proposed the use of amicarbazone on sugarcane grown in other countries

TABLE C.2.3.1. Summary of Characterization and Identification of Radioactive Residues in Rotational Crop Matrices Planted 47 Days Following Soil Application of Radiolabeled Amicarbazone at 0.42 lb ai/A.

Compound	Kale		Turnip root		Turnip top		Wheat forage		Wheat hay		Wheat straw	
	TRR = 1.59 ppm		TRR = 0.10 ppm		TRR = 7.42 ppm		TRR = 1.38 ppm		TRR = 2.42 ppm		TRR = 4.18 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Amicarbazone	40	0.64	–	–	30	2.2	40	0.55	26	0.64	22	0.94
DA MKH 3586	10	0.16	–	–	14	1.07	18	0.25	–	–	5	0.22
iPr-2-OH DA MKH 3586	16	0.25	–	–	11	0.80	19	0.26	16	0.38	17	0.70
Triazolinone MKH 3586	–	–	–	–	1	0.07	<1	<0.01	–	–	1	0.04
DA OH Glu I MKH 3586	2	0.03	–	–	1	0.05	5	0.07	15	0.36	11	0.47
tBu-OH DA MKH 3586	1	0.02	–	–	–	–	–	–	–	–	–	–
DA OH Glu II MKH 3586	4	0.07	–	–	2	0.14	4	0.05	15	0.36	14	0.59
Triazolinone MKH 3586 conjugate	–	–	–	–	–	–	1	0.02	–	–	–	–
N-Me DA MKH 3586	–	–	–	–	1	0.09	–	–	3	0.07	–	–
Unknowns	9	0.14	–	–	7	0.49	4	0.05	6	0.15	6	0.24
MeOH extract	–	–	100	0.1	–	–	–	–	–	–	–	–
Total identified	74	1.17	0	0	60	4.42	87	1.20	75	1.81	71	2.96
Total characterized	9	0.14	100	0.1	7	0.49	4	0.05	6	0.15	6	0.24
Total extractable	84	1.34	100	0.1	66	4.93	89	1.23	83	2.00	78	3.25
Unextractable (PES) [1]	13	0.20	10	0.01	22	1.64	7	0.09	20	0.49	20	0.85
Accountability [2]	97		110		88		96		103		98	

[1] Residues remaining after exhaustive extractions.
[2] Accountability = (Total extractable + Total unextractable)/(TRR from combustion analysis; see TABLE C.2.1) * 100.

for importation of sugar products into the U.S.; however, detailed information on this use was not available at the time of this review.

This tolerance petition was originally submitted by Bayer Corporation; however, Bayer Corporation has since sold amicarbazone to Arvesta Corporation, who is the petitioner for amicarbazone now.

In conjunction with the subject tolerance petition, Arvesta has submitted an application for Section 3 registration of a 70% dry flowable (DF) formulation (Amicarbazone DF Herbicide; EPA File Symbol No. 66330-UA). In the U.S., the 70% DF formulation is to be applied as either a preplant or preemergence broadcast spray at 0.22-0.45 lb ai/A or as an early postemergence broadcast spray at 0.10-0.15 lb ai/A. Applications are to be made using ground equipment only. Details of the proposed use on sugarcane grown outside the U.S. have not been provided; however, the maximum seasonal use rate is reported to be 1.35 lb ai/A.

Based on the submitted corn metabolism study, the major metabolic pathway in corn involves the deamination of the triazole amino group, to form DA amicarbazone, followed by hydroxylation at the secondary carbon of the isopropyl group, to form iPr-2-OH DA amicarbazone. Additional pathways involve hydroxylation of the isopropyl methyl to form iPr-1-OH DA MKH 3586, followed by glucosidation; hydroxylation of the t-butyl and isopropyl groups to form tBu-iPr-2-diOH DA MKH 3586; glucosidation of DA amicarbazone; and glucosidation of hydroxylated DA amicarbazone. The available data from the livestock metabolism studies indicate that metabolism is similar in ruminants and poultry, and that metabolism of amicarbazone proceeds via loss of the triazole amino group, yielding DA amicarbazone, followed by hydroxylation of the isopropyl methine carbon to form iPr-2-OH DA amicarbazone. Dehydration would then result in iPr-Ene DA MKH 3586, which may undergo epoxidation and hydrolysis to form iPr-1,2-diOH DA MKH 3586. Hydroxylation of DA amicarbazone at an isopropyl methyl carbon would yield iPr-1-OH DA MKH 3586, which may then undergo oxidation to form iPr-Acid DA MKH 3586. Minor pathways involve hydroxylation of the tert-butyl group of amicarbazone or DA amicarbazone to form tBu-OH MKH 3586 or tBu-OH DA MKH 3586, and hydrolytic cleavage of the carboxamide side chain of amicarbazone or DA amicarbazone to form triazolinone MKH 3586 or triazolinone DA MKH 3586. Additional sample storage information is required to support all metabolism studies.

The available analytical methodology (LC/MS/MS) is adequate for data collection, provided additional validation data for cattle liver are submitted. The proposed enforcement methods for corn (Method 108340) and livestock (Method 108111) commodities, and a revised plant method (Method 200258), have been forwarded to ACB for a petition method validation trial. Methods 108340 and 108111 appear to meet guideline requirements as acceptable enforcement methods (ACB/BEAD memo, 5/27/2005, A. Kamel). FDA multiresidue methods are not appropriate for determining amicarbazone residues.

Although additional field trial information and supporting storage stability data are required, an adequate number of corn field trials were conducted in the appropriate regions reflecting the

proposed use patterns for amicarbazone (DF) on field corn. In addition, adequate processing data have been submitted, indicating that tolerances for amicarbazone residues in corn processed commodities are not required. No field trial data have been submitted supporting the proposed tolerance for imported sugarcane products. However, a sugarcane processing study is available indicating that a separate tolerance will be required for sugarcane molasses, but not for refined sugar; storage stability data on cane are required to support the processing study.

An adequate ruminant feeding study has been submitted, and the results of the study indicate that tolerances for residues in livestock commodities are needed. A poultry feeding study has not been submitted; however, a poultry feeding study is required based on data from the poultry metabolism study and the calculated maximum theoretical dietary burden of amicarbazone residues for poultry.

An adequate confined rotational crop study is available, indicating that the metabolism of amicarbazone in rotational crops is similar to the metabolism in the primary corn crop, although the relative levels of the glucose conjugates were higher in rotational wheat commodities than in corn commodities. At plant-back intervals (PBIs) of approximately 1.5, 4 and 12 months, the major ^{14}C-residues identified in kale, turnips and wheat included amicarbazone, DA amicarbazone, and iPr-2-OH DA amicarbazone; two glucosyl conjugates of iPr-2-OH DA amicarbazone were also major residues in wheat hay, grain and straw.

A limited field rotational crop study is also available. Pending submission of supporting storage stability data, the study indicates that extended rotational crop field studies are required on crops routinely rotated with corn, with the exceptions of bulb vegetables and root and tuber vegetables. Data support a 1-month PBI for bulb vegetables and root and tuber vegetables.

Extended field rotational crop trials have been submitted supporting a 1-month PBI for soybeans, a 4-month PBI for alfalfa and wheat, and a 12-month PBI for cotton. For each rotational crop, an adequate number of field trials were conducted in the appropriate regions, reflecting exposure to the maximum seasonal rate of amicarbazone on field corn. The correct commodities were sampled in each test at the appropriate growth stages. Pending submission of supporting storage stability data and additional field trial information, the extended field rotational crop studies are adequate and support tolerances for inadvertent residues in/on alfalfa, cotton, soybean and wheat commodities. In conjunction with the rotational crop field trials, processing studies are available on cotton, soybean, and wheat. These studies indicate that separate tolerances will not be required for processed cotton, soybean or wheat commodities, with the exception of wheat milled byproducts. In addition, residue data on aspirated grain fractions (AGF) derived from soybeans and wheat grown as rotational crops are not required.

There are currently no Codex, Canadian or Mexican MRLs established for amicarbazone.

Regulatory Recommendations and Residue Chemistry Deficiencies

HED has evaluated the residue chemistry database for the new active ingredient amicarbazone. Provided a revised Section B/proposed label, Section F/proposed tolerances, and analytical reference standards are submitted, and ACB/BEAD determine that the proposed enforcement methods are adequate, there are no residue chemistry issues that would preclude granting a conditional registration for this herbicide for the proposed use on field corn in connection with the establishment of tolerances for the combined residues of amicarbazone [4-amino-N-(1,1-dimethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide] and its metabolites DA amicarbazone [N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide] and iPr-2-OH DA amicarbazone [N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide], calculated as parent equivalents, in/on the following raw agricultural commodities:

Corn, field, grain	0.05	ppm
Corn, field, forage	0.80	ppm
Corn, field, stover	1.0	ppm
Cattle, fat	0.01	ppm
Cattle, liver	1.0	ppm
Cattle, meat	0.01	ppm
Cattle, meat byproducts, except liver	0.10	ppm
Goat, fat	0.01	ppm
Goat, liver	1.0	ppm
Goat, meat	0.01	ppm
Goat, meat byproducts, except liver	0.10	ppm
Hog, fat	0.01	ppm
Hog, liver	0.10	ppm
Hog, meat	0.01	ppm
Hog, meat byproducts, except liver	0.01	ppm
Horse, fat	0.01	ppm
Horse, liver	1.0	ppm
Horse, meat	0.01	ppm
Horse, meat byproducts, except liver	0.10	ppm
Milk	0.01	ppm
Sheep, fat	0.01	ppm
Sheep, liver	1.0	ppm
Sheep, meat	0.01	ppm
Sheep, meat byproducts, except liver	0.10	ppm
Poultry, liver	0.01	ppm

and establishment for the indirect or inadvertent residues of amicarbazone and its metabolites DA amicarbazone and iPr-2-OH DA amicarbazone, calculated as amicarbazone, in/on the following raw agricultural commodities when present therein as a result of the application of amicarbazone to field corn:

Alfalfa, forage	0.05	ppm
Alfalfa, hay	0.10	ppm
Cotton, undelinted seed	0.07	ppm
Cotton, gin byproducts	0.30	ppm
Soybean, forage	1.50	ppm

Soybean, hay	5.0	ppm
Soybean, seed	0.80	ppm
Wheat, forage	0.50	ppm
Wheat, hay	1.0	ppm
Wheat, grain	0.10	ppm
Wheat, straw	0.50	ppm
Wheat, grain, milled byproducts	0.15	ppm

The deficiencies noted under 860.1300, 860.1340, 860.1380, 860.1480, 860.1500, and 860.1900 may be addressed as conditions of registration.

860.1200 Directions for Use

- The petitioner should specify an application rate or application rate range for the Fall Application use (described on the label under "Special Applications").
- Based on the available field trial data, the proposed use directions should specify that only one application may be made per season.
- The maximum seasonal rate for early post emergence treatment should be 0.25 lb ai/A.
- Based on the available field trial data, the proposed use directions should specify that early postemergence applications may not be made if preemergence applications have been made in the spring.
- The PBI for cotton should be amended to 12 months.
- Bulb vegetables and root and tuber vegetables may be planted provided a PBI of 1 month is imposed.
- Do not rotate to crops other than field corn and crops with specified PBI.

In addition, to support tolerances on sugarcane products imported into the U.S., the petitioner should submit labels (and translations) bearing use directions for sugarcane from the countries where this use is allowed.

860.1300 Nature of the Residue - Plants

- The dates of initial and final sample extraction and analysis should be submitted before it can be determined whether the storage stability data included in the study are adequate to support the corn metabolism study.

860.1300 Nature of the Residue - Livestock

- The dates of sample extraction and analysis should be provided for all samples from the goat and hen metabolism studies before HED can determine whether storage stability data are required to support the studies.

860.1340 Residue Analytical Methods

- Method 200258 may serve as an alternate plant enforcement method with submission of independent laboratory validation and radio-validation data (ACB/BEAD memo, 5/27/2005, A. Kamel).

860.1380 Storage Stability

- To support the corn field trials, the rotational crop studies on alfalfa, cotton, soybeans and wheat, and the processing studies on corn, sugarcane, cotton, soybean, and wheat, the petitioner should submit data demonstrating the stability of residues of amicarbazone, DA amicarbazone, and iPr-2-OH DA amicarbazone in/on field corn forage, stover, and grain, mustard greens, and turnip roots for up to 27 months of frozen storage. As processed fractions were generally analyzed within one month, storage stability data on processed commodities are not required.

860.1480 Meat, Milk, Poultry, and Eggs

- Method validation data should be submitted for cattle liver reflecting the range of residue levels observed in the cattle feeding study, up to 1.5 ppm for liver.

- Based on data from the poultry metabolism study and the calculated theoretical dietary burden for poultry, HED concludes that a poultry feeding study is required to support the proposed use of amicarbazone on field corn.

860.1500 Crop Field Trials

- Information pertaining to the weather conditions (including temperatures) over the course of the study, with comparison to historical averages (for both temperature and rainfall), should be submitted for the corn field trials; a discussion of any unusual weather conditions should be included. Because the proposed use pattern of amicarbazone on field corn is dependent on soil type, soil pH, and soil organic matter, information pertaining to these characteristics should be submitted for all field trials included in this submission.

- To support the proposed tolerances on sugarcane products imported into the U.S., sugarcane field trial data are required to support the proposed use of amicarbazone on sugarcane in other countries. In lieu of submitting the field trial data sugarcane commodities can be withdrawn from the petition.

860.1650 Submittal of Analytical Reference Standards

- Based on the proposed enforcement methods, the following analytical reference standards should be submitted to the EPA National Pesticide Standards Repository:

- DA amicarbazone
- iPr-2-OH DA amicarbazone
- amicarbazone-d_6
- DA amicarbazone-d_6
- iPr-2-OH DA amicarbazone-d_6

860.1900 Field Accumulation in Rotational Crops

- Pending submissions of the required storage stability data and information pertaining to soil characteristics and weather conditions, the submitted residue data from the field rotational crop studies are adequate to establish rotational crop tolerances.

- Information pertaining to the weather conditions (including temperatures) over the course of the study, with comparison to historical averages (for both temperature and rainfall), should be submitted for the rotational crop studies; a discussion of any unusual weather conditions should be included. Because the proposed use pattern of amicarbazone is dependent on soil type, soil pH, and soil organic matter, information pertaining to these characteristics should be submitted for all field trials included in this submission.

860.1550 Proposed Tolerances

- The proposed tolerance expression should be revised to be expressed in terms of combined residues of amicarbazone and its metabolites DA amicarbazone, and iPr-2-OH DA amicarbazone, calculated as parent equivalents.

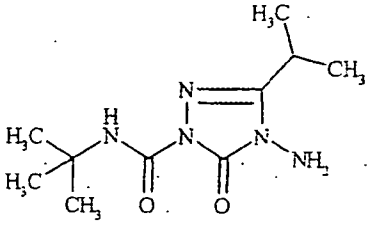
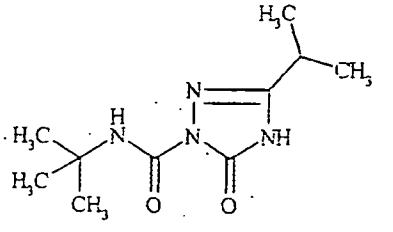
- Because the dry matter content of stover samples from the crop field trials were lower than the level specified in GLN 860.1000, the residue values for these samples are likely to be lower than expected. The proposed tolerance for stover should be increased to 1.0 ppm to account for this variability.

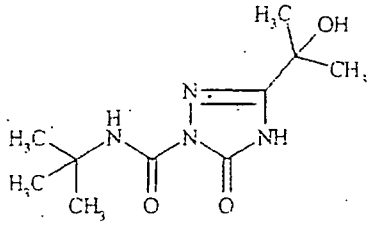
- The petitioner has proposed tolerances for milk and the meat and meat byproducts of cattle, sheep, goats, horses, and hogs. The available cattle feeding study data indicate that tolerances are required for milk and the fat, liver, meat, and meat byproducts (except liver) of cattle, goats, horses, sheep, and hogs. The petitioner should propose tolerances for the liver of cattle, goats, horses, sheep, and hogs, so that the proposed meat byproducts tolerances may then be reduced [the proposed tolerances for the meat byproducts of cattle, goats, horses, sheep and hogs should be reclassified as "meat byproducts, except liver"]. In addition, tolerances for the fat of cattle, goats, horses, sheep, and hogs should be proposed.

- The proposed tolerances should be revised to reflect recommended tolerance levels and the correct commodity definitions as specified in Table 7.

Background

The subject petition, PP#0F6131, represents the first food/feed use of amicarbazone proposed in the U.S. The chemical structure and nomenclature of amicarbazone and its metabolites proposed for regulation and the physicochemical properties of the technical grade of amicarbazone are presented in the tables below. A table of the names and chemical structures of amicarbazone and metabolites identified in the plant, livestock, and rotational crop studies associated with this petition is attached to this review as Appendix 1.

Amicarbazone Nomenclature.	
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-*tert*-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS registry number	129909-90-6
End-use formulation (EP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)
DA amicarbazone (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide

Amicarbazone Nomenclature.	
iPr-2-OH DA amicarbazone (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide

Physicochemical Properties of Technical Grade Amicarbazone.		
Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichlromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-6} Pa @ 25°C 1.30×10^{-6} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient. Log(K_{ow})	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003

860.1200 Directions for Use

Table 1. Summary of End-Use Amicarbazone Products.						
Trade Name	Reg. No.	ai (% of formulation)	Formulation Type	Target Crops in U.S.	Target Pests	Label Version
Amicarbazone DF Herbicide	66330-UA	70%	DF	Field corn and corn grown for silage	Annual broadleaf and grass weeds	Draft; not dated

Use directions are from a specimen label for the 70% dry flowable (DF) formulation (EPA File Symbol 66330-UA). The 70% DF formulation is proposed for use on field corn or corn grown

for silage; use on popcorn, sweet corn, high oil corn hybrids, or corn grown for seed is prohibited. Amicarbazone is to be used on medium- and fine-textured soils only; use on coarse-textured soils (sand, loamy sand, and sandy loam) is prohibited, and use on soils with a pH >7.4 is not recommended. Applications are to be made using ground equipment in a minimum of 10 gal/A spray; applications using aerial equipment or through irrigation systems are prohibited. The formulation is to be mixed with water or a sprayable fluid fertilizer, or impregnated or coated on dry bulk fertilizers.

Table 2. Summary of Directions for Use of Amicarbazone.[1]

Applic. Timing; Type; Equip.	Applic. Rate (lb ai/A)	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ai/A)	PHI (days)	Use Directions and Limitations
Field Corn					
Early preplant (≥30 days prior to planting); Broadcast; Ground	0.448	NS	0.448	N/A	Use limited to Eastern Corn Belt: MN, MO, AR, LA, and IA east of US Hwy 71, and points east.
Preplant, surface or soil incorporated (0-29 days prior to planting) OR **Preemergence** (planting to emergence); Broadcast; Ground	0.219-0.328 (soil OM <2%) 0.219-0.448 (soil OM >2%)		0.328 (soil OM <2%) 0.448 (soil OM >2%)		
Early preplant, surface or soil incorporated (≥30 days prior to planting); Broadcast; Ground	0.219-0.448	NS	0.448	N/A	Use limited to Western Corn Belt: ND, SD, NE, KS, OK, TX, and LA west of US Hwy 71, and points west. Application is to be made prior to a planned sequential soil applied or postemergence application, or in tank mixture with full rates of a broad spectrum herbicide.
Preplant, surface or soil incorporated (0-29 days prior to planting) OR **Preemergence** (planting to emergence); Broadcast; Ground	0.219		0.448		
Early Postemergence (emergence up to 10-leaf collar stage, V10); Broadcast or directed; Ground	0.098-0.153	NS	0.448	N/A	Application is to be made as a tank mix with recommended herbicides. Use of an adjuvant is recommended only when required for the tank mix herbicide; use of COC, MSO, or any adjuvant containing vegetable or petroleum oils is prohibited.

Table 2. Summary of Directions for Use of Amicarbazone.[1]

Applic. Timing; Type; Equip	Applic. Rate (lb ai/A)	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ai/A)	PHI (days)	Use Directions and Limitations
Field Corn					
Fall application, after October 15; Broadcast into crop stubble; Ground	NS	NS	0.448	N/A	Use limited to IA, MN, ND, SD, WI, north of Route 20 in NE, north of Route 136 in IL, and north of Interstate 70 in OH. Application is to be made in the fall prior to spring planting of corn. May be followed by a spring application; total rate not to exceed 0.448 lb ai/A.
Fall/winter application, from November to 30 days prior to spring planting; Broadcast into crop stubble; Ground	0.219-0.448	NS	0.448	N/A	Use limited to KS, OK, TX for winter weed control. Application is to be made in the fall prior to spring planting of corn. May be followed by a spring application; total rate not to exceed 0.448 lb ai/A.
Preplant/Preemergence Burndown; Broadcast; Ground	0.219-0.448	NS	0.448	N/A	To be used if weeds are present at time of treatment. Application is to be made as a tank mix with COC or MSO.

[1] NS = Not specified. OM = Organic matter. COC = Crop oil concentrate. MSO = Methylated seed oil.

The 70% DF formulation may be applied alone or tank mixed with additional registered herbicides in either conventional, conservation, or no-tillage crop management systems; tank mixing with metribuzin (Sencor®) is prohibited. The following tank mix partners are recommended: 2,4-D products; acetochlor (Harness® and TopNotch®); acetochlor + atrazine (Fultime® and Harness Xtra®); alachlor (Lasso®); alachlor + atrazine (Lariat®); atrazine products; atrazine + 2,4-D (Shotgun®); atrazine + dicamba (Marksman®); atrazine + dimethenamid (Guardsman® and Leadoff®); atrazine + S-metolachlor (Bicep II Magnum®); dicamba (Banvel® and Clarity®); dimethenamid (Frontier®); glyphosate products; paraquat dichloride (Gramoxone Extra®); pendimethalin (Prowl®); and S-metolachlor (Dual II Magnum®). Nicosulfuron (Accent®) is recommended for tank mixing for postemergence use only, and bentazon + atrazine (Laddok S-12®), flufenacet + metribuzin (Axiom®), flufenacet + metribuzin + atrazine (Axiom AT®), flumetsulam (Python®), and flumetsulam + clopyralid (Hornet®) are recommended for tank mixing for preplant or preemergence use only. All of the recommended herbicides have established tolerances for corn.

The general use directions specify a restricted entry interval (REI) of 12 hours. The following PBIs are proposed: 1 month for soybeans; 4 months for alfalfa, cotton, and wheat; corn may be replanted immediately following treatment with the 70% DF formulation. No other rotational crop restrictions are specified.

If a fall application is to be followed by a spring application, the maximum total application rate is 0.448 lb ai/A.

Conclusions: The proposed use directions on field corn are adequate for an assessment of whether the submitted residue data reflect the maximum residues likely to occur in field corn, provided that the proposed label is amended as specified below:

- The petitioner should specify an application rate or application rate range for the Fall Application use (described on the label under "Special Applications").
- Based on the available field trial data, the proposed use directions should specify that only one application may be made per season.
- The maximum seasonal rate for early post emergence treatment should be 0.25 lb ai/A.
- Based on the available field trial data, the proposed use directions should specify that early postemergence applications may not be made if preemergence applications have been made in the spring.
- Based on the available rotational crop data for cotton, the PBI for cotton should be amended to 12 months.
- Based on the available rotational crop data for turnip, bulb vegetables and root and tuber vegetables may be planted provided a PBI of 1 month is imposed.
- Do not rotate to crops other than field corn and crops with specified PBI.

As Arvesta is supporting the use of amicarbazone on sugarcane grown in other countries and this use could result in tolerances for residues in/on sugarcane products imported into the U.S., labels having this use should be submitted (along with translations) for review along with the requested field trial data.

860.1300 Nature of the Residue - Plants

45121633.der

Arvesta has submitted a study investigating the metabolism of [triazolinone-3-^{14}C]amicarbazone (208,500 dpm/µg) in corn. The radiolabeled test substance was applied to the soil at a rate equivalent of 0.72 lb ai/A (1.6x the proposed maximum seasonal rate) four hours after planting corn seed. The plants were grown in containers in a greenhouse.

The total radioactive residues were 0.988, 2.369, and 0.054 ppm in corn forage, fodder, and grain, respectively, harvested 104-126 days following a single preemergence application of [^{14}C]amicarbazone at 0.72 lb ai/A. The majority of the TRR (~82-92% TRR) was extracted from corn matrices using ACN/water/acetic acid or ACN/water/acetic acid and methanol. Additional residues were released by Soxhlet extraction with ACN/water/acetic acid (3-7% TRR), and hydrolysis with 2 N HCl at reflux (5% TRR; forage) or with 1 N or 2 N KOH at reflux (3-8% TRR; fodder and grain). Total extractable residues were 91-100% TRR.

Residues were characterized/identified primarily by HPLC analysis with confirmatory analysis by TLC and LC/MS/MS. Adequate storage stability data were submitted demonstrating frozen storage stability of the metabolite profiles in corn forage, fodder, and grain for ~8, 7, and 11 months, respectively; however, the dates of sample extraction and analysis were not included in the submission.

Total identified residues were 74-79% TRR in corn commodities. Parent, amicarbazone, was the major residue identified in forage (45%TRR) and fodder (20% TRR); amicarbazone was identified in smaller amounts in grain (13% TRR). The major residue identified in grain was iPr-2-OH DA MKH 3586 (38% TRR); this compound was also found in forage (15% TRR) and fodder (16% TRR). Other identified residues were DA MKH 3586 at 14% TRR in grain, 6% TRR in forage and 10% TRR in fodder; 4-*N*-glu-DA MKH 3586 at 4% in grain, 5% TRR in forage and 7% TRR in fodder; tBu-iPr-2-diOH DA MKH 3586 at 4% TRR in forage only; and iPr-2-O-glu-DA MKH 3586 at 4% TRR in forage, 5% TRR in fodder, and 7% TRR in grain. Four additional glucose/glucoside conjugates were identified in small amounts (≤5% TRR) in fodder.

Conclusions: Provided the storage intervals for the metabolism samples before extraction and analysis are supported by the storage stability data, HED concludes that the nature of the residue in plants is adequately understood for the purposes of the subject petition, in which use is only being proposed on field corn and sugarcane. Based on the results of the study, the petitioner proposed that the major metabolic pathway in corn involves the deamination of the triazole amino group followed by hydroxylation at the secondary carbon of the isopropyl group to form iPr-2-OH DA MKH 3586. Additional pathways involve hydroxylation of the isopropyl methyl to form iPr-1-OH DA MKH 3586, followed by glucosidation; hydroxylation of the t-butyl and isopropyl groups to form tBu-iPr-2-diOH DA MKH 3586. In addition, DA MKH 3586 formed an *N*-glucoside; and glucosidation of hydroxylated DA MKH 3586 formed several minor *O*-glucosides.

HED has determined that the residues of concern in field corn commodities are amicarbazone and its metabolites DA amicarbazone and iPr-2-OH DA amicarbazone for tolerance setting and risk assessment purposes. The corn metabolism study will also cover similar uses on sugarcane, given the physiological similarities between corn and sugarcane. However, additional plant metabolism studies would be required to support use on other crops.

860.1300 Nature of the Residue - Livestock

45121630.der (Goat; also includes review of MRID 45121632)
45121631.der (Hen)

Goat

Arvesta has submitted a study investigating the metabolism of [triazolinone-3-^{14}C]amicarbazone (100,000 dpm/µg) in goats. Radiolabeled amicarbazone was administered orally to two lactating goats at an average of 101 ppm in the diet (42x the maximum theoretical dietary burden to dairy cattle; see Table 3). The goats were dosed once per day for three consecutive days. Milk was collected twice daily throughout the study, and tissues (muscle, fat, liver, and kidney) were collected at sacrifice; milk and tissues from the two goats were pooled by matrix (and study day in the case of milk).

TRR were 0.202 ppm in Day-1 milk, 0.183 ppm in Day-2 milk, 1.066 ppm in muscle, 0.718 ppm in fat, 4.634 ppm in kidney, and 4.876 ppm in liver from two goats dosed orally with [^{14}C]amicarbazone. The majority of the TRR (~85-99% TRR) was extracted from goat tissues using methanol/acetic acid and acetone (liver, kidney, and muscle) or acetonitrile (ACN; milk and fat). Methanol/water extraction at reflux of the nonextractable residues of liver and kidney released small amounts of radioactivity (2-7% TRR); subsequent acid hydrolysis of nonextractable residues released an additional 2-4% TRR. These methods adequately extracted residues from goat matrices. Nonextractable residues accounted for <1-3% TRR (0.004-<0.049 ppm) in goat matrices. The petitioner normalized the extraction results; however, reported accountability prior to normalization was 80-103%. No storage stability data were submitted to support the study, and the actual storage intervals prior to final analysis were not reported.

Total residues amounting to 91-99% TRR were identified in goat matrices. Parent amicarbazone was a major residue in all matrices except kidney, accounting for 23-24% TRR (0.042-0.048 ppm) in milk, 29% TRR (0.309 ppm) in muscle, 45% TRR (0.323 ppm) in fat, and 11% TRR (0.537 ppm) in liver; amicarbazone was identified in kidney at 8% TRR (0.370 ppm). Metabolite DA amicarbazone was a major residue in all matrices, at 18% TRR (0.033-0.036 ppm) in milk, 40% TRR (0.426 ppm) in muscle, 42% TRR (0.302 ppm) in fat, 28% TRR (1.298 ppm) in kidney, and 60% TRR (2.926 ppm) in liver. Metabolite iPr-2-OH DA amicarbazone was a major residue in kidney, at 26% TRR (1.205 ppm), but was a minor residue in all other matrices (4-8% TRR, 0.015-0.244 ppm). Metabolite tBu-OH MKH 3586 was also identified as a major residue, at 28% TRR (0.051-0.057 ppm) in milk, 16% TRR (0.171 ppm) in muscle, and 10% TRR (0.463 ppm) in kidney, but was a minor residue at 4% TRR (0.029 ppm) in fat and 3% TRR (0.146 ppm) in liver. Metabolite iPr-Ene DA MKH 3586 was a major residue in liver, at 10% TRR (0.488 ppm), but was a minor residue in all other matrices (1-3% TRR, 0.002-0.093 ppm). Other identified metabolites were found at ≤9% TRR; these included triazolinone MKH 3586, triazolinone DA MKH 3586, tBu-Acid MKH 3586, tBu-iPr-2-diOH DA MKH 3586 (kidney and liver only), iPr-1,2-diOH DA MKH 3586, and tBu-OH DA MKH 3586. Based on the submitted data, the analytical methods used were adequate to determine the identity of the major components in goat milk and tissues treated with radiolabeled amicarbazone.

The petitioner proposed that amicarbazone is metabolized in goats via loss of the triazole amino group (deamination), yielding DA amicarbazone, followed by hydroxylation of the isopropyl methine carbon to form iPr-2-OH DA amicarbazone. Dehydration would then result in iPr-Ene DA MKH 3586, which may undergo epoxidation and hydrolysis to form iPr-1,2-diOH DA MKH 3586. Minor pathways involve hydroxylation of the t-butyl group of amicarbazone or DA amicarbazone to form tBu-OH MKH 3586 or tBu-OH DA MKH 3586, and hydrolytic cleavage of the carboxamide side chain of amicarbazone or DA amicarbazone to form triazolinone MKH 3586 or triazolinone DA MKH 3586.

Hen

Arvesta has submitted a study investigating the metabolism of [triazilinone-3-^{14}C]amicarbazone (100,000 dpm/µg) in hens. Radiolabeled amicarbazone was administered orally to ten laying

hens at an average of 15.3 ppm in the diet (49x the maximum theoretical dietary burden to poultry; see Table 3). The hens were dosed once per day for three consecutive days. Eggs were collected twice daily throughout the study, and tissues (muscle, fat, and liver) were collected at sacrifice; eggs and tissues were pooled by matrix (and study day in the case of eggs).

TRR were 0.009 ppm in Day-1 eggs, 0.023 ppm in Day-2 eggs, 0.034 ppm in Day-3 eggs, 0.061 ppm in muscle, 0.032 ppm in fat, and 0.476 ppm in liver from ten hens dosed orally with [^{14}C]amicarbazone. A large portion of the TRR (~58-91% TRR) was extracted from hen commodities, except fat, using ACN/water (muscle and liver) or ACN (eggs); extraction of fat with ACN yielded only 40% TRR. Hydrolysis using 1.5% trifluoroacetic acid released additional radioactivity from fat (44% TRR) and eggs (5-22% TRR). Protease hydrolysis released additional radioactivity from liver and muscle (34-39% TRR). These methods adequately extracted residues from hen matrices. Nonextractable residues accounted for 3-14% TRR (0.0004-0.019 ppm) in hen matrices. The petitioner normalized the extraction results, yielding material balances of 99-101%; however, reported accountability prior to normalization was 84-105%. No storage stability data were submitted to support the study, and the actual storage intervals prior to final analysis were not reported.

Parent amicarbazone was a minor residue in the extracts of all matrices, at 1-7% TRR (0.0006-0.010 ppm); however, amicarbazone was identified in the protease hydrolysate of liver at 30% TRR (0.143 ppm). The major residue identified in eggs and tissues was iPr-2-OH DA amicarbazone, at 24% TRR (0.015 ppm) in muscle, 9% TRR (0.043 ppm) in liver, 10% TRR (0.0032 ppm) in fat, and 20-35% TRR (0.0032-0.0068 ppm) in eggs. Metabolite DA amicarbazone was a significant residue in eggs (19-26% TRR, 0.0023-0.0065 ppm) but was found in lesser amounts in tissues (4-9% TRR, 0.0029-0.019 ppm). Metabolite iPr-1-OH DA MKH 3586 was a significant residue in liver (24% TRR, 0.114 ppm) and was also found in muscle (2% TRR, 0.001 ppm). Metabolite iPr-Ene DA MKH 3586 was a significant residue in fat and eggs (11-15% TRR, 0.0010-0.0048 ppm) but was found in lesser amounts in muscle and liver (≤8% TRR, ≤0.010 ppm). Metabolite iPr-Acid DA MKH 3586 was identified in liver only (11% TRR, 0.052 ppm). Other identified metabolites, each ≤7% TRR (≤0.005 ppm) were triazolinone MKH 3586, triazolinone DA MKH 3586, tBu-OH MKH 3586, and iPr-1,2-diOH MKH 3586 (muscle and liver only). The remainder of the radioactivity consisted of unknowns, which were generally polar in nature.

The petitioner proposed that amicarbazone is metabolized in hens via loss of the triazole amino group, yielding DA amicarbazone, followed by hydroxylation of the isopropyl methine carbon to form iPr-2-OH DA amicarbazone. Dehydration would then result in iPr-Ene DA MKH 3586, which may undergo epoxidation and hydrolysis to form iPr-1,2-diOH DA MKH 3586 Hydroxylation of DA amicarbazone at an isopropyl methyl carbon would yield iPr-1-OH DA MKH 3586, which may then undergo oxidation to form iPr-Acid DA MKH 3586. Minor pathways involve hydroxylation of the t-butyl group of amicarbazone to form tBu-OH MKH 3586, and hydrolytic cleavage of the carboxamide side chain of amicarbazone or DA amicarbazone to form triazolinone MKH 3586 or triazolinone DA MKH 3586.

Conclusions: Pending submission of additional sample storage information to support the study; and the dates of initial and final sample extraction and analysis should be submitted before it can be determined whether the storage stability data included in the study are adequate to support the corn metabolism study, HED concludes that the nature of the residue in livestock (goat and poultry) is adequately understood for the purposes of a conditional registration. The available data indicate that the metabolism of amicarbazone is similar in goats and hens.

HED has determined that the residues of concern in livestock commodities are amicarbazone and its metabolites DA amicarbazone and iPr-2-OH DA amicarbazone for tolerance setting and risk assessment purposes.

860.1340 Residue Analytical Methods

45121702.der (Plant commodity method; also includes review of MRIDs 45121708 and 45121709)
45121705.der (Livestock commodity method; also includes review of MRIDs 45121706 and 45121713)
46145302.der (Plant commodity method; also include data from MRIDs 46145303-46145310)

Plant commodity method

Proposed Enforcement method: Arvesta has proposed an LC/MS/MS method for the enforcement of tolerances for residues of amicarbazone, DA amicarbazone, and iPr-2-OH DA amicarbazone in plant commodities (MRID 45121702). The method is entitled "Analytical Residue Method for the Determination of MKH 3586 Residues in Plant Matrices" (Bayer Report Number 108340).

The method includes instructions for analysis of samples of corn forage, corn fodder, corn grain, and corn grain processed products. Briefly, samples are extracted using accelerated solvent extraction (ASE) with water or 0.05% phosphoric acid as the extraction solvent. The extracts are cleaned up by solid phase extraction (SPE) and dissolved in 2 mM ammonium acetate for LC/MS/MS analysis. The validated limit of quantitation (LOQ) is 0.01 ppm for each analyte in all matrices. The reported limits of detection (LODs) were 0.001 ppm for each analyte in corn forage and grain and 0.006 ppm for each analyte in corn fodder. The results for all analytes are reported as parent equivalents. The method includes instructions for confirmatory analysis, using LC/MS/MS with the same chromatographic system as the main method but different MS conditions.

Method validation data for the LC/MS/MS method demonstrated adequate method recoveries of residues of amicarbazone, DA amicarbazone, and iPr-2-OH DA amicarbazone at the LOQ and 5x the LOQ from field corn forage, fodder, and grain, at 60x the LOQ from field corn forage, at 50x the LOQ from field corn stover, and at the LOQ from field corn processed commodities starch, grits, meal, flour, and refined oil. Overall recovery ranges (and CVs) from these matrices were 70-114% (12%) for amicarbazone, 71-119% (12%) for DA amicarbazone, and 68-119% (10%) for iPr-2-OH DA amicarbazone. The fortification levels and samples used in method validation are adequate to bracket expected residue levels.

Adequate independent laboratory validation data have been submitted for this method using corn grain samples. The submitted radiovalidation data are adequate to demonstrate that the method adequately extracts incurred residues of amicarbazone, DA amicarbazone, and iPr-2-OH DA amicarbazone from corn fodder and grain.

Data collection method (Bayer Method 108340): Samples of field corn forage, stover, and grain from the field corn field trials and samples of field corn commodities from the processing study were analyzed for residues of amicarbazone and its metabolites DA amicarbazone and iPr-2-OH DA amicarbazone using the proposed LC/MS/MS enforcement method for plant commodities, with a validated LOQ of 0.01 ppm for each analyte in each matrix. Adequate concurrent method recovery data were included in the field trial and processing study submissions.

Data collection method (Bayer Method 200258): In conjunction with the field rotational crop trials and processing studies, the petitioner submitted method validation data for a newer LC/MS/MS method (Bayer Method 200258) for determining residues of amicarbazone, DA amicarbazone, and *i*Pr-2-OH DA amicarbazone in/on various plant commodities (MRID 46145302). This method is essentially a revised version of the earlier proposed enforcement method (Bayer Method 108340). However, the new method utilizes a simpler extraction procedure and the same extraction solvents that were used in the corn metabolism study, which has been shown to adequately recover all three residues of concern.

For Method 200258, residues are extracted from most plant matrices (except oil matrices) with 0.1% aqueous acetic acid in acetonitrile (ACN):water (4:1, v/v). Ground samples are initially soaked for 30 minutes in the extraction solvent and then homogenized. Celite is added and residues are filtered. A mixture of the deuterated internal standards are added at this point. For oil matrices, residues are extracted by mixing with hexane and then adding an equal volume of 0.1% aqueous acetic acid in ACN:water (4:1, v/v). Residue in the lower ACN phase are saved and the deuterated internal standards are added. For cleanup of all samples, residues are then eluted through a Bond Elut Certify II cartridge (mixed mode C_8 and SCX SPE) using 0.1% aqueous acetic acid in ACN:water (4:1, v/v). Residues are concentrated, diluted with a small amount of methanol, and then brought to the final volume with aqueous 5 mM ammonium bicarbonate. Residues are analyzed by LC/MS/MS and are quantified using the deuterated internal reference standards, monitoring the transition of parent ions to its daughter ions for each analyte.

The validated LOQ is 0.01 ppm for amicarbazone and its metabolites DA amicarbazone and *i*Pr-2-OH DA amicarbazone in/on all matrices. Depending on the plant matrix, the LOD is 0.001-0.010 ppm for amicarbazone, 0.001-0.009 ppm for DA amicarbazone, and 0.002-0.006 ppm for *i*Pr-2-OH DA amicarbazone.

In a method validation trial using control samples of mustard and turnip leaves and wheat forage, hay, straw and grain fortified with each analyte at 0.01 and 0.10 ppm, average recoveries for amicarbazone and both metabolites ranged from 87-113%, with standard deviations of ±1-14%. In addition, in the extensive rotational crop field trials and processing studies, average procedural

recoveries for each analyte were 89-114% with standard deviations of ±1-13% from alfalfa, cotton, soybean, sugarcane, and wheat RACs and processed commodities. Apparent residues of each analyte were <0.01 ppm in/on all control samples.

The HPLC/MS/MS method (Bayer Method 200258) is adequate for collecting data on residues of amicarbazone, DA amicarbazone, and *i*Pr-2-OH DA amicarbazone in/on various crop matrices.

Conclusions: The analytical methods for plant commodities are adequate. The proposed enforcement method (Bayer Method 108340) has undergone a successful ILV trial and has been forwarded to ACL for petition method validation (PMV).

Bayer Method 108340 appears adequate as an enforcement method for corn commodities. Method 200258 may serve as an alternate enforcement method for plant commodities with submissions of adequate independent laboratory validation (ILV) and radiovalidation data (ACB memo, 5/27/2005, A. Kamel).

HED notes that the chemical structures included in the method for the deuterated internal standards amicarbazone-d_9 and DA amicarbazone-d_9 are incorrect; the petitioner should correct these structures.

Livestock commodity method

Enforcement method: Arvesta has proposed an LC/MS/MS method for the enforcement of tolerances for residues of amicarbazone, DA amicarbazone, and iPr-2-OH DA amicarbazone in livestock commodities (MRID 45121705). The method is entitled "An Analytical Method for the Determination of MKH 3586 Residues in Livestock Matrices" (Bayer Report Number 108111).

The method includes instructions for the analysis of milk and tissue samples. Briefly, tissue samples are extracted using ASE with 0.05% phosphoric acid as the extraction solvent; milk samples are simply diluted with water. The extracts are oxidized using potassium permanganate to convert amicarbazone residues to a common moiety, iPr-2-OH DA amicarbazone. The oxidized extracts are then cleaned up by SPE and dissolved in water/methanol for LC/MS/MS analysis. The validated LOQ is 0.01 ppm in all matrices. The reported LOD was 0.005 ppm. All results are reported as parent equivalents.

The petitioner has not proposed any separate confirmatory analytical procedures for the method, stating that confirmation of analyte identity is made using two criteria: (1) co-chromatography of the sample peak with the internal standard; and (2) detection of the two daughter ions of the target analyte (iPr-2-OH amicarbazone) at the same retention time.

Method validation data for the LC/MS/MS method demonstrated adequate method recoveries at the LOQ for residues of amicarbazone, DA amicarbazone, and iPr-2-OH DA amicarbazone from milk and liver, and at the LOQ and 10x the LOQ for a mixture of amicarbazone, DA

amicarbazone and iPr-2-OH DA amicarbazone from milk, liver, kidney, muscle, and fat. Overall recovery ranges (and CVs) from these matrices were 79-112% (11%) for amicarbazone, 97-116% (7%) for DA amicarbazone, and 86-122% (15%) for iPr-2-OH DA amicarbazone, and 62-103% (12%) for the mixture of amicarbazone, DA amicarbazone and iPr-2-OH DA amicarbazone. The fortification levels and samples used in method validation are adequate to bracket expected residue levels.

Adequate independent laboratory validation data have been submitted for this method, using cattle liver samples. The submitted radiovalidation data are adequate to demonstrate that the method adequately extracts incurred residues of amicarbazone, DA amicarbazone, and iPr-2-OH DA amicarbazone from goat liver.

Data collection method: Cow tissues and milk from the feeding study were analyzed for amicarbazone-related residues using the proposed LC/MS/MS enforcement method for ruminant commodities. The concurrent method recovery data included in the submission reflected fortification at the method LOQ (0.01 ppm) only. These data are adequate for milk, muscle, and fat. Additional validation data, reflecting 10x the LOQ provided for kidney and liver in conjunction with validation of the method, adequately bracketing residues observed in kidney but are not adequate for liver. Residues greater than 100x the LOQ were observed in liver.

Conclusions: The proposed enforcement method (Bayer Method 108111) has undergone a successful ILV trial and has been forwarded to ACB for petition method validation (PMV). Bayer Method 108111 appears adequate as an enforcement method for livestock commodities (ACB memo, 5/27/2005, A. Kamel).

860.1360 Multiresidue Methods

45121703.der

Arvesta has submitted multiresidue method data for amicarbazone. Amicarbazone and its DA amicarbazone and iPr-2-OH DA amicarbazone metabolites were analyzed according to the FDA Multi-Residue Method Test guidelines in PAM Vol. I (dated 1/94).

Because the test substances are not N-methyl carbamates, naturally fluorescent, acids, phenols, or substituted ureas, testing under Protocols A, B, or G was not conducted. Protocol C is applicable to determine the GLC characteristics. Amicarbazone and its metabolite DA MKH 3586 chromatographed using Protocal C. Amicarbazone was not recovered using Protocol D, and interference caused high recoveries of DA MKH 3586 and iPr-2-OH MKH 3586 under Protocol D. DA MKH 3586 and iPr-2-OH MKH 3586 could not be recovered during Florisil column cleanup. Based on the results of Protocol D testing, testing under Protocol E was not conducted. No recovery of the test substances was obtained using the Florisil column cleanup procedures of Protocol F. The multiresidue test data will be forwarded to FDA for further evaluation.

Conclusions: Amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 were adequately evaluated for their recovery through FDA multiresidue methods. Based on the

results of the testing, the multiresidue methods are not appropriate for determining amicarbazone residues of concern.

860.1380 Storage Stability

The petitioner did not conduct storage stability studies in conjunction with the submitted field trials, processing studies, feeding studies, or field rotational crop studies.

In the corn field trials, the maximum storage intervals were ~27 months for field corn forage, stover, and grain. In support of the field corn field trials, the petitioner cited storage stability data submitted in conjunction with the corn metabolism study which demonstrated that the extraction profile and metabolite profiles were generally stable in corn forage, stover, and grain for 8, 7, and 11 months, respectively. These data are insufficient to support the current field trial study. The petitioner additionally stated that reanalysis of the 1997 field trial samples after up to 26 months of frozen storage demonstrated similar residue levels; however, no data to support this statement were included in the submission.

In the corn processing study, the maximum storage intervals from harvest/processing to analysis were 355 days (11.7 months) for corn grain, 21-37 days (~1 month) for starch, grits, and refined oil (wet-milled), 62 days (2 months) for meal, and 112 days (3.7 months) for refined oil (dry-milled). Samples of corn grain were processed ~1 year after harvest.

In the sugarcane processing study, the maximum storage intervals were 23 months for sugarcane RAC and ≤28 days for sugarcane bagasse, molasses, and refined sugar.

The maximum sample storage intervals in the limited field rotational crop studies were 27 months for mustard greens and turnip roots and tops and 21 months for wheat forage, hay, grain and straw. In the extensive field rotational crop trials and their associated processing studies, the maximum sample storage intervals were as follows: alfalfa forage and hay - 23 months; cotton seed and gin byproducts - 12 months; cottonseed processed fractions - ≤14 days; soybean forage, hay and seed - 26 months; soybean seed processed fractions - ≤14 days; wheat forage, hay, grain and straw - 19 months; and wheat grain processed fractions - ≤18 days.

The maximum storage intervals of samples from the cattle feeding study were 27 days for whole milk and 237 days for tissues. In support of the feeding study, the petitioner referenced data from the extraction efficiency study (MRID 45121713, reviewed in conjunction with the proposed enforcement method for livestock commodities), which indicated that residue levels of amicarbazone, DA amicarbazone, and iPr-2-OH DA amicarbazone in radiolabeled liver from a goat metabolism study were not significantly changed following frozen storage for up to ~45 months. These data are adequate to support the storage intervals and conditions of tissue samples from the cattle feeding study. Because milk samples were analyzed within one month of collection, storage stability data for milk will not be required.

Conclusions: The available storage stability data for livestock commodities are adequate to satisfy data requirements. However, data demonstrating the stability of residues of amicarbazone, DA amicarbazone, and iPr-2OH DA amicarbazone in/on representative plant matrices remain outstanding. The petitioner should submit data demonstrating the stability of residues of amicarbazone, DA amicarbazone, and iPr-2OH DA amicarbazone in/on field corn forage, stover, and grain, mustard greens, and turnip roots for up to 27 months of frozen storage. Stability data from these matrices stored for up to 27 months would support the storage intervals from the field rotational crop trials on alfalfa, cotton, soybeans, and wheat.

Because processed corn, cottonseed, sugarcane, soybeans, and wheat commodities were generally analyzed within one month of collection (except corn meal and dry-milled refined oil), no storage stability data for processed commodities are required. The storage stability data for field corn grain may be used to support the storage conditions and intervals for corn meal samples. Because no residues were observed in wet-milled refined oil, which were analyzed within 19 days of processing, the lack of detectable residues in dry-milled refined oil is not likely to be due to degradation upon storage; therefore, storage stability data for refined oil are not required.

860.1480 Meat, Milk, Poultry, and Eggs

45121707.der (Cattle feeding study)

The petitioner submitted a cattle feeding study with the subject petition. The maximum theoretical dietary burdens for livestock are presented in Table 3. The maximum exposure of livestock to residues in the diet occurs primarily from residues in the rotational crop commodities.

Table 3. Calculation of Maximum Theoretical Dietary Burdens of Amicarbazone to Livestock.

Feed Commodity	% Dry Matter[1]	% Diet[1]	Recommended Tolerance, ppm	Dietary Contribution, ppm[2]
Beef and Dairy Cattle				
Soybean, hay	85	30	5.0	1.76
Wheat, forage	25	25	0.5	0.50
Wheat, grain	89	30	0.1	0.03
Soybean, meal	92	15	0.8	0.13
TOTAL BURDEN		100		2.42
Poultry				
Wheat, grain	NA	70	0.1	0.07
Soybean, meal	NA	30	0.8	0.24
TOTAL BURDEN		100		0.31
Swine				
Wheat, grain	NA	75	0.1	0.075
Soybean, meal	NA	25	0.8	0.20
TOTAL BURDEN		100		0.28

[1] Table 1 (OPPTS Guideline 860.1000).

[2] Contribution = [tolerance / % DM (cattle)] x % diet). Poultry and swine diets are not corrected for % dry matter.

Cattle

Arvesta has submitted a ruminant feeding study with amicarbazone. Encapsulated amicarbazone was administered orally to three groups of lactating Holstein cows (3 cows/group) for 30 days. Cows were dosed at 0.374, 1.238, or 4.538 ppm in the diet; these dosing levels are equivalent to 0.15x, 0.5x, and 1.9x the maximum theoretical dietary burden to beef and dairy cattle, and 1.3x, 4.4x, and 16.2x the maximum theoretical dietary burden to swine. Milk was collected twice daily, and samples were composited daily for each cow. Milk samples were collected on study days 0, 4, 8, 12, 16, 18, 20, 22, 24, 26, and 28. Cows were sacrificed within 6 hours of the final dose, and samples of muscle, liver, kidney and fat were collected.

Amicarbazone-related residues were below the method LOQ (<0.010 ppm) in whole milk samples collected over the course of the dosing period from the high-dose group. Because no quantifiable residues were observed in milk from the high-dose group, milk samples from the low- and mid-dose group were not analyzed. Therefore, no trends in residue levels over time or dosing level could be determined in milk.

Amicarbazone-related residues were 1.165-1.193 ppm in liver, 0.080-0.127 ppm in kidney, 0.014-0.021 ppm in muscle, and <0.010-0.012 ppm in fat from the high-dose group. Residues were 0.305-0.440 ppm and 0.191-0.235 ppm in liver, and 0.033-0.039 ppm and 0.017-0.018 ppm in kidney from the mid- and low-dose groups, respectively. Residues were below the method LOQ (<0.010 ppm) in muscle and fat samples from the mid-dose group; therefore, samples of muscle and fat from the low-dose group were not analyzed. The relationship between dosing

level and amicarbazone-related residues appears to be linear in liver and kidney. Based upon the maximum residues observed in the 1.9x dose group, the maximum expected residues at a 1x feeding level would be 0.628 ppm in liver, 0.067 ppm in kidney, 0.011 ppm in muscle, and 0.006 ppm in fat from the high-dose group. The results are summarized in Table 3a below.

TABLE 3a. Summary of Residue Data from Ruminant Feeding Study with Amicarbazone.

Matrix	Feeding Level (ppm)	Pre-Slaughter Interval (days)	Residue Levels (ppm)					
			n	Min.	Max	Median	Mean	Std. Dev.
Whole milk	4.538	Not applicable (N/A)	30	<0.010	<0.010	0.005	0.006	0.001
Whey	4.538	N/A	1	<0.010	<0.010	0.006	0.006	N A
Cream	4.538	N/A	1	<0.010	<0.010	0.005	0.005	N A
Liver	4.538	0	3	1.165	1.193	1.184	1.181	0.014
	1.238	0	3	0.305	0.440	0.383	0.376	0.068
	0.374	0	3	0.191	0.235	0.197	0.208	0.024
Kidney	4.538	0	3	0.080	0.127	0.107	0.105	0.024
	1.238	0	3	0.033	0.039	0.036	0.036	0.003
	0.374	0	3	0.017	0.018	0.018	0.018	0.001
Muscle	4.538	0	3	0.014	0.021	0.019	0.018	0.004
	1.238	0	3	<0.010	<0.010	0.006	0.006	0.001
Fat	4.538	0	3	<0.010	0.012	0.009	0.010	0.002
	1.238	0		<0.010	<0.010	0.005	0.005	0

Conclusions: The cattle feeding study residue data are adequate to satisfy data requirements for this petition. The feeding study data indicate that tolerances for residues of amicarbazone, DA amicarbazone, and iPr-2OH DA amicarbazone should be established at 0.01 ppm (the LOQ) in/on milk and the fat and meat of cattle, goats, horses, and sheep, at 1.0 ppm in/on the liver of cattle, goats, horses, and sheep, and at 0.10 ppm in/on meat byproducts, except liver.

For swine commodities, tolerances for residues of amicarbazone, DA amicarbazone, and iPr-2OH DA amicarbazone are needed at 0.01 ppm in/on hog fat, meat and meat byproducts, except liver at 0.10 ppm in/on hog liver. If a petition is submitted that includes additional uses on livestock feed items, then a feeding study with higher feeding levels (up to and greater than 10x) should be submitted.

Poultry

The petitioner did not submit a poultry feeding study with this petition. The maximum combined residues of amicarbazone, DA amicarbazone, and iPr-2-OH DA amicarbazone observed in any matrix in the poultry metabolism study were 0.215 ppm in liver. Based on the 15.3 ppm dosing level (49x) used in the metabolism study, the expected combined residues of these metabolites at a 10x dosing level are 0.0439 ppm, which is above the method LOQ of 0.01 ppm. Considering that (i) hens in the metabolism study were dosed for only 3 days, (ii) residues in eggs increased

throughout the metabolism study, and (iii) quantifiable residues (>0.01 ppm) could be present in hens dosed at 10x. HED concludes that a poultry feeding study is required to support the proposed use of amicarbazone on field corn. However, for a conditional registration, HED recommends that the petitioner should propose a tolerance of 0.01 ppm (the LOQ) for poultry liver. The tolerances for poultry commodities will be reassessed after a poultry feeding study is submitted.

860.1500 Crop Field Trials

45121710.der. (corn field trials)

Avesta is supporting the use of amicarbazone (DF) on field corn in the U.S. and on sugarcane grown outside the U.S. for importation. The field trail data for field corn are summarized in Table 4 below. Field trial data on sugarcane were not submitted.

Table 4. Summary of Residues from the Field Corn Crop Field Trials with Amicarbazone.

Crop Matrix	Application Rate (lb ai/A)	PHI (days)	Analyte	Residues (ppm)					
				n	Min.	Max.	HAFT	Mean	Std. Dev.
Field corn (proposed use = single preemergence application at 0.448 lb ai/A)									
Forage	0.44-0.46	74-118	Amicarbazone	23	<0.010	0.1197	0.1197	0.017	0.024
			DA amicarbazone		<0.010	0.0634	0.0634	0.012	0.014
			iPr-2-OH DA amicarbazone		<0.010	0.0387	0.0387	0.012	0.011
			Total		<0.030	0.211	0.211	0.041	0.043
Stover	0.44-0.46	110-167	Amicarbazone	23	<0.010	0.0589	0.0589	0.014	0.013
			DA amicarbazone		<0.010	0.0423	0.0423	0.014	0.011
			iPr-2-OH DA amicarbazone		<0.010	0.0946	0.0946	0.019	0.024
			Total		<0.030	0.150	0.150	0.047	0.038
Grain	0.44-0.46	110-167	Amicarbazone	23	<0.010	<0.010	<0.010	0.005	0
			DA amicarbazone		<0.010	<0.010	<0.010	0.005	0
			iPr-2-OH DA amicarbazone		<0.010	0.0122	0.0122	0.006	0.003
			Total		<0.030	<0.032	<0.032	0.016	0.003
Field corn (proposed use = single preplant incorporated application at 0.448 lb ai/A)									
Forage	0.44-0.46	74-118	Amicarbazone	23	<0.010	0.0919	0.0919	0.019	0.020
			DA amicarbazone		<0.010	0.0575	0.0575	0.013	0.012
			iPr-2-OH DA amicarbazone		<0.010	0.0304	0.0304	0.013	0.008
			Total		<0.030	0.172	0.172	0.045	0.036

Table 4. Summary of Residues from the Field Corn Crop Field Trials with Amicarbazone.

Crop Matrix	Application Rate (lb ai/A)	PHI (days)	Analyte	Residues (ppm)					
				n	Min.	Max.	HAFT	Mean	Std. Dev.
Stover	0.44-0.46	110-167	Amicarbazone	23	<0.010	0.1332	0.1332	0.019	0.028
			DA amicarbazone		<0.010	0.0987	0.0987	0.017	0.020
			iPr-2-OH DA amicarbazone		<0.010	0.2001	0.2001	0.025	0.043
			Total		<0.030	0.432	0.432	0.061	0.086
Grain	0.44-0.46	110-167	Amicarbazone	23	<0.010	<0.010	<0.010	0.005	0
			DA amicarbazone		<0.010	<0.010	<0.010	0.005	0
			iPr-2-OH DA amicarbazone		<0.010	0.0138	0.0138	0.006	0.003
			Total		<0.030	<0.034	<0.034	0.016	0.003
Field corn (proposed use = single postemergence application at 0.153 lb ai/A, up to 10-leaf collar stage)									
Forage	0.24-0.26	19-78	Amicarbazone	46	<0.010	0.3956	0.3949	0.050	0.080
			DA amicarbazone		<0.010	0.1285	0.1252	0.015	0.025
			iPr-2-OH DA amicarbazone		<0.010	0.0343	0.0333	0.010	0.007
			Total		<0.030	0.557	0.553	0.075	0.110
Stover	0.24-0.26	67-125	Amicarbazone	46	<0.010	0.1690	0.1579	0.037	0.039
			DA amicarbazone		<0.010	0.0615	0.0549	0.015	0.012
			iPr-2-OH DA amicarbazone		<0.010	0.0796	0.0790	0.014	0.016
			Total		<0.030	0.240	0.226	0.066	0.059
Grain	0.24-0.26	67-125	Amicarbazone	46	<0.010	0.0250	0.0175	0.005	0.003
			DA amicarbazone		<0.010	<0.010	<0.010	0.005	0
			iPr-2-OH DA amicarbazone		<0.010	<0.010	<0.010	0.005	0.001
			Total		<0.030	<0.045	<0.038	0.016	0.003

Field Corn

Arvesta has submitted field trial data depicting amicarbazone residues in/on field corn forage, fodder (stover), and grain. The petitioner conducted a total of 23 field corn field trials in Regions 1 (NY and PA, 2 trials), 2 (GA; 1 trial), 5 (IA, IL, IN, KS, MI, MN, MO, NE, and OH; 19 trials), and 6 (TX; 1 trial) during the 1997 and 1998 growing seasons. The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 for field corn.

Each field trial consisted of three plots using different treatment regimens; a fourth untreated plot was used to generate control samples. At the first plot, a single broadcast preemergence (PRE) application of the 70% DF formulation was made at ~0.45 lb ai/A on the day of planting field corn seed (1x the maximum proposed rate for this type of application). At the second plot, a single broadcast preplant incorporated (PPI) application was made to the soil at ~0.45 lb ai/A on the day of planting (1x the maximum proposed rate for this type of application); the formulated solution was incorporated into the soil to a depth of ~2 inches prior to planting. At the third plot, a single early postemergence (POST) broadcast application was made at ~0.25 lb ai/A (1.6x the maximum proposed rate for this type of application), approximately 42 days after planting. Applications were made in 9.9-20.2 gal/A of water using ground equipment. Only limited information pertaining to weather conditions and soil characteristics was included in the submission.

Samples from all trials were collected at the following growth stages: forage was collected at the late dough to early dent stage, 74-118 days posttreatment from the PRE and PPI plots and 30-78 days posttreatment from the POST plot; and stover and grain were collected at crop maturity, 110-167 days posttreatment from the PRE and PPI plots and 67-125 days posttreatment from the POST plot.

In two field corn trials (in KS and IN), samples of forage, stover, and grain were collected at four intervals following the last application to evaluate residue decline: 5 ± 2 days apart for forage, beginning 5 days prior to normal harvest; and 7 ± 1 days apart for stover and grain, beginning 7 days prior to normal harvest. Residue decline data from the IN trial indicated that combined residues of amicarbazone and its metabolites DA amicarbazone and iPr-2-OH DA amicarbazone did not increase with increasing sampling intervals. All residues were below the method LOQ (<0.01 ppm) at each sampling interval in/on all samples (except one sample of forage) from the KS decline trial; therefore, decline could not be evaluated.

The petitioner reported the dry matter content for all samples collected in these trials. Dry matter content ranged 19-43% for forage, 29-71% for stover, and 63-95% for grain. We note that the values for stover were all lower than the value reported in Table 1 of OPPTS 860.1000 (83%); the forage and grain ranges were closer to expected values (40% and 88%, respectively).

Aspirated grain fractions were generated in conjunction with the corn processing study (MRID 45121711) in which field corn received a single postemergence foliar broadcast application of the 70% DF formulation at 1.24 lb ai/A (3x the maximum proposed seasonal rate). However, as application was made prior to ear formation, grain surface residues were not expected, and aspirated grain fractions samples were not analyzed.

Conclusions: The field corn field trial residue data are adequate, provided additional data/information are submitted. Storage stability data are needed to support this study. Information pertaining to the weather conditions (including temperatures) over the course of the study, with comparison to historical averages (for both temperature and rainfall), should be submitted. A discussion of any unusual weather conditions should be included. Because the

proposed use pattern of amicarbazone on field corn is dependent on soil type, soil pH, and soil organic matter, information pertaining to these characteristics should be submitted for all field trials included in this submission.

With respect to the low dry matter content of stover samples, an average of ≈40% dry matter content was estimated. Therefore, a factor of roughly 2x will be taken into account when determining an appropriate tolerance level for stover (see 860.1550).

The proposed use directions specify that application is not to be made to coarse-textured soils. We note that at least one field trial (1997, Tifton, GA) was conducted at a site with coarse-textured soil (sandy loam); information pertaining to soil type was only provided for three field trials.

Because no residue data were submitted reflecting preemergence/preplant applications in combination with postemergence applications, the proposed use pattern should be amended to specify that early postemergence applications may not be made if preemergence/preplant applications have been made.

The proposed use rate for early postemergence application is 0.098-0.153 lb ai/A with a maximum seasonal rate of 0.448 lb ai/A. Since the application rate used in the postemergence application field trials was 1x 0.25 lb ai/A, the maximum seasonal rate should be revised to reflect this rate.

Sugarcane

Arvesta is apparently supporting the use of amicarbazone on sugarcane grown in other countries, and this use could result in the importation into the U.S. of sugarcane products bearing amicarbazone residues. However, label directions and field trial data supporting the use on sugarcane were not submitted with the current data. The petitioner should submit field trial data that support the proposed use on sugarcane. Field trial data for sugarcane should be submitted from all regions where this use would be allowed.

860.1520 Processed Food and Feed

45121711.der (field corn)
46145305.der (processed field rotational cottonseed)
46145306.der (sugarcane)
46145308.der (processed field rotational soybeans)
46145310.der (processed field rotational wheat)

Field Corn

Arvesta has submitted a processing study with field corn grain. Residues of amicarbazone were nondetectable (<0.001 ppm) in/on field corn grain collected 113 days following a single

postemergence foliar broadcast application of the 70% DF formulation at 1.24 lb ai/A (3x the maximum proposed seasonal rate); detectable residues of metabolites DA amicarbazone and iPr-2-OH DA amicarbazone below the method LOQ (<0.01 ppm) were observed in corn grain, at 0.0011-0.0014 and 0.0037-0.0096 ppm, respectively. The average combined residues of amicarbazone and its metabolites, reported as amicarbazone equivalents, were <0.0097 ppm.

The processing factors for amicarbazone metabolites are summarized in Table 5. Processing factors for amicarbazone were not calculated because residues of amicarbazone were nondetectable (<0.001 ppm) in/on all subsamples of starch, meal, grits, flour, and refined oil (dry- and wet-milled) processed from treated corn grain.

Total combined residues of amicarbazone and its metabolites were found to concentrate slightly in corn meal (processing factor of 1.2x) but did not concentrate in starch (0.3x), grits (0.9x), flour (1.0x), or refined oil (0.3x).

Table 5. Processing Factors for Amicarbazone and its Metabolites in Field Corn Processed Commodities.

Processed Commodity	Processing Factor			
	Amicarbazone	DA amicarbazone	iPr-2-OH DA amicarbazone	Combined Residues
Starch	Not calculated (NC)	<1x	<0.2x	0.3x
Meal	NC	1.1x	1.3x	1.2x
Grits	NC	1.1x	0.8x	0.9x
Flour	NC	<1x	1.0x	1.0x
Refined oil (dry-milled)	NC	<1x	<0.2x	0.3x
Refined oil (wet-milled)	NC	<1x	<0.2x	0.3x

Sugarcane

In a single test conducted in LA during 2000, sugarcane was treated once with amicarbazone (70% WDG) at 1.35, 4.08, and 6.80 lb ai/A in three separate plots using ground equipment. These rates are reported to be 1x, 3x and 5x the maximum use rate proposed for amicarbazone on sugarcane grown outside the U.S. Sugarcane was harvested using a mechanical chopper at commercial maturity (139 days after treatment, DAT) from only the 5x treated plot. Triplicate samples of sugarcane (RAC) were collected and the remaining bulk samples were processed into bagasse, molasses, and refined sugar using simulated commercial procedures.

Prior to analysis, sugarcane samples were stored frozen for a maximum of 23 months, an interval not supported by available stability data, and processed sugarcane matrices were stored frozen for up to 28 days. Samples were analyzed for residues of amicarbazone and its metabolites DA amicarbazone and iPr-2-OH DA amicarbazone using an adequate LC-MS/MS method (Bayer Method 200258). Residues of metabolites were reported as parent equivalents. The LOQ was 0.01 ppm for each analyte in/on all sugarcane matrices, and the LOD for each analyte in each matrix ranged from 0.001-0.003 ppm.

Residues in/on 3 sugarcane samples were 0.013 ppm for amicarbazone, 0.027-0.028 ppm for DA amicarbazone, and 0.072-0.075 ppm for iPr-2-OH DA amicarbazone. Combined residues in/on sugarcane were 0.113-0.114 ppm and averaged 0.114 ppm. Average combined residues were <0.008 ppm in refined sugar, 0.782 ppm in molasses, and 0.435 ppm in bagasse. Based on these residues, the processing factors for combined amicarbazone residues were 0.07x for refined sugar, 6.9x for blackstrap molasses, and 3.8x for bagasse. The maximum theoretical concentration factor for sugarcane is >20x. These data indicate that a separate tolerance will be required for sugarcane, molasses; however, the appropriate tolerance cannot be determined until the sugarcane field trial data are available.

Sugarcane bagasse is no longer considered to be a significant livestock feed item in the U.S., thus a tolerance is not required.

Processed Rotational Crops

Cotton

In a single test conducted in GA during 2000, bare soil was treated once with amicarbazone (70% WDG) at 2.25 lb ai/A (5x) using ground equipment. The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. A rotational crop of cotton was planted at a 12-month PBI. Seed cotton was harvested using a mechanical picker at commercial maturity (175 days after planting, DAP), then ginned to generated undelinted cottonseed. Triplicate samples of undelinted cottonseed (RAC) were collected and the remaining bulk samples were processed into hulls, meal, and refined oil using simulated commercial procedures.

Prior to analysis, cotton undelinted seed samples were stored frozen for a maximum of 10 months, an interval not supported by available stability data, and processed cotton matrices were stored frozen for up to 14 days. Samples were analyzed for residues of amicarbazone and its metabolites DA amicarbazone and iPr-2-OH DA amicarbazone using an adequate LC/MS/MS method (Bayer Method 200258), with all residues being reported in parent equivalents. The LOQ was 0.01 ppm for each analyte in/on all cotton matrices and the LOD for each analyte in each matrix ranged from 0.001-0.009 ppm.

Amicarbazone and DA amicarbazone residues were each <0.005-<0.006 ppm (each <LOD) and iPr-2-OH DA amicarbazone residues were 0.004-0.005 ppm (each <LOQ) in/on 3 samples of cotton undelinted seed harvested at maturity from plants grown in soil treated at 5x. Amicarbazone and DA amicarbazone residues were each <LOD in all processed cotton matrices. Residues of iPr-2-OH DA amicarbazone were 0.004 ppm (<LOQ) ppm in 3 meal samples, <0.001 ppm (<LOD) in 3 refined oil samples, and ≤0.003 ppm (<LOQ) in 3 hulls samples. Combined residues were each <LOQ in/on cotton seed and all processed matrices: <0.015 ppm in undelinted seed, <0.016 ppm in meal, <0.009 ppm in refined oil, and <0.011 ppm in hulls. As residues were <LOQ from all cotton RAC and processed cotton samples, calculation of reliable processing factors was not possible. However, results from a study conducted at the 5x rate indicate tolerances in cotton processed commodities are not needed.

Soybean

In a single test conducted in KS during 2000, bare soil was treated once with amicarbazone (70% WDG) at 0.451 lb ai/A using ground equipment (~1x). A rotational crop of soybeans was planted immediately following treatment (0-day PBI) and soybean seed was harvested at commercial maturity (144 DAT). Triplicate samples of soybean seed (RAC) were collected and the remaining bulk samples were processed into hulls, meal, and deodorized oil using simulated commercial procedures.

Prior to analysis, soybean seed samples were stored frozen for a maximum of 24 months, an interval not supported by available stability data, and processed soybean matrices were stored frozen for up to 14 days. Samples were analyzed for residues of amicarbazone and its metabolites DA amicarbazone and iPr-2-OH DA amicarbazone using an adequate LC/MS/MS method (Bayer Method 200258), with residues being reported in parent equivalents. The LOQ was 0.01 ppm for each analyte in/on all matrices, and the LOD for each analyte in each soybean matrix ranged from 0.002-0.010 ppm.

Combined residues of amicarbazone, DA amicarbazone and iPr-2-OH DA amicarbazone were <0.167-<0.176 ppm in/on 3 soybean seed samples and averaged 0.170 ppm. Combined residues were <0.148-<0.158 ppm in hulls, <0.211-<0.219 ppm in meal, and <0.011 ppm in deodorized oil. Average combined residues were 0.153 ppm in hulls, 0.215 ppm in meal, and 0.011 ppm in deodorized oil. Based on these residues, the processing factors for combined amicarbazone residues were 0.9x for hulls, 1.3x for meal, and 0.06x for deodorized oil. The maximum theoretical concentration factor for soybeans is 12x.

Wheat

In a single test conducted in KS during 2000, bare soil was treated once with amicarbazone (70% WDG) at 0.45 lb ai/A (~1x) using ground equipment. A rotational crop of wheat was planted immediately following treatment (0-day PBI) and wheat grain was harvested at commercial maturity (273 DAT). Triplicate samples of wheat grain (RAC) were collected and the remaining bulk samples were processed into bran, middlings, shorts, flour, and germ using simulated commercial procedures.

Prior to analysis, wheat grain samples were stored frozen for a maximum of 15 months, an interval not supported by available stability data, and processed wheat matrices were stored frozen for up to 18 days. Samples were analyzed for residues of amicarbazone, DA amicarbazone, and iPr-2-OH DA amicarbazone using an adequate LC/MS/MS method (Bayer Method 200258), with residues reported in parent equivalents. The LOQ was 0.01 ppm for each analyte in/on all wheat matrices, and the LOD for each analyte in each wheat matrix ranged from 0.001-0.006 ppm.

Combined residues of amicarbazone and its metabolites in grain were <0.042-<0.046 ppm and averaged 0.044 ppm. Combined residues were <0.057-<0.065 ppm in bran, <0.047-<0.051 ppm in flour, <0.058-<0.061 ppm in shorts, <0.046-<0.048 ppm in middlings, and <0.039-<0.043 ppm in germ. Average combined residues were 0.062 ppm in bran, 0.049 ppm in flour, 0.059

ppm in shorts, 0.047 ppm in middlings, and 0.040 ppm in germ. Based on these residues, the processing factors for combined amicarbazone residues were 1.4x for bran, 1.1x for flour, 1.3x for shorts, 1.1x for middlings, and 0.9x for germ. The maximum theoretical concentration factor for wheat is 8x.

Conclusions: Pending submission of the required storage stability data for the various RACs, the processing studies are adequate to satisfy data requirements. Tolerances for amicarbazone residues are not required for the following processed fractions: corn starch, grits, flour and refined oil; sugarcane refined sugar; cotton oil, meal, and hulls; soybean oil and hulls; and wheat flour, middlings, and germ. Combined amicarbazone residues either did not concentrate or concentrated only slightly ($\leq$1.1x) in these processed fractions. Slightly higher concentrations of residues were observed in corn meal (1.2x), soybean meal (1.3x), and wheat bran (1.4x) and shorts (1.3x). Substantial concentrations of residues were observed in sugarcane molasses (6.9x)

Based on a processing factor of 1.2x for corn meal and a HAFT residue of <0.038 ppm for field corn grain, the expected combined residues in corn meal following treatment at 1x are <0.046. Because these residues are less than the proposed tolerance of 0.05 ppm for field corn grain, no tolerance for corn meal is needed.

The residue data indicate that amicarbazone residues concentrated substantially in sugarcane molasses (6.9x). However, field trial residue data on sugarcane (RAC) are not available; therefore, a decision regarding the appropriate tolerance for sugarcane molasses cannot be made at this time.

Based on the 1.3x processing factor for soybean meal and HAFT residues of <0.589 ppm in/on soybean seed, the maximum expected residues in meal would be ~0.766 ppm, which is within the recommended 0.8 ppm tolerance for soybean seed. Therefore, a tolerance for soybean meal is not needed.

Based on the processing factors for wheat bran (1.4x) and shorts (1.3x) and HAFT residues of <0.09 ppm in/on wheat grain, the maximum expected combined residues would be ~0.126 ppm in wheat bran and 0.117 ppm in wheat shorts. The recommended tolerance for wheat grain is 0.1 ppm. Based on these data, a separate tolerance should be established at 0.15 ppm for wheat milled byproducts, which will cover residues in bran, middlings and shorts.

860.1650 Submittal of Analytical Reference Standards

As of September, 2004, an analytical reference standard for amicarbazone was available at the EPA National Pesticide Standards Repository. However, based on the proposed enforcement methods, the following additional analytical reference standards are needed and should be submitted to the Repository:

- DA amicarbazone [N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide]

- iPr-2-OH DA amicarbazone [N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide]
- amicarbazone-d_9 (deuterated internal standard)
- DA amicarbazone-d_9 (deuterated internal standard)
- iPr-2-OH DA amicarbazone-d_6 (deuterated internal standard)

The reference standards should be sent to the Analytical Chemistry Lab, which is located at Fort. Meade. You should send them to the attention of either Theresa Cole or Frederic Siegelman at the following address:

USEPA
National Pesticide Standards Repository/Analytical Chemistry Branch/OPP
701 Mapes Road
Fort George G. Meade, MD 20755-5350

The extended zip code should be used or the mail will be returned to you.

860.1850 Confined Accumulation in Rotational Crops

45121704.der

Arvesta has submitted a confined rotational crop study with [triazolinone-3-^{14}C]amicarbazone (specific activity 209,000 dpm/µg). The radiolabeled test substance was applied directly to silt loam soil in metal tubs at 0.42 lb ai/A (0.9x the maximum proposed seasonal rate), and rotational kale (leafy vegetable), turnip (root crop), and wheat (grain crop) were planted 47, 138, and 364 days after treatment (DAT). The petitioner had intended to include a 30-day plant-back interval (PBI); however, crops which had been planted 30 days following treatment failed due to amicarbazone phytotoxicity.

Total radioactive residues (TRR) accumulated at ≥0.01 ppm in all rotated crops from all PBIs. Only small amounts of sample could be collected at the 47-day PBI due to phytotoxicity. TRR were generally highest in samples from the 47-day PBI and lower in those from the 138- and 364-day PBIs. For the 47-day PBI, TRR were highest in turnip tops (7.42 ppm) and wheat hay and straw (2.42 and 4.18 ppm); residues in other commodities were 0.10-1.59 ppm. In crop matrices from the 138-day PBI, TRR were highest in wheat hay and straw (2.78 and 3.49 ppm); residues in other commodities were 0.01-0.72 ppm. In crop matrices from the 364-day PBI, TRR were again highest in wheat hay and straw (1.53 and 1.72 ppm), and residues in other commodities were 0.02-0.74 ppm.

Methanol (MeOH) extraction released 60->100% TRR from rotational crop matrices, with the exception of wheat grain. For wheat grain, MeOH extraction released 28-29% TRR, and extraction with MeOH at reflux released 53-57% TRR. For 138- and 364-DAT samples, additional extraction and hydrolysis procedures were conducted for selected crop matrices; however, these procedures released ≤9% TRR, except for MeOH extraction at reflux of 138-DAT turnip roots, which released 15% TRR. Nonextractable residues in 138- and 364-DAT

rotational crop commodities were <1-9% TRR (<0.01-0.03 ppm). The extraction procedures extracted sufficient residues from rotational crop matrices from the 138- and 364-day PBIs. The procedures did not extract sufficient residues from rotational crop matrices from the 47-day PBI; nonextractable residues in rotational crop matrices from the 47-day PBI were 7-22% TRR (0.01-1.64 ppm). The petitioner stated that the small sample sizes for the 47-DAT samples prevented further extraction of the samples. Adequate supporting storage stability data were submitted to support the storage conditions and intervals of samples from this study.

No characterization/identification of residues could be conducted for 47-DAT turnip root and wheat grain because of sample size and low radioactivity levels. Total identified residues were 22->100% TRR in remaining rotated crop commodities. Residues were characterized/identified primarily by HPLC analysis with confirmatory LC/MS analysis. In 47-DAT matrices, amicarbazone was the major identified residue, at 22-40% TRR (0.55-2.2 ppm). Metabolite iPr-2-OH DA amicarbazone was also a significant residue in all rotational commodities, at 11-19% TRR (0.25-0.80 ppm). Metabolite DA amicarbazone was identified in kale, turnip top, wheat forage, and wheat straw at 5-18% TRR (0.16-1.07 ppm). Glucose conjugates DA OH Glu I MKH 3586 and DA OH Glu II MKH 3586 were major residues in wheat hay and straw (each at 11-15% TRR; 0.36-0.59 ppm), but were found in smaller quantities in kale, turnip top, and wheat forage (≤5% TRR, ≤0.14 ppm). Other identified residues, each present at ≤3% TRR, included triazolinone MKH 3586 (turnip top and wheat forage and straw), tBu-OH DA amicarbazone (kale), N-Me DA amicarbazone (turnip top and wheat hay), and a conjugate of triazolinone MKH 3586 (wheat forage).

In 138-DAT matrices, amicarbazone was a major residue in all commodities except turnip root and wheat grain at 10-37% TRR (0.16-0.64 ppm); amicarbazone was found in turnip root at 2% TRR (<0.01 ppm) and was not found in wheat grain. Metabolite iPr-2-OH DA amicarbazone was found to be a significant residue in all rotational commodities at 12-42% TRR (<0.01-0.78 ppm). Metabolite DA amicarbazone was identified in all commodities except wheat grain, at <1-18% TRR (<0.01-0.13 ppm). Glucose conjugates DA OH Glu I MKH 3586 and DA OH Glu II MKH 3586 were major residues in wheat hay, grain, and straw (each at 11-28% TRR; 0.02-0.96 ppm), but were found in smaller quantities in kale, turnip top, and wheat forage (≤7% TRR, ≤0.05 ppm). Other identified residues, each present at ≤5% TRR, included triazolinone MKH 3586 (all commodities except turnip root), tBu-OH DA amicarbazone (kale), N-Me DA amicarbazone (kale, turnip top, and wheat hay), and a conjugate of triazolinone MKH 3586 (wheat forage).

In 364-DAT matrices, amicarbazone was a major residue in kale, turnip top, and wheat forage, at 12-18% TRR (0.02-0.09 ppm); amicarbazone was found in wheat hay and straw at ≤7% TRR (≤0.10 ppm) and was not found in turnip root or wheat grain. Metabolite iPr-2-OH DA amicarbazone was found to be a significant residue in all rotational commodities at 12-41% TRR (<0.01-0.47 ppm). Metabolite DA amicarbazone was identified in all commodities except wheat grain at <1-35% TRR (<0.01-0.22 ppm). Glucose conjugates DA OH Glu I MKH 3586 and DA OH Glu II MKH 3586 were major residues in wheat hay, grain, and straw (each at 13-39% TRR; <0.01-0.67 ppm), but were found in smaller quantities in kale, turnip top, and wheat forage (≤7%

TRR, ≤0.05 ppm). Other identified residues, each present at ≤10% TRR, included triazolinone MKH 3586 (turnip top and wheat forage, grain, and straw), tBu-OH DA amicarbazone (kale), N-Me DA amicarbazone (kale, turnip top, and wheat hay), and a conjugate of triazolinone MKH 3586 (wheat forage).

The petitioner did not propose a metabolic profile for rotational crops, but noted that the metabolites identified in rotational crops were similar to those found in the primary crop metabolism study and that the major metabolites were those in which the triazole ring had undergone deamination and the tertiary carbon of the isopropyl moiety had undergone oxidation. Two minor metabolites identified in rotational crops, triazolinone MKH 3586 and N-Me DA amicarbazone, were not found in the corn metabolism study.

Conclusions. Although the characterization/identification of the TRR in turnip roots and wheat grain from the 47-day PBI was inadequate, the confined rotational crop study is classified as acceptable based on the extensive identification of ^{14}C-residues in the remaining samples from the 47-day PBI and in all crop samples from the 138- and 364-day PBIs.

With the exceptions of turnip roots and wheat grain, which had lower residues (≤0.2 ppm), 60-87% of the TRR were identified in crops samples from the 47-day PBI, and the majority of these residues (42-77% TRR) were accounted for by amicarbazone, DA amicarbazone, and iPr-2-OH DA amicarbazone. The only other major (>10%) residues were identified in wheat hay and straw as two glucose conjugates of iPr-2-OH DA amicarbazone (25-30% TRR). Similar ^{14}C-residue profiles were observed in crops at the 138- and 364-day PBIs.

At the 138-day PBI, 74-85% of the TRR were identified in all commodities, except turnip roots (TRR = 0.01 ppm), and the majority of these residues (33-76% TRR) were accounted for by the proposed regulated residues. The only other major (>10%) residues were again the two glucose conjugates of iPr-2-OH DA amicarbazone, accounting for 26-47% of the TRR in wheat hay, grain and straw.

At the 364-day PBI, 78-104% of the TRR were identified in all commodities, except turnip roots (TRR = 0.02 ppm), and the majority of these residues (17-82% TRR) were accounted for by the proposed regulated residues. As at the other intervals, the only other major (>10%) residues were the two glucose conjugates of iPr-2-OH DA amicarbazone, accounting for 29-62% of the TRR in wheat hay, grain and straw.

Overall the metabolism of amicarbazone in rotational kale, turnips and wheat was similar to corn, although the relative levels of the glucose conjugates were higher in wheat commodities than corn commodities. The residues of concern in rotational crops for tolerance enforcement are the same as those in the treated crop field corn: parent and the metabolites DA MKH 3586 and iPr-2-OH DA MKH. Based on the levels of the proposed regulated residues in plant commodities in the confined study, field rotational crop studies are required at PBIs up to 12 months.

860.1900 Field Accumulation in Rotational Crops

46145301.der (limited field rotational mustard greens, turnip, and wheat)
46145303.der (extended field rotational alfalfa)
46145304.der (extended field rotational cotton)
46145307.der (extended field rotational soybean)
46145309.der (extended field rotational wheat)

The confined rotational crop data indicated that field rotational crop studies were needed. The petitioner submitted limited field rotational crop studies on mustard greens, turnips and wheat, and extended field rotational crop studies on alfalfa, cotton, soybeans, and wheat. In addition, the petitioner requested that the requirement for residue data on AGF from soybean and wheat be waived because the application was a preplant soil application and residues would not be expected in the outer portion of wheat grain or soybeans. The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. The following rotational crop restrictions have been proposed: a 1-month PBI for soybeans, mustard greens and turnips, and a 4-month PBI for alfalfa, cotton, and wheat. Corn may be replanted immediately following treatment with the 70% DF formulation of amicarbazone.

Limited field rotational crop studies

Mustard, turnip, and wheat
A series of limited field rotational crop trials were conducted at field sites in GA, IN, and KS during 1997-1998. At each of the test sites, bare soil was treated once with amicarbazone (70% WDG) at 0.42-0.46 lb ai/A (~1x) using ground equipment. At each site, representative rotational crops of mustard greens, wheat, and turnips were planted at ~1, 4, 8, and 10-months PBIs. Two of the wheat plantings in KS (8-month and 10-month PBI) failed due to adverse weather conditions, and one planting each of mustard greens (1-month PBI) and turnips (1-month PBI) failed due to phytotoxicity. For the remaining plantings, duplicate treated samples and a single control sample of mustard green leaves, turnip tops and roots, and wheat forage, hay, grain, and straw were harvested at intervals reflecting normal agricultural practices at each site. Samples were harvested 42-143 DAP for mustard greens and turnips, 51-251 DAP for wheat forage, 67-259 days after planting (DAP) for wheat hay, and 95-280 DAP for wheat grain and straw. Samples were stored frozen for a maximum of 27 months, an interval that is not supported by the available storage stability data.

Samples were analyzed for residues of amicarbazone and its metabolites DA amicarbazone and *i*Pr-2-OH DA amicarbazone using the LC/MS/MS method Bayer Method 108340 described above. This method is adequate for data collection based on acceptable concurrent recovery data. The validated LOQ is 0.01 ppm for each analyte in/on all matrices. The limits of detection (LODs) were calculated to be 0.001 ppm for wheat straw, 0.002 ppm for turnip tops, wheat forage, and wheat grain, 0.005 ppm for mustard greens and wheat hay, and 0.008 ppm for turnip roots.

Quantifiable residues were observed in all rotated commodities, except turnip roots. Residues of all analytes were <0.01 ppm in/on turnip roots at all PBIs. At the 1-month PBI, maximum residues of amicarbazone were 0.063 ppm in/on mustard greens, 0.037 ppm in/on turnip tops, 0.088 ppm in/on wheat forage, 0.235 ppm in/on wheat hay, <0.01 ppm in/on wheat grain, and 0.205 ppm in/on wheat straw. Residues of DA amicarbazone were <0.01 ppm in/on all samples from all matrices, except two turnip tops samples with residues of 0.020 and 0.026 ppm and two wheat straw samples each with residues of 0.022 ppm. Maximum residues of *i*Pr-2OH DA amicarbazone were 0.028 ppm in/on mustard greens, 0.083 ppm in/on turnip tops, 0.113 ppm in/on wheat forage, 0.170 ppm in/on hay, 0.049 ppm in/on grain, and 0.260 ppm in/on straw. Maximum combined residues of amicarbazone and its metabolites DA amicarbazone and *i*Pr-2-OH DA amicarbazone (expressed as parent equivalents) from the 1-month PBI were <0.095 ppm in/on mustard greens, 0.120 ppm in/on turnip tops, <0.211 ppm in/on wheat forage, <0.397 ppm in/on hay, <0.069 ppm in/on grain, and 0.485 ppm in/on straw.

At the 4-month PBI, maximum residues of amicarbazone were 0.011 ppm in/on mustard greens, 0.019 ppm in/on turnip tops, <0.01 ppm in/on wheat forage, 0.018 ppm in/on wheat hay, <0.01 ppm in/on wheat grain, and 0.013 ppm in/on wheat straw. Residues of DA amicarbazone were <0.01 ppm in/on all samples from all matrices, except one turnip tops sample with residues of 0.014 ppm. Maximum residues of *i*Pr-2OH DA amicarbazone were 0.025 ppm in/on mustard greens, 0.035 ppm in/on turnip tops, 0.080 ppm in/on wheat forage, 0.104 ppm in/on hay, 0.026 ppm in/on grain, and 0.163 ppm in/on straw. Maximum combined residues were <0.045 ppm in/on mustard greens,<0.059 ppm in/on turnip tops, <0.100 ppm in/on wheat forage, <0.130 ppm in/on hay, <0.046 ppm in/on grain, and <0.186 ppm in/on straw.

At the 8-month PBI, maximum residues of amicarbazone were 0.016 ppm in/on mustard greens, 0.028 ppm in/on turnip tops, 0.015 ppm in/on wheat forage, 0.028 ppm in/on wheat hay, and <0.01 ppm in/on wheat grain and straw. Residues of DA amicarbazone were <0.01 ppm in/on all samples from all matrices, except three mustard greens samples with residues of 0.010-0.016 ppm and two turnip tops samples with residues of 0.018 and 0.026 ppm. Maximum residues of *i*Pr-2OH DA amicarbazone were 0.142 ppm in/on mustard greens, 0.183 ppm in/on turnip tops, 0.050 ppm in/on wheat forage, 0.093 ppm in/on hay, 0.024 ppm in/on grain, and 0.056 ppm in/on straw. Maximum combined residues were 0.174 ppm in/on mustard greens, 0.230 ppm in/on turnip tops, <0.073 ppm in/on wheat forage, <0.131 ppm in/on hay, <0.044 ppm in/on grain, and <0.076 ppm in/on straw.

At the 10-month PBI, maximum residues of amicarbazone were <0.01 ppm in/on all mustard greens, turnip tops, turnip roots, wheat forage, and wheat straw, and 0.014 ppm in/on wheat hay and 0.010 ppm in/on wheat grain. Residues of DA amicarbazone were <0.01 ppm in/on all samples from all matrices. Maximum residues of *i*Pr-2OH DA amicarbazone were 0.024 ppm in/on mustard greens, 0.025 ppm in/on turnip tops, 0.041 ppm in/on wheat forage, 0.097 ppm in/on hay, 0.014 ppm in/on grain, and 0.025 ppm in/on straw. Maximum combined residues were <0.044 ppm in/on mustard greens, <0.045 ppm in/on turnip tops, <0.055 ppm in/on wheat forage, <0.121 ppm in/on hay, <0.034 ppm in/on grain, and <0.045 ppm in/on straw.

Conclusions. Pending submission of the required storage stability data, the residue data from the limited field rotational crop study are adequate and indicate that extended rotational crop field studies are required on crops routinely rotated with corn, except for bulb vegetables. Provided a 1-month PBI is specified, tolerances for bulb vegetables and root and tuber vegetables will not be required.

Extended field rotational crop studies

Residue data from the extended field rotational crop studies on alfalfa, cotton, soybean, and wheat are summarized in Table 6. The current label proposes a 1-month PBI for soybeans, and a 4-month PBI for alfalfa, cotton, and wheat.

Table 6. Summary of Residue Data in Rotational Crops Following Primary Treatment of Bare Soil with Amicarbazone (70% WDG).

Rotational Crop	Commodity	Total Rate (lb ai/A)	PBI (days)	Combined Amicarbazone Residue Levels (ppm) [1]						
				n	Min.	Max.	HAFT[2]	Median (STMdR[3])	Mean (STMR[3])	Std. Dev.
Alfalfa	Forage	0.43-0.45	99-123	24	<0.03	<0.037	<0.037	0.015	0.017	0.004
	Hay			24	<0.03	<0.074	<0.068	0.019	0.025	0.013
Cotton	Undelinted Seed	0.43-0.45	329-367	24	<0.03	<0.053	<0.047	0.015	0.017	0.006
	Gin Byproducts			12	<0.031	0.210	<0.168	0.068	0.084	0.053
Soybean	Forage	0.44-0.47	22-57 [4]	37	<0.268	1.264	1.179	0.654	0.750	0.289
	Hay			37	<0.629	4.569	4.354	2.744	2.569	1.038
	Seed			38	<0.051	<0.602	<0.589	0.187	0.225	0.161
Wheat	Forage	0.40-0.46	115-130	40	<0.03	0.485	0.466	0.120	0.136	0.112
	Hay			40	<0.03	0.878	0.872	0.094	0.159	0.178
	Grain			40	<0.03	<0.095	<0.090	0.029	0.033	0.018
	Straw			40	<0.03	0.450	0.391	0.080	0.109	0.090

[1] The LOQ is 0.01 ppm for each analyte in/on each matrix. Total residues are the sum of amicarbazone, DA amicarbazone, and iPr-2-OH DA amicarbazone residues in parent equivalents; the LOQ was used for individual residues reported at <LOQ in calculating total residues.

[2] HAFT = Highest Average Field Trial.

[3] STMdR = Supervised Trial Median Residue; STMR = Supervised Trial Mean Residue. For calculation of the median, mean and standard deviation, ½ the LOQ (0.005 ppm) was used for residues reported at <LOD.

[4] Residue values from the soybean test with the 366-day PBI were excluded from the soybean data set.

Alfalfa

Twelve field rotational crop trials were conducted at field sites throughout the US during 2000-2001. At each of the test sites, bare soil was treated once with amicarbazone (70% WDG) at 0.434-0.454 lb ai/A using ground equipment. The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. At each site, a rotational crop of alfalfa was planted 99-123 DAT (~4 months). Duplicate treated samples and a single control sample of alfalfa forage and hay were harvested at the first cutting of alfalfa, 41-286 DAP. Hay samples from three of the tests were allowed to dry in the field for 2-4 days prior to sampling. Samples from the second and third cutting of alfalfa were not collected.

Samples were stored frozen from collection to analysis for up to 23 months, an interval that is not supported by the available storage stability data. Samples were analyzed for residues of amicarbazone and its metabolites DA amicarbazone and iPr-2-OH DA amicarbazone using an adequate LC-MS/MS method (Bayer Method 200258), with metabolite residues being reported in parent equivalents. The LOQ was 0.01 ppm each analyte in/on forage and hay, and the LODs for each analyte in each matrix ranged from 0.001-0.004 ppm.

Residues of amicarbazone were <0.01-0.017 ppm in/on forage and <0.01-0.054 ppm in/on hay samples from alfalfa planted at a ~4-month PBI. Residues of DA amicarbazone were <0.01 ppm in/on all alfalfa forage and hay samples. Residues of iPr-2OH DA amicarbazone were <0.01 ppm in/on alfalfa forage and <0.01-0.014 ppm in/on hay. Combined residues were <0.030-<0.037 ppm (ave. = 0.017 ppm) in/on alfalfa forage and <0.030-<0.074 ppm (ave. = 0.025 ppm) in/on hay.

Cotton

Twelve field rotational crop trials were conducted at field sites throughout the US during 2000. At each of the test sites, bare soil was treated once with amicarbazone (70% WDG) at 0.396-0.463 lb ai/A using ground equipment (~1x). At each site, a rotational crop of cotton was planted 329-367 days (11-12 months) after treatment. Duplicate treated samples and a single control sample of cotton bolls were harvested at intervals reflecting normal agricultural practices at each site, 123-213 DAP. Cotton was harvested using a picker at three locations, a stripper at three locations, and by hand at the other six locations. Whole cotton seed samples were ginned and undelinted seed samples were collected from all tests and gin byproducts samples were collected from six tests (picker and stripper only).

Samples were stored frozen from collection to analysis for up to 12 months, an interval that is not supported by the available storage stability data. Samples were analyzed for residues of amicarbazone, DA amicarbazone and iPr-2-OH DA amicarbazone using an adequate LC/MS/MS method (Bayer Method 200258), with all residues being reported in parent equivalents. The LOQ was 0.01 ppm each analyte in/on all matrices, and the LOD for each analyte in each matrix ranged from 0.002-0.006 ppm.

Residues of amicarbazone were <0.01 ppm in/on all cotton undelinted seed samples and <0.01-0.015 ppm in/on cotton gin byproducts samples from cotton planted at a 12-month PBI. Residues of DA amicarbazone were <0.01 ppm in/on all cotton seed samples and <0.01-0.026 ppm in/on gin byproducts samples. Residues of iPr-2OH DA amicarbazone were <0.01-0.033 ppm in/on cotton seed and <0.01-0.169 ppm in/on gin byproducts. Combined residues were <0.030-<0.053 ppm (ave. = 0.017 ppm) in/on cotton undelinted seed and <0.031-0.210 ppm (ave. = 0.084 ppm) in/on gin byproducts.

Soybean

Twenty field rotational crop trials on soybeans were conducted at field sites throughout the US during 2000. At each of the test sites, bare soil was treated once with amicarbazone (70% WDG) at 0.44-0.47 lb ai/A using ground equipment (~1x rate). A rotational crop of soybeans was

planted 22-57 DAT at 19 of the 20 test sites. Due to phytotoxicity, soybeans from one test site were replanted the following season (366-day PBI). Duplicate treated samples and a single control sample of soybean forage, hay, and seed were harvested at intervals reflecting normal agricultural practices at each site. Hay samples were allowed to dry in the field for 1-12 days prior to sampling.

Samples were stored frozen from collection to analysis for up to 26 months, an interval that is not supported by the available storage stability data. Samples were analyzed for residues of amicarbazone and its metabolites DA amicarbazone and iPr-2-OH DA amicarbazone using an adequate LC/MS/MS method (Bayer Method 200458), with residues being reported in parent equivalents. The LOQ was 0.01 ppm each analyte in/on all matrices, and the LOD for each analyte in each matrix ranged from 0.001-0.004 ppm.

For soybeans planted ~1 month post-treatment (proposed PBI), residues of amicarbazone were <0.010-0.430 ppm in/on soybean forage, 0.015-1.326 ppm in/on soybean hay, and <0.01 (<LOQ) in/on soybean seed. Residues of DA amicarbazone were <0.01-0.089 ppm in/on forage, <0.01-0.270 ppm in/on hay, and <0.01 ppm in/on seed samples. Residues of iPr-2-OH DA amicarbazone were 0.143-1.132 ppm in/on forage, 0.562-4.236 ppm in/on hay, and 0.031-0.582 ppm in/on seed. Combined residues were <0.268-1.264 ppm (ave. = 0.750 ppm) in/on soybean forage, <0.629-4.569 ppm (ave. = 2.569 ppm) in/on hay, and <0.051-<0.602 ppm (ave. = 0.225 ppm) in/on seed.

Wheat

Twenty field rotational crop trials on winter wheat were conducted at field sites throughout the US during 2000-2001. At each of the test sites, bare soil was treated once with amicarbazone (70% WDG) at 0.400-0.458 lb ai/A (~1x rate) using ground equipment. A rotational crop of wheat was planted 115-130 days after treatment (~4-month PBI). Duplicate treated samples and a single control sample of wheat forage, hay, grain, and straw were harvested at intervals reflecting normal agricultural practices at each site. Hay samples were allowed to dry in the field for 2-19 days prior to sampling.

Samples were stored frozen from collection to analysis for up to 19 months, an interval that is not supported by the available storage stability data. Samples were analyzed for residues of amicarbazone and its metabolites DA amicarbazone and iPr-2-OH DA amicarbazone using an adequate LC/MS/MS method (Bayer Method 200458), with residues being reported in parent equivalents. The LOQ was 0.01 ppm each analyte in/on all wheat matrices, and the LOD for each analyte in each matrix ranged from 0.001-0.005 ppm.

Residues of amicarbazone were <0.010-0.249 ppm in/on 40 wheat forage samples, <0.010-0.566 ppm in/on 40 wheat hay samples, and <0.010 (all <LOQ) in/on 40 wheat grain samples, and <0.010-0.280 ppm in/on 40 wheat straw samples. Residues of DA amicarbazone were <0.01-0.184 ppm in/on forage, <0.01-0.029 ppm in/on hay, <0.01 ppm in/on grain, and <0.01-0.045 ppm in/on straw. Residues of iPr-2-OH DA amicarbazone were <0.10-0.166 ppm in/on forage, <0.027-0.308 ppm in/on hay, <0.010-0.0.075 ppm in/on grain, and <0.01-0.236 ppm in/on straw

Combined residues were <0.040-0.485 ppm (ave. = 0.136 ppm) in/on wheat forage, <0.030-0.878 ppm (ave. = 0.159 ppm) in/on hay, <0.030-<0.095 ppm (ave. = 0.033 ppm) in/on grain, and <0.030-0.450 ppm (ave. = 0.109 ppm) in/on straw.

Conclusions. Pending submission of the required storage stability data and information on weather and soil characteristics in each study, the submitted residue data from the extended field rotational crop studies are adequate.

Based on the data from the extended field rotational crop trials, the proposed 1-month PBI for soybeans and 4-month PBI for alfalfa and wheat are supported by the available residue data. However, the labeled PBI for cotton should be amended to 12 months, which is supported by the available cotton field rotational crop data. For each rotational crop, an adequate number of tests were conducted in the appropriate geographic regions.

At the 1-month PBI, maximum combined amicarbazone residues were 1.264 ppm in/on soybean forage, 4.569 ppm in/on soybean hay, and <0.602 ppm in/on soybean seed. At the 4-month PBI, maximum combined amicarbazone residues were <0.037 and <0.074 ppm in/on alfalfa forage and hay, 0.485 ppm in/on wheat forage, 0.878 ppm in/on wheat hay, <0.095 ppm in/on wheat grain, and 0.450 ppm in/on wheat straw. At the 12-month PBI, maximum combined amicarbazone residues were <0.053 and 0.210 ppm in/on cotton undelinted seed and gin byproducts. The recommended rotational crop tolerances are summarized in Table 7.

In conjunction with the rotational soybean and wheat field trials and processing studies, the petitioner has also requested waivers for residue data on AGF generated from soybean seed and wheat grain. The petitioner's basis for these requests is that there is no direct exposure of seed/grain to the herbicide during the later stages of growth.

With regard to soybeans, residue data for AGF are not required as the available soybean processing study indicates that residues are unlikely to concentrate in the outer seed coat; the processing factor for soybean hulls was 0.9x. For wheat, while residues in hulls were higher (0.062 ppm) than wheat grain (0.044 ppm), residues also distributed in flour (0.049 ppm), shorts (0.059 ppm), middlings (0.047 ppm), and germ (0.040 ppm). In view of sample variability and uncertainty in method analysis, residue data on AGF derived from wheat grain grown as a rotational crop are not required.

860.1550 Proposed Tolerances

The petitioner has proposed the establishment of permanent tolerances for residues of the herbicide amicarbazone [4-amino-4,5-dihydro-N-(1,1-dimethylethyl)-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide] and its metabolites DA amicarbazone [N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide] and iPr-2-OH DA amicarbazone [N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide] in/on corn and livestock commodities.

Because the proposed enforcement methods calculate residues of all analytes as parent equivalents, the proposed tolerance expression should be revised to be expressed in terms of:

> the combined residues of the herbicide amicarbazone [4-amino-N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide] and its metabolites DA amicarbazone [N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide] and iPr-2-OH DA amicarbazone [N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide], calculated as parent equivalents

There are currently no established Codex, Canadian, or Mexican MRLs for amicarbazone. An International Residue Limit Status sheet is attached to this review.

Pending submission of adequate storage stability data, the proposed tolerance levels for field corn grain and forage are adequate. Because the dry matter content of stover samples from the crop field trials were lower than the level specified in the GNL 860.1000 for residue analysis, the residue values for these samples are likely to be lower than expected. The proposed tolerance for stover should be adjusted by a factor of 2x to account for this variability.

The petitioner has proposed tolerances for milk and the meat and meat byproducts of cattle, sheep, goats, horses, and hogs. The available cattle feeding study data indicate that tolerances are required for milk and the fat, liver, meat, and meat byproducts (except liver) of cattle, goats, horses, sheep, and hogs. The petitioner should propose tolerances for the liver of cattle, goats, horses, sheep, and hogs, as that the proposed meat byproducts tolerances may then be reduced [the proposed tolerances for the meat byproducts of cattle, goats, horses, sheep and hogs should be reclassified as "meat byproducts, except liver"]. In addition, tolerances for the fat of cattle, goats, horses, sheep, and hogs should be proposed.

With regards to inadvertent tolerances on rotational crop commodities, the proposed tolerances for alfalfa forage, cotton gin byproducts, and wheat hay are adequate, pending submission of the requested storage stability data and field trial information. However, the available residue data indicate that higher tolerances are required for alfalfa hay, undelinted cottonseed, soybean seed, and wheat grain and straw, and lower tolerances are needed for soybean forage and hay and wheat forage. The available processing studies on rotational crops indicate that tolerances are not required for cottonseed meal, oil and hulls, soybean meal, soybean oil and hulls, and wheat flour, middlings and germ. However, based on concentrations in wheat bran and shorts a separate tolerance should be established on wheat grain milled byproducts, which will cover residues in these two commodities.

The sugarcane processing study indicates that amicarbazone residues concentrate in sugarcane molasses (6.9x) and bagasse (3.8x). Bagasse is not imported into the U.S. and is no longer a regulated feed item; therefore, a tolerance is not required on this commodity. However, a separate tolerance will be required for sugarcane molasses. As field trial residue data on

sugarcane have not been submitted, a decision regarding tolerances for sugarcane and molasses cannot be made at this time.

The proposed tolerances should be revised to reflect the correct commodity definitions as specified in Table 7.

Table 7. Tolerance Summary for Amicarbazone.

Commodity	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments (*correct commodity definition*)
Primary Crop and Livestock Tolerances			
Corn, grain	0.05	0.05	*Corn, field, grain*
Corn, forage	0.8	0.80	*Corn, field, forage*
Corn, stover	0.5	1.0	*Corn, field, stover*
Sugarcane	0.15	Additional data required	The available processing study indicates that amicarbazone residues can concentrate in sugarcane molasses by 6.9x. However, field trial residue data on sugarcane were not available for review. A decision regarding tolerances for sugarcane, and sugarcane, molasses can not be made at this time.
Sugarcane, molasses	0.80		
Meat (cattle, sheep, goats, horses, hogs)	0.01	0.01	*Cattle, meat*
		0.01	*Goat, meat*
		0.01	*Horse, meat*
		0.01	*Sheep, meat*
		0.01	*Hog, meat*
Meat byproducts (cattle, sheep, goats, horses, hogs)	0.2	0.10	*Cattle, meat byproducts, except liver*
		0.10	*Goat, meat byproducts, except liver*
		0.10	*Horse, meat byproducts, except liver*
		0.10	*Sheep, meat byproducts, except liver*
		0.01	*Hog, meat byproducts, except liver*
Milk	0.01	0.01	
Cattle, fat	Not proposed	0.01	
Goat, fat	Not proposed	0.01	
Horse, fat	Not proposed	0.01	
Sheep, fat	Not proposed	0.01	
Hog, fat	Not proposed	0.01	
Cattle, liver	Not proposed	1.0	
Goat, liver	Not proposed	1.0	
Horse, liver	Not proposed	1.0	
Sheep, liver	Not proposed	1.0	
Hog, liver	Not proposed	0.10	
Poultry, liver	Not proposed	0.01	

Table 7. Tolerance Summary for Amicarbazone.

Commodity	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments (*correct commodity definition*)
Rotational Crop Tolerances			
Alfalfa, forage	0.04	0.05	The available residue data are adequate.
Alfalfa, hay	0.06	0.10	The available residue data indicate that the proposed tolerance is too low. Maximum residues from the field trials were 0.074 ppm in/on alfalfa hay.
Cotton, undelinted seed	0.04	0.07	The available residue data indicate that the proposed tolerance is too low. Maximum residues from the field trials were 0.053 ppm in/on undelinted cottonseed.
Cotton, gin by-product	0.2	0.30	The available residue data are adequate *Cotton, gin byproducts*
Cottonseed, meal	0.01	None	The available residue data indicate that tolerances for processed cotton matrices will not be required.
Cottonseed, refined oil	0.01		
Cottonseed, hulls	0.01		
Soybean, forage	2.5	1.50	The available residue data indicate that the proposed tolerance is too high. Maximum residues from the field trials were 1.26 ppm in/on soybean forage.
Soybean, hay	7.0	5.0	The available residue data indicate that the proposed tolerance is too high. Maximum residues from the field trials were 4.57 ppm in/on soybean hay.
Soybean, seed	0.6	0.80	The available residue data are adequate.
Soybean, meal	0.25	None	Residues concentrated in soybean meal by a factor of 1.3x. Based on HAFT residues of 0.589 ppm for soybean seed, the maximum expected residues in meal would be 0.766 ppm. As this value is within the recommended tolerance for seed, a separate tolerance is not needed for soybean, meal
Soybean, hulls	0.2	None	Residues did not concentrate in soybean hulls (0.9x) and oil (<0.1x) Therefore, tolerances are not required.
Soybean, oil	0.01		
Wheat, forage	0.6	0.50	The available residue data indicate that the proposed tolerance is too high. Maximum residues from the field trials were 0.485 ppm in/on wheat forage.
Wheat, hay	0.9	1.0	The available residue data are adequate.

Table 7. Tolerance Summary for Amicarbazone.

Commodity	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments (*correct commodity definition*)
Wheat, grain	0.09	0.10	The available residue data indicate that the proposed tolerance is too low. Maximum residues from the field trials were 0.095 ppm in/on wheat grain.
Wheat, straw	0.4	0.50	The available residue data indicate that the proposed tolerance is too low. Maximum residues from the field trials were 0.450 ppm in/on wheat straw
Wheat, bran	0.08	0.15	Residues concentrated by factors of 1.3x and 1.4x in wheat shorts and bran . Based on HAFT residues for wheat grain of 0.09 ppm, the maximum expected residues would be 0.126 ppm in bran and 0.117 ppm in shorts. Therefore, a separate tolerance should be established at 0.15 ppm for *wheat, grain, milled byproducts*, which would cover residue in both commodities
Wheat, shorts	0.06		
Wheat, flour	0.05	None	As residues did not concentrate substantially ($\leq$1.1x)in wheat flour, middlings and germ, tolerances are not required for these commodities.
Wheat, middlings	0.05		
Wheat, germs	0.05		

Attachments:
International Residue Limit Status sheet
Appendix 1 - Chemical Name and Structure Table

Template Version September 2003

INTERNATIONAL RESIDUE LIMIT STATUS

Chemical Name: 4-amino-4,5-dihydro-*N*-(1,1-dimethylethyl)-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	Common Name: Amicarbazone	☐ Proposed tolerance ☐ Reevaluated tolerance X Other (US EPA recommended tolerances)	Date: 04/07/2005

Codex Status (Maximum Residue Limits)	U. S. Tolerances
X No Codex proposal step 6 or above ☐ No Codex proposal step 6 or above for the crops requested	Petition Number: PP#0F6131 DP Barcodes: D288216 and D309766 Other Identifier: Decision Numbers 303867 and 303868
Residue definition (step 8/CXL): N/A	Reviewer/Branch: Manying Xue/RAB3
	Residue definition: Amicarbazone and its metabolites DA amicarbazone [*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide] and *i*Pr-2-OH DA amicarbazone [*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide]

Crop (s)	MRL (mg/kg)	Crop(s)	Tolerance (ppm)
		Corn, grain	0.05
		Corn, forage	0.8
		Corn, stover	1.0
		Fat (cattle, goats, horses, sheep hogs)	0.1
		Meat (cattle, goats, horses, sheep, hogs)	0.01
		Meat byproducts (cattle, goats, horses, sheep, hogs)	0.1
		Liver (cattle, goats, horses, sheep)	1.0
		Meat byproducts (hogs)	0.01
		Liver (hogs)	0.10
		Milk	0.01
		Liver (poultry)	0.0
		Alfalfa, forage	0.05
		Alfalfa, hay	0.10
		Cotton, undelinted seed	0.07
		Cotton, gin by-product	0.3
		Soybean, forage	1.50
		Soybean, hay	5.0
		Soybean, seed	0.80
		Wheat, forage	0.5
		Wheat, hay	1.0

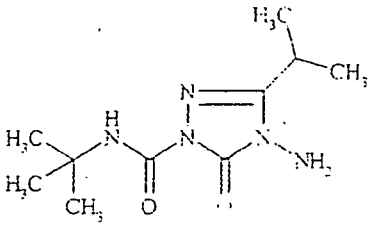
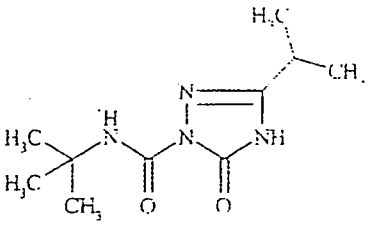
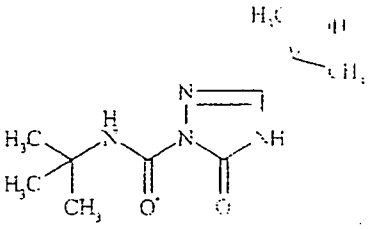
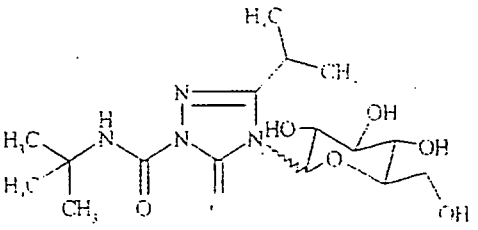
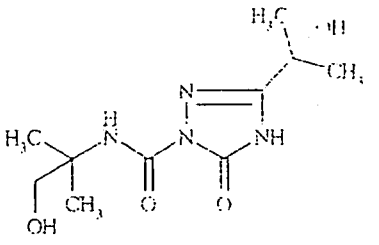
		Wheat, grain	0.1
		Wheat, straw	0.5
		Wheat, grain, milled byproducts	0.15

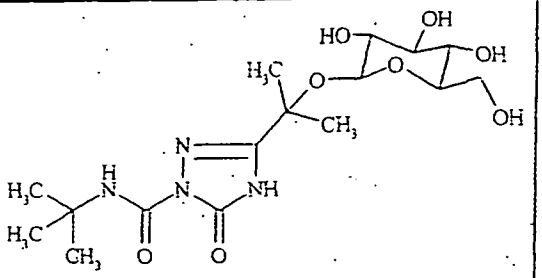
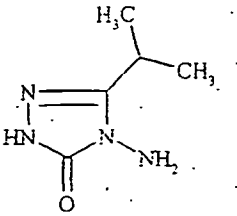
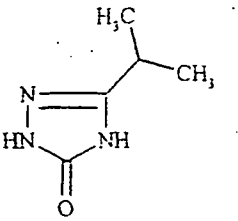
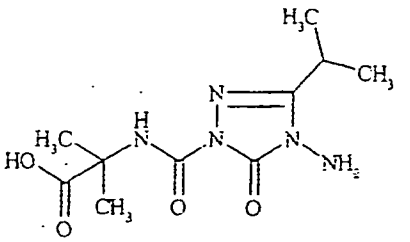
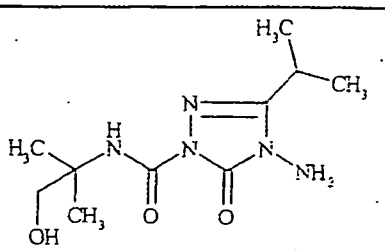
Limits for Canada	Limits for Mexico
X No Limits ☐ No Limits for the crops requested	X No Limits ☐ No Limits for the crops requested
Residue definition: N/A	Residue definition: N/A

Crop(s)	MRL (mg/kg)	Crop(s)	MRL (mg/kg)

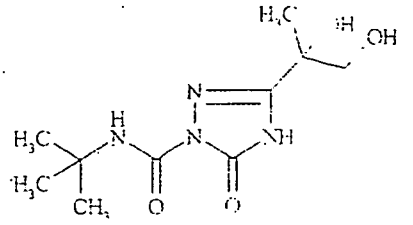
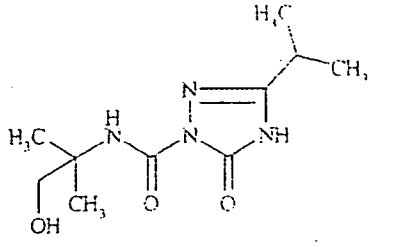
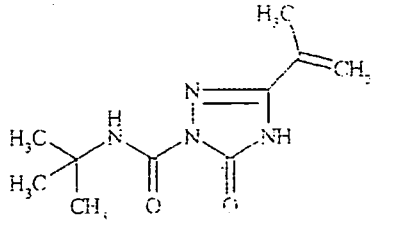
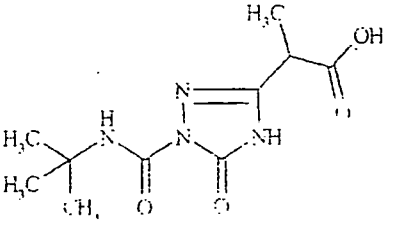
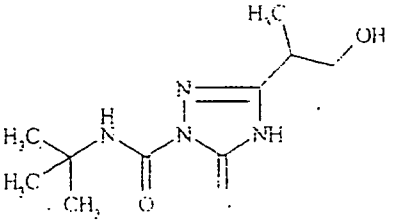
Notes/Special Instructions:S.Funk, 09/02/04.

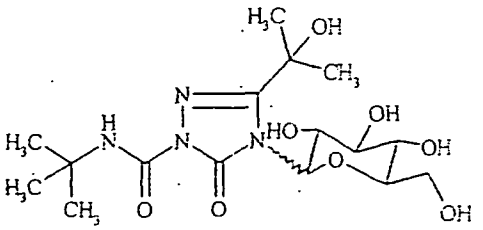
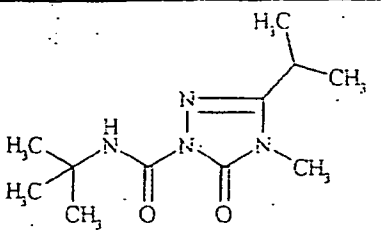
Rev. 1998

Appendix 1. Chemical Name and Structure of Amicarbazone and its Transformation Products.		
Company Name	Chemical Name	Structure
Amicarbazone; MKH 3586	4-amino-N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide	
DA amicarbazone; DA MKH 3586	N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide	
iPr-2-OH DA amicarbazone; iPr-2-OH DA MKH 3586	N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide	
4-N-Glucosyl-DA MKH 3586	N-(1,1-dimethylethyl)-4,5-dihydro-4-N-glucosyl-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide	
tBu-iPr-2-diOH DA MKH 3586	4,5-dihydro--N-(2-hydroxy-1,1-dimethyl)-3-(1-hydroxy-1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide	

Appendix 1. Chemical Name and Structure of Amicarbazone and its Transformation Products.		
Company Name	Chemical Name	Structure
iPr-2-O-Glucosyl-DA MKH 3586; DA OH Glu I MKH 3586	3-[1-Methyl-1-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-ethyl]-5-oxo-4,5-dihydro-[1,2,4]triazole-1-carboxylic acid tert-butylamide	
Triazolinone MKH 3586	4-amino-2,4-dihydro-5-(1-methylethyl)-3H-1,2,4-triazol-3-one	
Triazolinone DA MKH 3586; DCA DA MKH 3586	1,2-dihydro-5-(1-methylethyl)-3H-1,2,4-triazol-3-one	
tBu-Acid MKH 3586	N-[[4-amino-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-yl]carbonyl]-2-methylalanine	
tBu-OH MKH 3586	4-amino-4,5-dihydro-N-(2-hydroxy-1,1-dimethylethyl)-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide	

Appendix 1. Chemical Name and Structure of Amicarbazone and its Transformation Products.

Company Name	Chemical Name	Structure
iPr-1,2-diOH DA MKH 3586	3-(1,2-dihydroxy-1-methylethyl)-N-(1,1-dimethylethyl)-4,5-dihydro-5-oxo-1H-1,2,4-triazole-1-carboxamide	
tBu-OH DA MKH 3586	4,5-dihydro-N-(2-hydroxy-1,1-dimethylethyl)-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide	
iPr-Ene DA MKH 3586	N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethenyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide	
iPr-Acid DA MKH 3586	2-(1-tert-butylcarbamoyl-5-oxo-4,5-dihydro-1H-[1,2,4]triazol-3-yl)propionic acid	
iPr-1-OH DA MKH 3586	3-(2-hydroxy-1-methylethyl)-5-oxo-4,5-dihydro-[1,2,4]triazole-1-carboxylic acid tert-butylamide	

Appendix 1. Chemical Name and Structure of Amicarbazone and its Transformation Products.		
Company Name	Chemical Name	Structure
DA OH Glu II MKH 3586	3-(1-hydroxy-1-methyl-ethyl)-5-oxo-4-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yl)-4,5-dihydro-[1,2,4]triazole-1-carboxylic acid tert-butylamide [1]	
N-Me DA MKH 3586	N-(1,1-dimethylethyl)-4,5-dihydro-4-methyl-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazol-1-carboxamide [1]	

[1] Chemical name generated using naming software of ISIS/Draw.

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.4.4/OPPTS 860.1900/OECD IIA 6.6.3, 6.8.7 and IIIA 8.6
Limited Field Accumulation in Rotational Crops - Mustard Greens, Turnips, and Wheat

Primary Evaluator	Manying Xue, Chemist RAB3/HED (7509C)	Date: 03/23/05
Approved by	Leung Cheng, Senior Chemist RAB3/HED (7509C)	Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (1910 Sedwick Rd., Suite B; Durham, NC 27713; submitted 1/07/2005). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46145301 Krolski, M. (2003) MKH 3586 70 WG - Magnitude of the Residues in Rotational Crops. Lab Project Number: 109560: TMN-0258, TMN-0284, TMN-0287. Unpublished study prepared by Bayer CropScience. 456 p.

EXECUTIVE SUMMARY:

A series of limited field rotational crop trials were conducted at field sites in GA, IN, and KS during 1997-1998. At each of the test sites, bare soil was treated once with amicarbazone (70% WDG) at 0.42-0.46 lb ai/A using ground equipment. The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. At each site, representative rotational crops of mustard greens, wheat, and turnips were planted ~1, 4, 8, and 10 months after treatment (PBI). Two of the wheat plantings in KS (8-month and 10-month PBI) failed due to adverse weather conditions, and one planting each of mustard greens (1-month PBI) and turnips (1-month PBI) failed due to phytotoxicity. For the remaining plantings, duplicate treated samples and a single control sample of mustard green leaves, turnip tops and roots, and wheat forage, hay, grain, and straw were harvested at intervals reflecting normal agricultural practices at each site. Samples were harvested 42-143 days after planting (DAP) for mustard greens and turnips, 51-251 DAP for wheat forage, 67-259 DAP for wheat hay, and 95-280 DAP for wheat grain and straw. Samples were stored frozen for a maximum of 27 months, an interval that is not supported by the available storage stability data.

Samples were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 using a LC/MS/MS method (Bayer Method 108340) that has been proposed as the enforcement method for plant commodities. Briefly, samples were extracted with either water or 0.05% aqueous phosphoric acid, concentrated and cleaned up by solid phase extraction. Residues were quantitated by LC/MS/MS, using deuterated internal reference standards of amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586. Residues of metabolites were reported as parent equivalents. The validated LOQ is 0.01 ppm each analyte in/on all matrices. The limits of detection (LODs) were calculated to be 0.001 ppm

for wheat straw, 0.002 ppm for turnip tops, wheat forage, and wheat grain, 0.005 ppm for mustard greens and wheat hay, and 0.008 ppm for turnip roots.

Quantifiable residues were observed in all rotated crops, except turnip roots. Residues of all analytes were <0.01 ppm in/on turnip roots at all PBIs. At the 1-month PBI, maximum residues of amicarbazone were 0.063 ppm in/on mustard greens, 0.037 ppm in/on turnip tops, 0.088 ppm in/on wheat forage, 0.235 ppm in/on wheat hay, <0.01 ppm in/on wheat grain, and 0.205 ppm in/on wheat straw. Residues of DA MKH 3586 were <0.01 ppm in/on all samples from all matrices, except two turnip tops samples with residues of 0.020 and 0.026 ppm and two wheat straw samples each with residues of 0.022 ppm. Maximum residues of *i*Pr-2OH DA MKH 3586 were 0.028 ppm in/on mustard greens, 0.083 ppm in/on turnip tops, 0.113 ppm in/on wheat forage, 0.170 ppm in/on hay, 0.049 ppm in/on grain, and 0.260 ppm in/on straw. Maximum combined residues of amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 (expressed as parent equivalents), were <0.095 ppm in/on mustard greens, 0.120 ppm in/on turnip tops, <0.211 ppm in/on wheat forage, <0.397 ppm in/on hay, <0.069 ppm in/on grain, and 0.485 ppm in/on straw.

At the 4-month PBI, maximum residues of amicarbazone were 0.011 ppm in/on mustard greens, 0.019 ppm in/on turnip tops, <0.01 ppm in/on wheat forage, 0.018 ppm in/on wheat hay, <0.01 ppm in/on wheat grain, and 0.013 ppm in/on wheat straw. Residues of DA MKH 3586 were <0.01 ppm in/on all samples from all matrices, except one turnip tops sample with residues of 0.014 ppm. Maximum residues of *i*Pr-2OH DA MKH 3586 were 0.025 ppm in/on mustard greens, 0.035 ppm in/on turnip tops, 0.080 ppm in/on wheat forage, 0.104 ppm in/on hay, 0.026 ppm in/on grain, and 0.163 ppm in/on straw. Maximum combined residues were <0.045 ppm in/on mustard greens, <0.059 ppm in/on turnip tops, <0.100 ppm in/on wheat forage, <0.130 ppm in/on hay, <0.046 ppm in/on grain, and <0.186 ppm in/on straw.

At the 8-month PBI, maximum residues of amicarbazone were 0.016 ppm in/on mustard greens, 0.028 ppm in/on turnip tops, 0.015 ppm in/on wheat forage, 0.028 ppm in/on wheat hay, <0.01 ppm in/on wheat grain and straw. Residues of DA MKH 3586 were <0.01 ppm in/on all samples from all matrices, except three mustard greens samples with residues of 0.010-0.016 ppm and two turnip tops samples with residues of 0.018 and 0.026 ppm. Maximum residues of *i*Pr-2OH DA MKH 3586 were 0.142 ppm in/on mustard greens, 0.183 ppm in/on turnip tops, 0.050 ppm in/on wheat forage, 0.093 ppm in/on hay, 0.024 ppm in/on grain, and 0.056 ppm in/on straw. Maximum combined residues were 0.174 ppm in/on mustard greens, 0.230 ppm in/on turnip tops, <0.073 ppm in/on wheat forage, <0.131 ppm in/on hay, <0.044 ppm in/on grain, and <0.076 ppm in/on straw.

At the 10-month PBI, maximum residues of amicarbazone were <0.01 ppm in/on all mustard greens, turnip tops, turnip roots, wheat forage, and wheat straw, 0.014 ppm in/on wheat hay and 0.010 ppm in/on wheat grain. Residues of DA MKH 3586 were <0.01 ppm in/on all samples from all matrices. Maximum residues of *i*Pr-2OH DA MKH 3586 were 0.024 ppm in/on mustard greens, 0.025 ppm in/on turnip tops, 0.041 ppm in/on wheat forage, 0.097 ppm in/on hay, 0.014 ppm in/on grain, and 0.025 ppm in/on straw. Maximum combined residues were

<0.044 ppm in/on mustard greens, <0.045 ppm in/on turnip tops, <0.055 ppm in/on wheat forage, <0.121 ppm in/on hay, <0.034 ppm in/on grain, and <0.045 ppm in/on straw.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in this study, these limited field rotational crop data are classified as scientifically acceptable, pending submission of adequate supporting storage stability data. The limited rotational crop studies indicate that extended rotational crop field studies will be required. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document DP Barcode D309766.

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

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. There are currently no permanent tolerances established for amicarbazone. The original petitioner was Bayer Corporation; however, Bayer Corporation has since sold amicarbazone to Arvesta Corporation, which is now the petitioner for amicarbazone. In conjunction with the subject tolerance petition, Arvesta has submitted an application for Section 3 registration of a 70% dry flowable (DF) formulation (Amicarbazone DF Herbicide; EPA File Symbol No. 66330-UA). The 70% DF formulation is to be applied to corn as preplant, preemergence, or early postemergence broadcast sprays at 0.22-0.45 lb ai/A for preplant/preemergence application, or 0.10-0.15 lb ai/A for postemergence application. The petitioner has submitted limited field rotational crop trial data on mustard greens, turnips and wheat.

The nomenclature and physicochemical properties of amicarbazone are presented below in Tables A.1 and A.2.

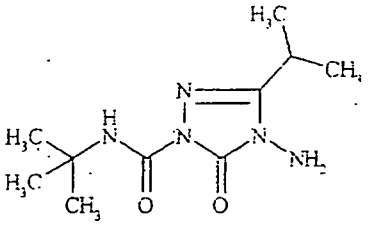
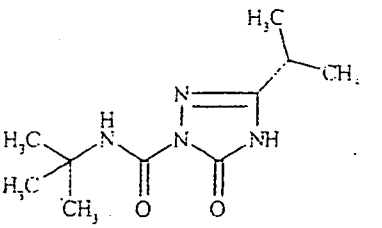
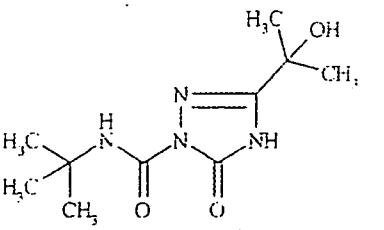
TABLE A.1. Amicarbazone Nomenclature.	
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-*tert*-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS registry number	129909-90-6
End-use formulation (EP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)
DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
*i*Pr-2-OH DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-*H*-1,2,4-triazole-1-carboxamide

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.

Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00 x 10^{-4} Pa @ 25°C 1.30 x 10^{-4} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, Log(K_{ow})	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

A series of limited field rotational crop trials were conducted at field sites in GA, IN, and KS during 1997-1998. At each of the test sites, bare soil was treated once with amicarbazone (70% WDG) at 0.42-0.46 lb ai/A using ground equipment (~1x maximum rate). At each site, rotational crops of mustard greens, wheat, and turnips were planted ~1, 4, 8, and 10 months after treatment. At each test site, separate plots were treated for each representative rotational crop at each PBI.

TABLE B.1.1. Trial Site Conditions.

Trial Identification (City, State, Year)	Soil characteristics				Meteorological data	
	Type	%OM	pH	CEC (meq/g)	Total rainfall during study period (inches)[2]	Overall monthly temperature range (°C)
Mustard Greens						
Tifton, GA, 1997	NR = not reported	NR	NR	NR	20-47	NR
Oxford, IN, 1997	NR	NR	NR	NR	24-45	NR
Stilwell, KS, 1997	Silt Loam or Silty Clay Loam	3.3	6.3	NR	6-32	NR
Turnips						
Tifton, GA, 1997	NR	NR	NR	NR	31-69	NR
Oxford, IN, 1997	NR	NR	NR	NR	21-45	NR
Stilwell, KS, 1997	Silt Loam or Silty Clay Loam	3.3	6.3	NR	19-44	NR
Wheat						
Tifton, GA, 1997	Gravelly Sandy Loam	1.1	5.9	NR	41-89	NR
Oxford, IN, 1997	NR	NR	NR	NR	49-58	NR
Stilwell, KS, 1997	Silt Loam	1.5	6.0	NR	29-41	NR

[1] Detailed meteorological data were not provided
[2] Range of rainfall covers differences between the various PBIs.

Detailed meteorological data were not provided. Two wheat tests in KS (8-month and 10-month PBI) failed due to adverse weather conditions, and one planting each of mustard greens (1-month PBI) and turnip tests (1-month PBI) failed due to phytotoxicity from the test substance. No other unusual conditions that would affect the integrity of the study were reported. Rainfall was supplemented with irrigation as needed.

TABLE B.1.2. Study Use Pattern.

Location (County, State) Year	EP[1]	Application				Tank Mix Adjuvants
		Method; Timing	Vol. (GPA[2])	Single Rate (lb ai/A)[3]	No. of Appl.	
Mustard Greens						
Tifton, GA, 1997/1998	70% WDG	broadcast; bare ground	11.2-12.5	0.45	1	None
Oxford, IN, 1997	70% WDG	broadcast; bare ground	14.5-14.7	0.44-0.45	1	None
Stilwell, KS, 1997	70% WDG	broadcast; bare ground	9.9-11.3	0.43-0.45	1	None

TABLE B.1.2. Study Use Pattern.

Location (County, State) Year	EP[1]	Application				Tank Mix Adjuvants
		Method; Timing	Vol. (GPA[2])	Single Rate (lb ai/A)[3]	No. of Appl.	
Turnips						
Tifton, GA, 1997/1998	70% WDG	broadcast; bare ground	11.2-12.5	0.45	1	None
Oxford, IN, 1997	70% WDG	broadcast; bare ground	14.7	0.45	1	None
Stilwell, KS, 1997	70% WDG	broadcast; bare ground	9.7-11.2	0.42-0.45	1	None
Wheat						
Tifton, GA, 1997/1998	70% WDG	broadcast; bare ground	10.8-12.5	0.45	1	None
Oxford, IN, 1997	70% WDG	broadcast; bare ground	14.6-14.7	0.45	1	None
Stilwell, KS, 1997	70% WDG	broadcast; bare ground	10.0-10.4	0.45-0.46	1	None

1 EP = End-use Product
2 All applications were made using ground equipment.
3 Range of application rates covers the various PBIs.

Duplicate treated samples and a single control sample of mustard green leaves (>5 lbs each), turnip tops and roots (>5 lbs each), and wheat forage, hay, grain (>2.5 lbs each), and straw (>1.5 lbs each) were harvested at intervals reflecting normal agricultural practices at each site. Planting to harvest intervals (DAP) were 42-143 DAP for mustard greens and turnips, 51-251 DAP for wheat forage, 67-259 DAP for wheat hay, and 95-280 DAP for wheat grain and straw. After collection, plant samples were placed in frozen storage at the test facility, stored frozen for 0-45 days, then shipped frozen by ACDS freezer truck to the analytical laboratory, Bayer Research Park (BRP), Stilwell, KS and stored frozen (<-15° C) prior to analysis. Samples were stored frozen from collection to analysis for up to 27 months.

B.2. Analytical Methodology

Samples of mustard greens, turnip, and wheat matrices were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 using the LC/MS/MS method proposed as the enforcement method for plant commodities (Bayer Method 108340). Only a brief description of the method was included in the submission. For a complete description of the method, refer to the DER for MRID 45121708, reviewed in 45121702.der.

Briefly, homogenized samples were extracted with either water or 0.05% aqueous phosphoric acid using an accelerated solvent extractor. The extract, following centrifugation, was cleaned up by solid phase extraction (C_{18}). Residues were analyzed and quantitated by LC/MS/MS, using deuterated internal reference standards of amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586. Residues of metabolites were reported as parent equivalents. The validated LOQ was 0.01 ppm each for amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 in/on all matrices. The limits of detection (LODs) were calculated to be

0.001 ppm for wheat straw, 0.002 ppm for turnip tops, wheat forage, and wheat grain, 0.005 ppm for mustard greens and wheat hay, and 0.008 ppm for turnip roots (three times the standard deviation of the average control response).

In the current submission, the LC/MS/MS method was validated using concurrent method recoveries of the various RAC samples fortified separately with amicarbazone, DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 at 0.01-0.30 ppm.

C. RESULTS AND DISCUSSION

The total frozen (< -15° C) storage intervals were 7-27 months for mustard greens, wheat, and turnip samples (Table C.1). In support of the field rotational crop trials, the petitioner cited storage stability data (45121704.der) submitted in conjunction with a confined rotational crop study. In that review, it was also stated that LC/MS analyses of some metabolites were conducted up to 30 months following sample collection; however, the petitioner noted that these analyses were not quantitative. These data are insufficient to support the current field rotational crop study because quantitative data were not included in MRID 45121704.

TABLE C.1. Summary of Storage Conditions

Matrix	Storage Temp. (°C)	Actual Storage Duration (months)	Limit of Demonstrated Storage Stability (months) [1]
Mustard greens	<-15	7-27	NA
Turnip tops and roots		7-27	
Wheat forage, hay, grain and straw		10-21	

[1] NA = not available. Adequate storage stability data are not available for frozen plant commodities.

The LC/MS/MS method (Bayer Method 108340) used to determine residues of amicarbazone and it two metabolites in/on mustard greens, turnip, and wheat matrices is adequate for data collection. Average concurrent recoveries from all matrices were 77-104% with standard deviations of ±2-16% (Table C.2). Apparent residues of amicarbazone were <LOQ in/on all control samples. The LOQ was 0.01 ppm each for amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 in/on all matrices. The limits of detection (LODs) were calculated to be 0.001 ppm for wheat straw, 0.002 ppm for turnip tops, wheat forage, and wheat grain, 0.005 ppm for mustard greens and wheat hay, and 0.008 ppm for turnip roots. Adequate sample calculations and chromatograms were provided.

TABLE C.2. Summary of Concurrent Recoveries of Amicarbazone, DA MKH 3586, and *i*Pr-2-OH DA MKH 3586 from Turnip, Wheat, and Mustard Greens Matrices.

Matrix	Analyte	Spike level (mg/kg)	Sample size (n)	Recoveries (%)	Mean ± std dev (%)
Mustard greens	Amicarbazone	0.01	5	90-102	94 ± 4
		0.20	3	92	
	DA MKH 3586	0.01	5	92-114	96 ± 10

TABLE C.2. Summary of Concurrent Recoveries of Amicarbazone, DA MKH 3586, and *i*Pr-2-OH DA MKH 3586 from Turnip, Wheat, and Mustard Greens Matrices.

Matrix	Analyte	Spike level (mg/kg)	Sample size (n)	Recoveries (%)	Mean ± std dev (%)
		0.20	3	83-89	
	*i*Pr-2-OH DA MKH 3586	0.01	5	89-113	95 ± 10
		0.20	3	84-92	
Turnip tops	Amicarbazone	0.01	5	79-111	98 ± 10
		0.20	3	95-108	
	DA MKH 3586	0.01	5	85-113	96 ± 11
		0.20	3	85-94	
	*i*Pr-2-OH DA MKH 3586	0.01	5	97-114	101 ± 9
		0.20	3	88-98	
Turnip roots	Amicarbazone	0.01	4	98-103	100 ± 2
	DA MKH 3586	0.01	4	98-117	104 ± 9
	*i*Pr-2-OH DA MKH 3586	0.01	4	90-108	99 ± 9
Wheat forage	Amicarbazone	0.01	4	70-90	77 ± 7
		0.25	3	74-81	
	DA MKH 3586	0.01	4	78-90	86 ± 5
		0.25	3	83-91	
	*i*Pr-2-OH DA MKH 3586	0.01	4	74-104	93 ± 10
		0.25	3	86-95	
Wheat hay	Amicarbazone	0.01	5	71-86	78 ± 5
		0.25	3	75-82	
	DA MKH 3586	0.01	5	89-116	96 ± 10
		0.25	3	88-95	
	*i*Pr-2-OH DA MKH 3586	0.01	5	75-117	97 ± 16
		0.25	3	99-104	
Wheat grain	Amicarbazone	0.01	6	74-96	82 ± 9
		0.05	3	72-86	
	DA MKH 3586	0.01	6	83-117	99 ± 10
		0.05	3	96-103	
	*i*Pr-2-OH DA MKH 3586	0.01	6	88-112	101 ± 8
		0.05	3	88-107	
Wheat straw	Amicarbazone	0.01	5	80-97	91 ± 7
		0.30	3	90-96	
	DA MKH 3586	0.01	5	85-101	94 ± 5
		0.30	3	92-96	
	*i*Pr-2-OH DA MKH 3586	0.01	5	86-110	101 ± 10
		0.30	3	92-112	

At the 1-month PBI, residues of amicarbazone were <0.01-0.063 ppm in/on 4 mustard greens samples, <0.01-0.037 ppm in/on 4 turnip tops samples, <0.01 ppm in/on all 4 turnip root

samples, <0.01-0.088 ppm in/on 6 wheat forage samples, <0.01-0.235 ppm in/on 6 wheat hay samples, <0.01 ppm in/on 6 wheat grain samples, and <0.01-0.205 ppm in/on 6 wheat straw samples (Table C.3). Residues of DA MKH 3586 were <0.01 ppm in/on all samples from all matrices, except two turnip tops samples with residues of 0.020 and 0.026 ppm and two wheat straw samples each with residues of 0.022 ppm. Residues of *i*Pr-2OH DA MKH 3586 were 0.015-0.028 ppm in/on mustard greens, <0.01-0.083 ppm in/on turnip tops, <0.01 in/on all turnip roots, 0.030-0.113 ppm in/on wheat forage, 0.038-0.170 ppm in/on hay, 0.023-0.049 ppm in/on grain, and 0.025-0.260 ppm in/on straw. Combined residues of amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 (expressed as parent equivalents) were <0.048-<0.095 ppm (ave. = 0.064 ppm) in/on mustard greens, <0.030-0.120 ppm (ave. = 0.079 ppm) in/on turnip tops, <0.030 ppm in/on all turnip roots, <0.057-<0.211 ppm (ave. = 0.111 ppm) in/on wheat forage, <0.070-<0.397 ppm (ave. = 0.183 ppm) in/on hay, <0.043-<0.069 ppm (ave. = 0.051 ppm) in/on grain, and <0.045-0.485 ppm (ave. = 0.184 ppm) in/on straw (Table C.4).

At the 4-month PBI, residues of amicarbazone were <0.01-0.011 ppm in/on mustard greens, <0.01-0.019 ppm in/on turnip tops, <0.01 ppm in/on turnip roots, <0.01 ppm in/on wheat forage, <0.01-0.018 ppm in/on wheat hay, <0.01 ppm in/on wheat grain, and <0.01-0.013 ppm in/on wheat straw (Table C.3). Residues of DA MKH 3586 were <0.01 ppm in/on all samples from all matrices, except one turnip tops sample with residues of 0.014 ppm. Residues of *i*Pr-2OH DA MKH 3586 were 0.011-0.025 ppm in/on mustard greens, 0.011-0.035 ppm in/on turnip tops, <0.01 in/on all turnip roots, 0.025-0.080 ppm in/on wheat forage, 0.031-0.104 ppm in/on hay, 0.013-0.026 ppm in/on grain, and 0.015-0.163 ppm in/on straw. Combined residues were <0.031-<0.045 ppm (ave. = 0.038 ppm) in/on mustard greens, <0.031-<0.059 ppm (ave. = 0.045 ppm) in/on turnip tops, <0.030 ppm in/on turnip roots, <0.045-<0.100 ppm (ave. = 0.062 ppm) in/on wheat forage, <0.051-<0.130 ppm (ave. = 0.082 ppm) in/on hay, <0.033-<0.046 ppm (ave = 0.039 ppm) in/on grain, and <0.035-<0.186 ppm (ave. = 0.091 ppm) in/on straw (Table C.4).

At the 8-month PBI, residues of amicarbazone were <0.01-0.016 ppm in/on mustard greens, <0.01-0.028 ppm in/on turnip tops, <0.01 ppm in/on turnip roots, <0.01-0.015 ppm in/on wheat forage, <0.01-0.028 ppm in/on wheat hay, <0.01 ppm in/on wheat grain, and <0.01 ppm in/on wheat straw (Table C.3). Residues of DA MKH 3586 were <0.01 ppm in/on all samples from all matrices, except three mustard greens samples with residues of 0.010-0.016 ppm and two turnip tops samples with residues of 0.018 and 0.026 ppm. Residues of *i*Pr-2OH DA MKH 3586 were 0.028-0.142 ppm in/on mustard greens, 0.029-0.183 ppm in/on turnip tops, <0.01 in/on all turnip roots, 0.032-0.050 ppm in/on wheat forage, 0.051-0.093 ppm in/on hay, 0.012-0.024 ppm in/on grain, and 0.035-0.056 ppm in/on straw. Combined residues were <0.049-0.174 ppm (ave. = 0.092 ppm) in/on mustard greens, <0.061-0.230 ppm (ave. = 0.108 ppm) in/on turnip tops, <0.030 ppm in/on turnip roots, <0.052-<0.073 ppm (ave. = 0.063 ppm) in/on wheat forage, <0.071-<0.131 ppm (ave. = 0.101 ppm) in/on hay, <0.032-<0.044 ppm (ave. = 0.039 ppm) in/on grain, and <0.055-<0.076 ppm (ave. = 0.065 ppm) in/on straw (Table C.4)

At the 10-month PBI, residues of amicarbazone were <0.01 ppm in/on all mustard greens, turnip tops, turnip roots, wheat forage, and wheat straw, and <0.01-0.014 ppm in/on wheat hay and <0.01-0.010 ppm in/on wheat grain (Table C.3). Residues of DA MKH 3586 were <0.01

ppm in/on all samples from all matrices. Residues of *i*Pr-2OH DA MKH 3586 were 0.011-0.024 ppm in/on mustard greens, 0.018-0.025 ppm in/on turnip tops, <0.01 in/on all turnip roots, <0.01-0.041 ppm in/on wheat forage, 0.021-0.097 ppm in/on hay, <0.01-0.014 ppm in/on grain, and 0.013-0.025 ppm in/on straw. Combined residues were <0.031-<0.044 ppm (ave. = 0.040 ppm) in/on mustard greens, <0.038-<0.045 ppm (ave. = 0.041 ppm) in/on turnip tops, <0.030 ppm in/on turnip roots, <0.030-<0.055 ppm (ave. = 0.045 ppm) in/on wheat forage, <0.041-<0.121 ppm (ave. = 0.079 ppm) in/on hay, <0.030-<0.034 ppm (ave. = 0.032 ppm) in/on grain, and <0.033-<0.045 ppm (ave. = 0.041 ppm) in/on straw (Table C.4).

Residues of parent and the metabolite *i*Pr-2OH DA MKH 3586 were significantly higher than metabolite DA MKH 3586. Also, residues were higher, in some cases nearly 10x higher, in/on samples from the Tifton GA site. No explanation for this variation was provided by the petitioner.

Common cultural practices were used to maintain plants, and the weather conditions and the maintenance chemicals and fertilizer used in the study did not have a notable impact on the residue data.

TABLE C.3. **Residues of Amicarbazone in Rotational Crops Following a Single Application of a 70% WDG.**

Location (County, State, Year)	EPA Region	Variety	Total Rate (lb a.i./A)	PBI [1] (days)	Harvest Interval (DAP) [2]	Matrix	Residues (ppm) [3]			
							Amicarbazone	DA MKH 3586	i-Pr-2-OH MKH 3586	Total
Mustard Greens										
Tifton, GA, 1997	2	Florida	0.45	28	112	Leaves	0.036, 0.063	ND[4], (0.006)[4]	0.015, 0.022	<0.061, <0.095
Stilwell, KS, 997	5	Bloomsdale	0.45	39	83	Leaves	ND, ND	ND, ND	0.028, 0.031	<0.048, <0.051
Tifton, GA, 1997	2	Florida	0.45	120	112	Leaves	(0.008), 0.011	ND, ND	0.013, 0.020	<0.033, <0.041
Oxford, IN, 1997	5	Tendergreen	0.44	125	51	Leaves	ND, ND	ND, ND	0.011, 0.025	<0.031, <0.045
Stilwell, KS, 1997	5	Curly	0.43	119	42	Leaves	ND, ND	ND, ND	0.015, 0.020	<0.035, <0.040
Tifton, GA, 1998	2	Florida	0.45	241	73	Leaves	0.014, 0.016	0.016, 0.016	0.119, 0.142	0.150, 0.174
Oxford, IN, 1997	5	Tendergreen	0.44	262	51	Leaves	0.012, 0.011	(0.006), ND	0.035, 0.028	<0.057, <0.049
Stilwell, KS, 1997	5	Curly	0.45	249	64	Leaves	(0.006), (0.005)	0.010, (0.009)	0.041, 0.042	<0.061, <0.062
Tifton, GA, 1997	2	Florida	0.45	300	143	Leaves	ND, ND	ND, ND	0.011, 0.017	<0.031, <0.037
Oxford, IN, 1997	5	Tendergreen	0.45	319	51	Leaves	ND, ND	(0.005), ND	0.021, 0.023	<0.041, <0.043
Stilwell, KS, 1997	5	Curly	0.45	308	64	Leaves	ND, ND	ND, ND	0.021, 0.024	<0.041, <0.044

TABLE C.3. **Residues of Amicarbazone in Rotational Crops Following a Single Application of a 70% WDG.**

Location (County, State, Year)	EPA Region	Variety	Total Rate (lb a.i./A)	PBI [1] (days)	Harvest Interval (DAP) [2]	Matrix	Residues (ppm) [3]			
							Amicarbazone	DA MKH 3586	i-Pr-2-OH MKH 3586	Total
Turnips										
Tifton, GA, 1997	2	Purple Top	0.45	28	112	Tops	0.010, 0.037	ND, (0.005)	(0.005), 0.014	<0.030, 0.061
						Roots	ND, ND	ND, ND	ND, ND	<0.030, <0.030
Stilwell, KS, 1997	5	Purple Top	0.45	39	84	Tops	0.011, (0.009)	0.026, 0.020	0.083, 0.075	0.120, 0.105
						Roots	ND, ND	ND, ND	ND, ND	<0.030, <0.030
Tifton, GA, 1997	2	Purple Top	0.45	120	112	Tops	0.014, 0.019	(0.005, 0.007)	0.020, 0.026	<0.044, <0.055
						Roots	ND, ND	ND, ND	ND, ND	<0.030, <0.030
Oxford, IN, 1997	5	Purple Top	0.45	125	69	Tops	(0.004, 0.006)	ND, (0.006)	0.011, 0.019	<0.031, <0.039
						Roots	ND, ND	ND, ND	ND, ND	<0.030, <0.030
Stilwell, KS, 1997	5	Purple Top	0.42	119	42	Tops	(0.006, 0.006)	(0.005), 0.014	0.024, 0.035	<0.042, 0.056
						Roots	ND, ND	ND, ND	ND, ND	<0.030, <0.030
Tifton, GA, 1998	2	Purple Top	0.45	253	61	Tops	0.022, 0.017	0.026, 0.018	0.183, 0.124	0.230, 0.159
						Roots	ND, ND	ND, ND	ND, ND	<0.030, <0.030
Oxford, IN, 1997	5	Purple Top	0.45	262	62	Tops	0.022, 0.028	(0.006, 0.008)	0.029, 0.028	<0.051, <0.067
						Roots	ND, ND	ND, ND	ND, ND	<0.030, <0.030
Stilwell, KS, 1997	5	Purple Top	0.45	249	79	Tops	(0.005, 0.006)	(0.007, 0.007)	0.043, 0.046	<0.063, <0.066
						Roots	ND, ND	ND, ND	ND, ND	<0.030, <0.030
Tifton, GA, 1997	2	Purple Top	0.45	300	143	Tops	(0.003, 0.003)	(0.002), ND	0.018, 0.025	<0.038, <0.045
						Roots	ND, ND	ND, ND	ND, ND	<0.030, <0.030
Oxford, IN, 1997	5	Purple Top	0.45	381	62	Tops	(0.009, 0.008)	(0.006, 0.006)	0.021, 0.018	0.041, 0.038
						Roots	ND, ND	ND, ND	ND, ND	<0.030, <0.030
Stilwell, KS, 1997	5	Purple Top	0.45	308	79	Tops	(0.002, 0.003)	(0.005, 0.003)	0.022, 0.022	<0.042, <0.042
						Roots	ND, ND	ND, ND	ND, ND	<0.030, <0.030
Wheat										
Tifton, GA, 1997	2	Pioneer	0.45	29	119	Forage	0.074, 0.088	ND, (0.002)	0.099, 0.113	<0.183, <0.211
					139	Hay	0.235, 0.217	ND, ND	0.142, 0.170	<0.387, <0.397
					172	Grain	ND, ND	ND, ND	0.035, 0.049	<0.055, <0.06
						Straw	0.144, 0.205	0.022, 0.022	0.260, 0.258	0.425, 0.485
Oxford, IN, 1997	5	Stewart	0.45	34	251	Forage	0.028, 0.038	ND, ND	0.030, 0.034	<0.068, <0.082
					259	Hay	0.027, 0.022	ND, ND	0.038, 0.038	<0.075, <0.070
					280	Grain	ND, ND	ND, ND	0.025, 0.023	<0.045, <0.043
						Straw	(0.009, 0.007)	(0.003, 0.003)	0.030, 0.025	<0.050, <0.045
Stilwell, KS, 1997	5	Karl 92	0.45	31	214	Forage	(0.003, 0.003)	ND, ND	0.037, 0.043	<0.057, <0.063
					232	Hay	ND, ND	ND, (0.006)	0.057, 0.072	<0.077, <0.092
					270	Grain	ND, ND	ND, ND	0.026, 0.028	<0.046, 0.048
						Straw	(0.002, 0.001)	(0.002, 0.002)	0.030, 0.026	<0.050, <0.046

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.4.4/OPPTS 860.1900/OECD IIA 6.6.3, 6.8.7 and IIIA 8.6
Limited Field Accumulation in Rotational Crops - Mustard Greens, Turnips, and Wheat

TABLE C.3. Residues of Amicarbazone in Rotational Crops Following a Single Application of a 70% WDG.

Location (County, State, Year)	EPA Region	Variety	Total Rate (lb a.i./A)	PBI [1] (days)	Harvest Interval (DAP) [2]	Matrix	Residues (ppm) [3]			
							Amicarbazone	DA MKH 3586	i-Pr-2-OH MKH 3586	Total
Tifton, GA, 1997	2	Pioneer	0.45	122	119	Forage	(0.008, 0.007)	ND, ND	0.080, 0.064	<0.100, <0.084
					139	Hay	0.016, 0.018	ND, ND	0.104, 0.097	<0.130, <0.125
					172	Grain	(0.006), ND	(0.003), ND	0.026, 0.023	<0.046, <0.043
						Straw	0.013, (0.010)	(0.003, 0.002)	0.163, 0.154	<0.186, <0.174
Oxford, IN, 1997	5	Stewart	0.45	122	251	Forage	(0.005, 0.005)	ND, ND	0.028, 0.025	<0.048, <0.045
					259	Hay	ND, ND	ND, ND	0.037, 0.031	<0.057, <0.051
					280	Grain	(0.004, 0.004)	ND, ND	0.013, 0.016	<0.033, <0.036
						Straw	(0.003, 0.003)	(0.003, 0.002)	0.039, 0.029	<0.059, <0.049
Stilwell, KS, 1997	5	Karl 92	0.46	123	207	Forage	ND, ND	ND, ND	0.026, 0.027	<0.046, <0.047
					225	Hay	ND, ND	ND, ND	0.046, 0.041	<0.066, <0.061
					263	Grain	ND, ND	ND, ND	0.015, 0.021	<0.035, <0.041
						Straw	ND, ND	(0.001, 0.002)	0.015, 0.020	<0.035, <0.040
Tifton, GA, 1998	2	Pioneer	0.45	237	127	Forage	ND, (0.002)	ND, ND	0.034, 0.032	<0.054, <0.052
					165	Hay	ND, ND	ND, ND	0.055, 0.051	<0.075, <0.071
					189	Grain	ND, ND	ND, ND	0.012, 0.014	<0.032, <0.034
						Straw	(0.002, 0.001)	(0.003, 0.002)	0.049, 0.056	<0.069, <0.076
Oxford, IN, 1997	5	Penawana	0.45	262	51	Forage	0.015, 0.013	ND, ND	0.048, 0.050	<0.073, <0.073
					67	Hay	0.022, 0.028	ND, ND	0.093, 0.093	<0.125, <0.131
					95	Grain	(0.006, 0.004)	ND, ND	0.024, 0.024	<0.044, <0.044
						Straw	(0.005, 0.004)	(0.006, 0.006)	0.041, 0.035	<0.061, <0.055
Tifton, GA, 1998	2	Pioneer	0.45	297	127	Forage	ND, ND	ND, ND	0.022, (0.009)	<0.042, <0.030
					165	Hay	ND, ND	ND, ND	0.021, 0.043	<0.041, <0.063
					189	Grain	ND, *ND*, ND	ND, *ND*, ND	ND, *ND*, (0.006)	<0.030, <0.030
						Straw	(0.002, 0.003)	(0.003), ND	0.020, 0.013	<0.040, <0.033
Oxford, IN, 1997	5	Penawana	0.45	319	51	Forage	(0.007, 0.004)	ND, ND	0.032, 0.035	<0.052, <0.055
					67	Hay	0.014, 0.014	ND, ND	0.065, 0.097	<0.089, <0.121
					95	Grain	0.010, (0.007)	ND, ND	0.014, 0.014	<0.034, <0.034
						Straw	(0.003, 0.003)	(0.007, 0.006)	0.025, 0.025	<0.045, <0.045

[1] PBI = Plant Back Interval
[2] DAP = Days After Planting.
[3] The LOQ is 0.01 ppm for each analyte in/on each matrix; the LOD was 0.001 ppm for wheat straw, 0.002 ppm for turnip tops, wheat forage, and wheat grain, 0.005 ppm for mustard greens and wheat hay, and 0.008 ppm for turnip roots. Total residues are the sum of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 residues in parent equivalents; the LOQ was used for individual residues reported at <LOQ in calculating total residues.
[4] ND = not detected; residues were <LOD. Residues <LOQ but >LOD are reported in parentheses. Values in italics are from repeat analysis of the sample.

TABLE C.4. Summary of Residue Data in Representative Rotational Crops Following Primary Treatment of Bare Soil with Amicarbazone (70% WDG).

Commodity	Total Rate (lb ai/A)	PBI (days)	Total Residue Levels (ppm) [1]						
			n	Min.	Max.	HAFT[2]	Median (STMdR[3])	Mean (STMR[3])	Std. Dev
Mustard Greens	0.43-0.45	28-39	4	<0.048	<0.095	<0.078	0.056	0.064	0.022
		119-125	6	<0.031	<0.045	<0.038	0.038	0.038	0.005
		241-262	6	<0.049	0.174	0.162	0.062	0.092	0.055
		300-319	6	<0.031	<0.044	<0.042	0.041	0.040	0.005
Turnip Tops	0.42-0.45	28-39	4	<0.030	0.120	<0.113	0.083	0.079	0.041
		119-125	6	<0.031	<0.059	<0.051	0.043	0.045	0.010
		249-262	6	<0.061	0.230	0.195	0.067	0.108	0.071
		300-381	6	<0.038	<0.045	<0.042	0.042	0.041	0.003
Turnip Roots		28-39	4	<0.030	<0.030	<0.030	<0.030	<0.030	NA[4]
		119-125	6	<0.030	<0.030	<0.030	<0.030	<0.030	NA
		249-262	6	<0.030	<0.030	<0.030	<0.030	<0.030	NA
		300-381	6	<0.030	<0.030	<0.030	<0.030	<0.030	NA
Wheat Forage	0.45-0.46	29-34	6	<0.057	<0.211	<0.197	0.075	0.111	0.068
		122-123	6	<0.045	<0.100	<0.092	0.048	0.062	0.024
		237-262	4	<0.052	<0.073	<0.073	0.064	0.063	0.012
		297-319	4	<0.030	<0.055	<0.054	0.047	0.045	0.011
Wheat Hay		29-34	6	<0.070	<0.397	<0.392	0.085	0.183	0.162
		122-123	6	<0.051	<0.130	<0.128	0.064	0.082	0.036
		237-262	4	<0.071	<0.131	<0.128	0.100	0.101	0.032
		297-319	4	<0.041	<0.121	<0.105	0.076	0.079	0.034
Wheat Grain		29-34	6	<0.043	<0.069	<0.062	0.047	0.051	0.010
		122-123	6	<0.033	<0.046	<0.045	0.039	0.039	0.005
		237-262	4	<0.032	<0.044	<0.044	0.039	0.039	0.006
		297-319	4	<0.030	<0.034	<0.034	0.032	0.032	0.002
Wheat Straw		29-34	6	<0.045	0.485	0.455	0.050	0.184	0.211
		122-123	6	<0.035	<0.186	<0.180	0.054	0.091	0.070
		237-262	4	<0.055	<0.076	<0.073	0.065	0.065	0.009
		297-319	4	<0.033	<0.045	<0.045	0.043	0.041	0.006

[1] The LOQ is 0.01 ppm for each analyte in/on each matrix; the LOD were 0.001 ppm for wheat straw, 0.002 ppm for turnip tops, wheat forage, and wheat grain, 0.005 ppm for mustard greens and wheat hay, and 0.008 ppm for turnip roots. Total residues are the sum of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 residues in parent equivalents; the LOQ was used for individual residues reported at <LOQ in calculating total residues.

[2] HAFT = Highest Average Field Trial.

[3] STMdR = Supervised Trial Median Residue; STMR = Supervised Trial Mean Residue.

[4] NA=Not Applicable.

D. CONCLUSION

Pending submission of adequate supporting storage stability data, the limited field rotational crop data are adequate. As quantifiable residues were observed in all rotated crops, the data indicate that extended rotational crop field studies will be required.

E. REFERENCES

None

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: 0F6131
DP Barcode: D309766
PC Code: 114004

Template Version September 2003

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.2.1, 7.2.2, 7.2.3 and 7.4/OPPTS 860.1340/OECD IIA 4.2.5; 4.2.6 and 4.3
Residue Analytical Method - Various Plant Commodities

Primary Evaluator	Manying Xue, Chemist RAB3/HED (7509C)	Date: 05/31/05
Approved by	Leung Cheng, Senior Chemist RAB3/HED (7509C)	Date: 05/31/05

This DER was originally prepared under contract by Dynamac Corporation (1910 Sedwick Rd., Building 100, Suite B; Durham, NC 27713; submitted 1/07/2005). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46145302 Moore, S. & Harbin, A. (2003) An Analytical Method for the Determination of MKH 3586, DA MKH 3586, and iPr-2OH DA MKH 3586 Residues in Various Plant Matrices by LC-MS/MS. Lab Project Number: 200258: TMN-226. Unpublished study prepared by Bayer CropScience. 185 p. {OPPTS 860.1340}

EXECUTIVE SUMMARY:

In conjunction with the field rotational crop trials and processing studies, the petitioner submitted method validation data for a newer HPLC/MS/MS method (Bayer Method 200258) for determining residues of amicarbazone, DA MKH 3586, and *i*Pr-2-OH DA MKH 3586 in/on various plant commodities. This method is essentially a revised version of the earlier proposed enforcement method (Bayer Method 108340). The new method utilizes a simpler extraction procedure and the same extraction solvents that were used in the corn metabolism study, which has been shown to adequately recover all three residues of concern.

For Method 200258, residues are extracted from most plant matrices (except oil matrices) with 0.1% aqueous acetic acid in acetonitrile (ACN):water (4:1, v/v). Ground samples are initially soaked for 30 minutes in the extraction solvent and then homogenized. Celite is added and residues are filtered. A mixture of the deuterated internal standards are added at this point. For oil matrices, residues are extracted by mixing with hexane and then adding an equal volume of 0.1% aqueous acetic acid in ACN:water (4:1, v/v). Residue in the lower ACN phase are saved and the deuterated internal standards are added. For cleanup of all samples, residues are then eluted through a Bond Elut Certify II cartridge (mixed mode C_8 and SCX SPE) using 0.1% aqueous acetic acid in ACN:water (4:1, v/v). Residues are concentrated, diluted with a small amount of methanol, and then brought to the final volume with aqueous 5 mM ammonium bicarbonate. Residues are analyzed by LC/MS/MS and are quantified using the deuterated internal reference standards, monitoring the transition of parent ions to its daughter ions for each analyte.

The validated LOQ is 0.01 ppm for amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 in/on all matrices. Depending on the plant matrix, the LOD is 0.0011-0.0097 ppm for amicarbazone, 0.0013-0.0087 ppm for DA MKH 3586, and 0.0017-0.0058 ppm for *i*Pr-2-OH DA MKH 3586.

In a method validation trial using control samples of mustard and turnip leaves and wheat forage, hay, straw and grain fortified with each analyte at 0.01 and 0.10 ppm, average recoveries for amicarbazone and both metabolites ranged from 87-113%, with standard deviations of ±1-14% In addition, in a series of extensive rotational crop field trials and processing studies, average procedural recoveries for each analyte were 89-114% with standard deviations of ±1-13% from alfalfa, cotton, soybean, sugarcane, and wheat RACs and processed commodities. Apparent residues of each analyte were <0.01 ppm in/on all control samples.

The HPLC/MS/MS method (Bayer Method 200258) is adequate for collecting data on residues of amicarbazone, DA MKH 3586, and *i*Pr-2-OH DA MKH 3586 in/on various crop matrices.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in this study, the method validation data are classified as scientifically acceptable. The new method (Method 200258) may serve as an alternate enforcement method, provided that supporting independent laboratory validation (ILV) and radiolabeled extraction efficiency data be submitted to demonstrate method performance (ACB memo, 5/27/05, A. Kamel).

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D309766].

COMPLIANCE:

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations were noted that would impact the acceptability the study results or their interpretation

A. BACKGROUND INFORMATION

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis. There are currently no permanent tolerances established for amicarbazone. The original petitioner was Bayer Corporation; however, Bayer Corporation has since sold amicarbazone to Arvesta Corporation, who is now the petitioner for amicarbazone. In conjunction with the subject tolerance petition, Arvesta has submitted an application for Section 3 registration of a 70% dry flowable (DF) formulation (Amicarbazone DF Herbicide; EPA File Symbol No. 66330-UA).

The nomenclature and physicochemical properties of amicarbazone are presented below in Tables A.1 and A.2.

TABLE A.1. Amicarbazone Nomenclature.

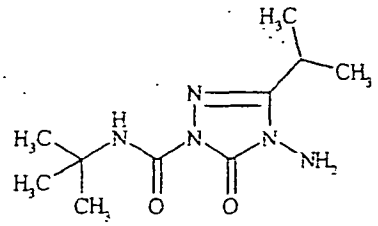
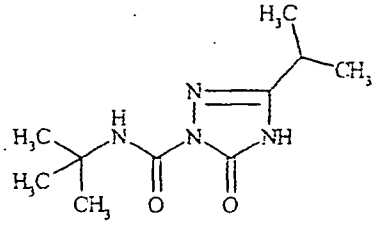
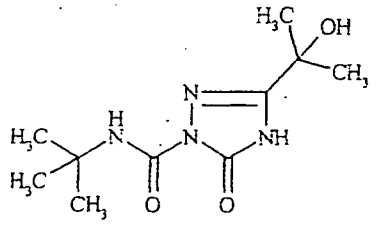
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-N-tert-butyl-4,5-dihydro-3-isopropyl-5-oxo-1H-1,2,4-triazole-1-carboxamide
CAS name	4-amino-N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide
CAS registry number	129909-90-6
End-use formulation (EP)	70% DF
DA MKH 3586 (metabolite proposed for regulation)	 N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide
*i*Pr-2-OH DA MKH 3586 (metabolite proposed for regulation)	 N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.

Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C.	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-6} Pa @ 25°C 1.30×10^{-6} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, Log(K_{ow})	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. MATERIALS AND METHODS

B.1. Data-Gathering Method (Bayer Method 200258)

The petitioner submitted method validation data for a new LC/MS/MS method (Bayer Method 200258) that is similar to the earlier proposed tolerance enforcement method (Bayer Method 108340). This revised method is reported to be more rapid than the earlier method and yet still maintains the same level of sensitivity. The principal differences between the two methods are in the initial extraction and cleanup procedures. The new method extracts samples by homogenization in 0.1% aqueous acetic acid in ACN:water (4:1, v/v) rather than using an accelerated solvent extractor with aqueous phosphoric acid. The extraction solvent used in the new method is also the same extraction solvent used in the earlier corn metabolism study. In addition, the new method uses a different type of SPE cartridge (mixed mode C_8 and SCX) for cleanup than the earlier method (C_{18}). The newer method was used to determine residues in the extensive field rotational crop trials on alfalfa, cotton, soybeans, wheat, and the processing studies on cotton, soybeans, wheat, and sugarcane.

B.1.1. Principle of the Method:

For most plant matrices (except oil matrices), residues are extracted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v). Ground samples are initially soaked for 30 minutes in the extraction solvent and then homogenized. Celite is added and residues are filtered. A mixture of the deuterated standards of each analyte are added at this point to serve as internal standards. For

oil matrices, residues are extracted by mixing with hexane and then adding an equal volume of 0.1% aqueous acetic acid in ACN:water (4:1, v/v). Residue in the lower ACN phase are saved and the deuterated internal standards are added. For cleanup of all samples, residues are then eluted through a Bond Elut Certify II cartridge (mixed mode C_8 and SCX SPE) using 0.1% aqueous acetic acid in ACN:water (4:1, v/v). Residues are concentrated, diluted with a small amount of methanol, and then brought to the final volume with aqueous 5 mM ammonium bicarbonate. Residues are analyzed by LC/MS/MS using electrospray ionization. Residues are quantified using deuterated internal reference standards by monitoring the following transition of parent ions to its daughter ion for each analyte:

amicarbazone: m/z 242 to m/z 143
[$^2H_9$]amicarbazone: m/z 251 to m/z 143
DA MKH 3586: m/z 227 to m/z 128
[$^2H_9$]DA MKH 3586: m/z 236 to m/z 128
*i*Pr-2-OH DA MKH 3586: m/z 243 to m/z 144
[$^2H_6$]*i*Pr-2-OH DA MKH 3586: m/z 249 to m/z 150.

The validated LOQ is 0.01 ppm for amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 in/on all matrices. Depending on the plant matrix, the LOD is 0.0011-0.0097 ppm for amicarbazone, 0.0013-0.0087 ppm for DA MKH 3586, and 0.0017-0.0058 ppm for *i*Pr-2-OH DA MKH 3586.

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

Method ID	Bayer Method 200258
Analytes	Amicarbazone, DA MKH 3586, and *i*Pr-2-OH DA MKH 3586
Extraction solvent/technique	Most plant matrices: 0.1% aqueous acetic acid in ACN:water (4:1, v/v) Oil matrices: equal volumes of hexane and 0.1% aqueous acetic acid in ACN:water (4:1, v/v)
Cleanup strategies	Mixed C_8 and SCX SPE cartridge eluted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v)
Instrument/Detector	HPLC with Waters X-terra RPC-18 column (150 mm x 4.6 mm, 5 µm film thickness) and an MS/MS detector equipped with an electrospray interface operating in the positive ion mode. Residues are quantified by monitoring the transition of parent to its daughter ion for each analyte: amicarbazone (m/z 242 to 143) [$^2H_9$]amicarbazone (m/z 251 to 143) DA MKH 3586 (m/z 227 to 128) [$^2H_9$]DA MKH 3586 (m/z 236 to 128) *i*Pr-2-OH DA MKH 3586 (m/z 243 to 144) [$^2H_6$]*i*Pr-2-OH DA MKH 3586 (m/z 249 to 150)
Standardization method	Internal standards (Deuterated standards of each analyte)
Stability of std solutions	All neat standards were stored at <-15° C and all primary and secondary standards were stored at <10° C. Storage duration for the std samples and stability data were not reported.
Retention times	Amicarbazone: ~9 minutes; DA MKH 3586: ~8 minutes; *i*Pr-2-OH DA MKH 3586: ~6 minutes

B.1.2. Method Validation

For method validation, three control samples of mustard leaves, turnip tops, and wheat forage, hay, grain, and straw from the limited field rotational crop study were fortified separately with amicarbazone, DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 at 0.01 and 0.10 ppm. Fortified

samples were analyzed for each analyte along with control samples using the procedures described above.

In addition, concurrent method recovery data were provided in conjunction with the extensive rotational crop field trials and processing studies on alfalfa, cotton, soybeans, sugarcane and wheat. In each study, control samples of the various raw and processed plant commodities samples were fortified with amicarbazone, DA MKH 3586, and *i*Pr-2-OH DA MKH 3586 at 0.01-5.0 ppm and analyzed along with treated samples using LC/MS/MS Method 200258.

B.2. Enforcement Method (Bayer Method 108340)

A LC/MS/MS method (Method 108340) has been proposed for the enforcement of tolerances for amicarbazone, DA MKH 3586, and *i*Pr-2-OH DA MKH 3586 residues in/on plant commodities. This method has been validated and found to be adequate for data collection and tolerance enforcement.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method (Bayer Method 200258)

Recoveries of amicarbazone, DA MKH 3586, and *i*Pr-2-OH DA MKH 3586 for all fortification levels from all plant matrices were within the acceptable 70-120% range (Table C.1.1). Average recoveries for amicarbazone ranged from 90-112%, with standard deviations of ±1.7-13.6%. Average recoveries for DA MKH 3586 ranged from 87-113%, with standard deviations of ±1.5-12.5%. Average recoveries for iPr-2-OH DA MKH 3586 ranged from 89-111%, with standard deviations of ±1.0-8.1%.

Field residue studies conducted in 1997 were analyzed for residues of amicarbazone using Method 108340. Representative samples of mustard green leaves, turnip tops, and wheat forage, wheat hay, wheat grain and wheat straw from three 1997 rotational crop studies were re-analyzed using Method 200258. Similar results were obtained (Table C.1.2).

TABLE C.1.1. Recovery Results from Method Validation Trial for HPLC/MS/MS Method (Bayer Method 200258) using Various Plant Matrices.

Matrix	Spiking Level (mg/kg)	Sample size	Amicarbazone		DA MKH 3586		iPr-2-OH DA MKH 3586	
			Recoveries (%)		Recoveries (%)		Recoveries (%)	
			Range	Mean ± std dev	Range	Mean ± std dev	Range	Mean ± std dev
Wheat forage	0.01	3	100-105	103 ± 2.6	103-115	108 ± 6.4	109-115	111 ± 3.5
	0.10	3	104-109	107 ± 2.6	91-94	93 ± 1.7	103-110	107 ± 3.5
Wheat hay	0.01	3	102-112	108 ± 5.5	103-106	104 ± 1.5	103-113	109 ± 5.1
	0.10	3	105-119	112 ± 7.0	98-111	106 ± 6.8	98-104	102 ± 3.2
Wheat grain	0.01	3	99-111	107 ± 6.9	92-102	96 ± 5.3	103-107	105 ± 2.1
	0.10	3	105-119	111 ± 7.1	96-107	100 ± 6.4	104-107	106 ± 1.7
Wheat straw	0.01	3	79-106	92 ± 13.6	73-96	87 ± 12.5	82-96	91 ± 8.1
	0.10	3	84-94	90 ± 5.5	84-92	87 ± 4.4	85-94	89 ± 4.6
Mustard greens	0.01	3	100-106	102 ± 3.2	92-106	101 ± 7.8	98-104	102 ± 3.2
	0.10	3	95-105	100 ± 5.0	97-106	101 ± 4.5	98-100	99 ± 1.0
Turnip tops	0.01	3	106-109	107 ± 1.7	110-117	113 ± 3.5	96-108	103 ± 6.4
	0.10	3	104-117	109 ± 7.2	100-106	104 ± 3.5	102-104	103 ± 1.0

TABLE C.1.2. Comparative Residue Levels in Parts per Million using HPLC/MS/MS Methods (Bayer Method 108340 and 200258) with Various Plant Matrices (BASF Report No. 109560).

Matrix	Sample	Method	Amicarbazone	DA MKH 3586	iPr-2-OH-DA MKH 3586	Total[1]
Wheat forage	105298	108340	0.07394	0.00109	0.09852	0.174
		200258	0.09648,0.11676	0.00172,0.00152	0.09275,0.10548	0.207
	105299	108340	0.0882	0.00199	0.11305	0.203
		200258	0.12415,0.13995	0.00215,0.00228	0.11135,0.12737	0.254
Wheat hay	105253	108340	0.00405	ND[2]	0.05646	0.0605
		200258	0.00509,0.00450	ND,0.00010	0.07691,0.07283	0.0797
	105254	108340	0.00329	0.00064	0.07238	0.0763
		200258	0.00439,0.00493	0.00032,0.00018	0.06987,0.07690	0.0783
Wheat grain	105304	108340	ND	ND	0.03518	0.0352
		200258	0.00106,0.00015	0.00119,ND	0.05113,0.04880	0.0512
	105305	108340	ND	ND	0.04851	0.0485
		200258	0.00019,0.00020	ND,ND	0.05487,0.05374	0.0545
Wheat straw	105391	108340	0.00342	0.00665	0.02506	0.0351
		200258	0.00516,0.00503	0.00730,0.00666	0.02683,0.02616	0.0386
	105392	108340	0.00269	0.00573	0.02463	0.0331
		200258	0.00544,0.00453	0.00650,0.00686	0.02768,0.02699	0.0390
Mustard greens	104578	108340	0.03551	0.00302	0.01465	0.0532
		200258	0.03467,0.03385	0.00271,0.00243	0.01498,0.01386	0.0513
	104579	108340	0.06321	0.00576	0.02196	0.0909
		200258	0.07396,0.05569	0.00374,0.00377	0.02229,0.02209	0.0908

TABLE C.1.2. Comparative Residue Levels in Parts per Million using HPLC/MS/MS Methods (Bayer Method 108340 and 200258) with Various Plant Matrices (BASF Report No. 109560).

Matrix	Sample	Method	Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total[1]
Turnip tops	105418	108340	0.01021	ND	0.00522	0.0154
		200258	0.00868,0.00957	0.00226,0.00229	0.00535,0.00577	0.0170
	105419	108340	0.03697	0.00515	0.01437	0.0565
		200258	0.04118,0.04261	0.00494,0.00559	0.01741,0.01631	0.0640

[1] Rounded off to 3 significant figures. Denote average values if duplicate samples were analyzed.
ND=not detected; residues were <LOD. LOD was 0.001 ppm for wheat straw, 0.002 ppm for turnip tops, wheat forage, and wheat grain, 0.005 ppm for mustard greens and wheat hay. See DER for MRID 46145302. "ND" in this table was treated as zero when summing the total residues.

In addition, control samples fortified with each analyte were analyzed concurrently with treated and control samples using Method 200258 during the analysis of samples from the extensive rotational crop field trials and processing studies on alfalfa, cotton, soybeans, sugarcane and wheat. Recovery at the method LOQ was evaluated for each analyte in all matrices, and a second higher fortification (0.05-5.0 ppm) was evaluated in most matrices.

Individual recoveries for each analyte from each matrix were adequate, ranging from 75-119% (Table C.1.3). For all three analytes, average procedural recoveries (±S.D.) were 97-100% (±3-6%) from alfalfa forage and hay, 92-105% (±1-13%) from cotton RACs and processed fractions, 90-114% (±1-8%) from sugarcane and its processed fractions, 89-106% (±2-13%) from soybean RACs and processed commodities, and 91-105% (±1-9%) from wheat RACs and processed commodities. Apparent residues of each analyte were <0.01 ppm in/on all control samples.

TABLE C.1.3. Summary of Concurrent Recoveries of Amicarbazone, DA MKH 3586, and *i*Pr-2-OH DA MKH 3586 from Various Plant Matrices using LC/MS/MS Method 200258.

Matrix	Spiking Level (mg/kg)	Sample size	Amicarbazone		DA MKH 3586		*i*Pr-2-OH DA MKH 3586	
			Recoveries (%)		Recoveries (%)		Recoveries (%)	
			Range	Mean ± std dev	Range	Mean ± std dev	Range	Mean ± std dev
46145303.der[1]								
Alfalfa forage	0.01	7	93-108	99 ± 6	93-110	100 ± 6	96-105	99 ± 3
Alfalfa hay	0.01	6	91-106	100 ± 6	90-105	97 ± 5	97-107	99 ± 5
	0.10	3	103-107		91-98		94-99	
46145304.der[1]								
Cotton undelinted seed	0.01	3	100-113	104 ± 5	102-119	104 ± 7	100-105	98 ± 6
	0.10	6	96-106		96-104		86-99	
Cotton gin byproducts	0.01	4	95-108	97 ± 9	81-105	92 ± 8	100-104	99 ± 4
	0.20	3	84-96		93-96		94-98	
46145305.der[1]								
Cotton undelinted seed	0.01	4	95-112	103 ± 7	97-111	104 ± 7	83-100	92 ± 7
Cottonseed meal	0.01	3	96-103	100 ± 4	94-117	102 ± 13	101-107	105 ± 3
Cottonseed refined oil	0.01	3	88-92	90 ± 2	95-109	101 ± 7	87-89	98 ± 1
Cottonseed hulls	0.01	3	103-107	105 ± 2	88-104	96 ± 8	100-106	103 ± 3
46145306.der[1]								
Sugarcane	0.01	3	91-98	96 ± 4	97-100	98 ± 3	94-98	97 ± 3
	0.10	3	96-100		94-101		94-101	
Refined sugar	0.01	3	100-103	102 ± 2	105-118	114 ± 8	98-99	98 ± 1
Blackstrap molasses	0.01	3	99-104	97 ± 5	96-103	96 ± 5	97-100	97 ± 2
	0.70	3	92-95		91-94		93-96	
Bagasse	0.01	3	88-93	93 ± 4	98-101	94 ± 6	89-92	90 ± 2
	0.20	3	92-98		85-91		87-91	
46145307.der[1]								
Soybean forage	0.01	8	87-107	96 ± 5	91-105	98 ± 5	88-112	100 ± 7
	2.0	3	90-98		93-102		94-97	
Soybean hay	0.01	8	93-116	104 ± 10	84-116	98 ± 9	84-107	98 ± 7
	5.0	3	84-115		92-104		92-96	
Soybean seed	0.01	8	86-118	100 ± 11	96-116	99 ± 13	91-105	98 ± 4
	0.60	3	90-109		75-85		93-99	

TABLE C.1.3. Summary of Concurrent Recoveries of Amicarbazone, DA MKH 3586, and *i*Pr-2-OH DA MKH 3586 from Various Plant Matrices using LC/MS/MS Method 200258.

Matrix	Spiking Level (mg/kg)	Sample size	Amicarbazone		DA MKH 3586		*i*Pr-2-OH DA MKH 3586	
			Recoveries (%)		Recoveries (%)		Recoveries (%)	
			Range	Mean ± std dev	Range	Mean ± std dev	Range	Mean ± std dev
46145308.der[1]								
Soybean seed	0.01	3	101-109	106 ± 4	98-108	100 ± 6	86-96	95 ± 5
	0.20	3	100-111		94-99		97-100	
Soybean hulls	0.01	3	80-103	98 ± 9	81-111	93 ± 13	79-104	[illegible]
	0.20	3	97-105		82-94		95-103	
Soybean meal	0.01	3	92-101	101 ± 5	106-110	101 ± 7	97-100	98 ± 2
	0.30	3	100-105		92-96		95-98	
Deodorized oil	0.01	3	94-100	97 ± 3	93-104	97 ± 6	87-93	89 ± 3
46145309.der[1]								
Wheat forage	0.01	8	87-104	97 ± 6	94-112	100 ± 5	93-103	98 ± 4
	0.20	3	96-102		97-101		99-102	
Wheat hay	0.01	9	85-101	95 ± 4	88-103	94 ± 5	89-99	95 ± 3
	0.70	3	92-96		89-96		90-94	
Wheat grain	0.01	8	97-105	101 ± 3	92-104	100 ± 5	92-105	100 ± 4
	0.10	3	100-107		103-105		99-103	
Wheat straw	0.01	8	81-97	91 ± 5	83-104	93 ± 7	86-99	93 ± 4
	0.30	3	90-98		91-92		90-93	
46145310.der[1]								
Wheat grain	0.01	3	99-102	95 ± 7	96-97	97 ± 1	102-105	100 ± 5
	0.05	3	84-93		95-99		93-97	
Wheat bran	0.01	3	95-102	97 ± 4	99-101	99 ± 2	97-100	98 ± 2
	0.10	3	92-101		94-99		95-99	
Wheat flour	0.01	3	103-111	101 ± 9	98-109	97 ± 7	103-108	101 ± 6
	0.10	3	85-110		89-94		94-97	
Wheat shorts	0.01	3	105-113	105 ± 7	95-99	98 ± 2	104-105	99 ± 6
	0.10	3	95-104		96-99		92-96	
Wheat middlings	0.01	3	93-108	100 ± 5	99-105	99 ± 5	97-100	98 ± 1
	0.10	3	99-102		94-99		97-98	

[1] Field rotational crop trial and processed commodity data are reviewed in 46145303.der through 46145310.der currently under review.

TABLE C.1.4. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Amicarbazone, DA MKH 3586, and *i*Pr-2-OH DA MKH 3586 Residues in Plant RAC and Processed Commodities. [1]

Analytes	Amicarbazone, DA MKH 3586, and *i*Pr-2-OH DA MKH 3586
Equipment ID	HPLC with an MS/MS detector equipped with an ion electrospray operating in the positive ion mode. Residues are quantified by monitoring the transition of parent to its daughter ion for each analyte: amicarbazone (m/z 242 to 143) — $[^2H_6]$amicarbazone (m/z 251 to 143) DA MKH 3586 (m/z 227 to 128) — $[^2H_5]$DA MKH 3586 (m/z 236 to 128) *i*Pr-2-OH DA MKH 3586 (m/z 243 to 144) — $[^2H_6]$*i*Pr-2-OH DA MKH 3586 (m/z 249 to 150)
Limit of quantitation (LOQ)	0.01 ppm for each analyte in each matrix.
Limit of detection (LOD)	Matrix — Amicarbazone — DA MKH 3586 — *i*Pr-2-OH DA MKH 3586 Wheat forage — 0.0019 — 0.0047 — 0.0027 Wheat hay — 0.0038 — 0.0013 — 0.0036 Wheat grain — 0.0046 — 0.0037 — 0.0017 Wheat straw — 0.0097 — 0.0087 — 0.0058 Mustard greens — 0.0021 — 0.0052 — 0.0024 Turnip tops — 0.0011 — 0.0026 — 0.0045
Accuracy/Precision	For fortifications at 0.01 and 0.10 ppm, average method recoveries were 89-112% for all three analytes from all matrices, with standard deviations of ±1.0-13.6%.
Linearity	Example standard curves for amicarbazone DA MKH 3586, and *i*Pr-2-OH DA MKH 3586 from all matrices at concentrations from 0.0005-0.100 ppm had coefficients of determination ≥0.9958.
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.

[1] The data for this table are based on data from the method validation trial using control samples of mustard and turnips leaves and wheat forage, hay, straw and grain.

C.2. Enforcement Method

As indicated above, the newer LC/MS/MS Method (Bayer Method 200258) is essentially a revised version of the earlier proposed enforcement method. The new method utilizes a simpler extraction procedure and the same extraction solvent that was used in the corn metabolism study, which has been shown to adequately recover all three residues of concern. Method 200258 may serve as an alternate enforcement method, provided that supporting independent laboratory validation (ILV) and radiolabeled extraction efficiency data be submitted to demonstrate method performance (ACB memo, 5/27/2005, A. Kamel).

D. CONCLUSION

The HPLC/MS/MS Method (Bayer Method 200258) was successfully validated using various plant matrices and is adequate for data collection. The validated LOQ for amicarbazone, DA MKH 3586, and *i*Pr-2-OH DA MKH 3586 is 0.01 ppm in/on all matrices tested.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: 0F6131
DP Barcode: D309766
PC Code: 114004

Template Version September 2003

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.4.4/OPPTS 860.1900/OECD IIA 6.6.3, 6.8.7 and IIIA 8.6
Field Accumulation in Rotational Crops - Alfalfa

Primary Evaluator	Manying Xue, Chemist RAB3/HED (7509C)	Date: 03/23/05
Approved by	Leung Cheng, Senior Chemist RAB3/HED (7509C)	Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (1910 Sedwick Rd., Suite B; Durham, NC 27713; submitted 1/07/2005). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46145303 Krolski, M. (2003) MKH 3586 70 WG - Magnitude of the Residues in the Rotational Crop of Alfalfa. Lab Project Number: 200456: TMN-0246. Unpublished study prepared by Bayer CropScience. 190 p.

EXECUTIVE SUMMARY:

Twelve field rotational crop trials were conducted at field sites throughout the US during 2000-2001. At each of the test sites, bare soil was treated once with amicarbazone (70% WDG) at 0.434-0.454 lb ai/A using ground equipment. The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. At each site, a rotational crop of alfalfa was planted 99-123 days (~4 months) after treatment. Duplicate treated samples and a single control sample of alfalfa forage and hay were harvested at the first cutting of alfalfa, 41-286 days after planting (DAP). Hay samples from three of the tests were allowed to dry in the field for 2-4 days prior to sampling. Samples from the second and third cutting of alfalfa were not collected. Samples were stored frozen from collection to analysis for up to 23 months, an interval that is not supported by the available storage stability data. Data demonstrating the stability of residues of amicarbazone, DA MKH 3586, and iPr-2OH DA MKH 3586 in/on representative plant matrices remain outstanding.

Samples were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 using an adequate LC-MS/MS method (Bayer Method 200258) which is similar to the proposed enforcement method for plant commodities (Bayer Method 108340). Briefly, samples were extracted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v), concentrated and cleaned up by solid phase extraction. Residues were quantified by LC-MS/MS, using deuterated internal standards of each analyte. Residues of metabolites were reported in parent equivalents. The LOQ was 0.01 ppm each for amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on all matrices. The limits of detection (LODs) for amicarbazone were calculated to be 0.002 and 0.004 ppm for amicarbazone in/on forage and hay, 0.002 ppm for DA MKH 3586 in/on alfalfa forage and hay, and 0.001 and 0.002 ppm for iPr-2-OH DA MKH 3586 in/on forage and hay.

Residues of amicarbazone were <0.01-0.017 ppm in/on forage and <0.01-0.054 ppm in/on hay samples from alfalfa planted at a ~4-month PBI. Residues of DA MKH 3586 were <0.01 ppm in/on all alfalfa forage and hay samples. Residues of iPr-2OH DA MKH 3586 were <0.01 ppm in/on alfalfa forage and <0.01-0.014 ppm in/on hay. Combined residues were <0.030-<0.037 ppm (ave. = 0.017 ppm) in/on alfalfa forage and <0.030-<0.074 ppm (ave. = 0.025 ppm) in/on hay.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in this study, the field rotational crop data are classified as scientifically acceptable, pending submission of acceptable supporting storage stability data. In addition, information pertaining to the weather conditions (including temperatures) over the course of the study, with comparison to historical averages (for both temperature and rainfall), should be submitted for the rotational crop field trials; a discussion of any unusual weather conditions should be included. Because the proposed use pattern of amicarbazone is dependent on soil type, soil pH, and soil organic matter, information pertaining to these characteristics should be submitted for all field trials. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document DP Barcode D309766.

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

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis. There are currently no permanent tolerances established for amicarbazone. The original petitioner was Bayer Corporation; however, Bayer Corporation has since sold amicarbazone to Arvesta Corporation, which is now the petitioner for amicarbazone. In conjunction with the subject tolerance petition, Arvesta has submitted an application for Section 3 registration of a 70% water dispersible granule (WDG, DF) formulation (Amicarbazone DF Herbicide; EPA File Symbol No. 66330-UA). The 70% DF formulation is to be applied as preplant, preemergence, or early postemergence broadcast sprays at 0.22-0.45 lb ai/A for preplant/preemergence application, or 0.10-0.15 lb ai/A for postemergence application. The petitioner has submitted extended field rotational crop trial data on alfalfa.

The nomenclature and physicochemical properties of amicarbazone are presented below in Tables A.1 and A.2.

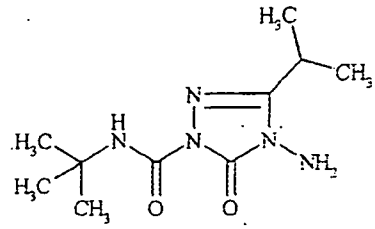
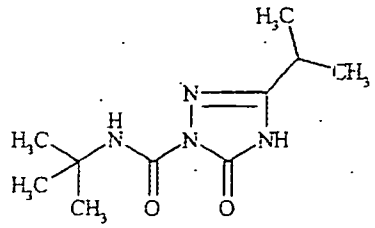
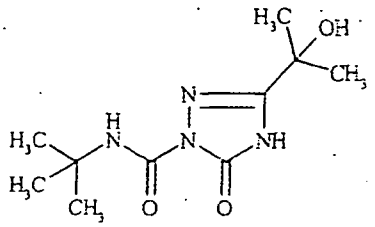
TABLE A.1. Amicarbazone Nomenclature.	
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-*tert*-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS registry number	129909-90-6
End-use formulation (EP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)
DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
*i*Pr-2-OH DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-*H*-1,2,4-triazole-1-carboxamide

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.

Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-4} Pa @ 25°C 1.30×10^{-4} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, Log(K_{OW})	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Twelve field rotational crop trials were conducted at field sites throughout the US during 2000-2001. At each of the test sites, bare soil was treated once with amicarbazone (70% WDG) at 0.434-0.454 lb ai/A using ground equipment. The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. At each site, a rotational crop of alfalfa was planted 99-123 days (~4 months) after treatment.

TABLE B.1.1. Trial Site Conditions.

Trial Identification (City, State, Year)	Soil characteristics				Meteorological data	
	Type	%OM	pH	CEC (meq/g)	Total rainfall during study period (inches)	Overall monthly temperature range (°C)
North Rose, NY, 2000	Not reported = NR	NR	NR	NR	48.57	NR
Suffolk, VA, 2000	NR	NR	NR	NR	41.58	NR
Saginaw, MI, 2000	NR	NR	NR	NR	33.97	NR
Campbell, MN, 2001	NR	NR	NR	NR	13.91	NR
Leonard, MO, 2001	NR	NR	NR	NR	34.95	NR
Dow, IL, 2000	NR	NR	NR	NR	45.84	NR
Carlyle, IL, 2000	NR	NR	NR	NR	46.93	NR
York, NE, 2001	NR	NR	NR	NR	18.24	NR
Grande Island, NE, 2001	NR	NR	NR	NR	10.78	NR
Smithfield, UT, 2000	NR	NR	NR	NR	6.75	NR
Fresno, CA, 2000	NR	NR	NR	NR	7.95	NR
Ephrata, WA, 2000	NR	NR	NR	NR	1.62	NR

[1] Detailed meteorological data were not provided.

Detailed soil characteristics data and meteorological data were not provided. Rainfall was supplemented with irrigation as needed.

TABLE B.1.2. Study Use Pattern.

Location (County, State) Year	EP[1]	Application				Tank Mix Adjuvants
		Method; Timing	Vol. (GPA[2])	Single Rate (lb ai/A)	No. of Appl.	
North Rose, NY, 2000	70% WDG	broadcast; bare ground	19.8	0.451	1	None
Suffolk, VA, 2000	70% WDG	broadcast; bare ground	14.7	0.448	1	None
Saginaw, MI, 2000	70% WDG	broadcast; bare ground	17.5	0.451	1	None
Campbell, MN, 2001	70% WDG	broadcast, bare ground	14.9	0.451	1	None
Leonard, MO, 2001	70% WDG	broadcast, bare ground	16.3	0.453	1	None
Dow, IL, 2000	70% WDG	broadcast; bare ground	14.8	0.445	1	None
Carlyle, IL, 2000	70% WDG	broadcast; bare ground	16.4	0.445	1	None
York, NE, 2001	70% WDG	broadcast; bare ground	19.6	0.448	1	None
Grande Island, NE, 2001	70% WDG	broadcast; bare ground	19.5	0.447	1	None
Smithfield, UT, 2000	70% WDG	broadcast; bare ground	15.0	0.454	1	None
Fresno, CA, 2000	70% WDG	broadcast; bare ground	18.1	0.434	1	None
Ephrata, WA, 2000	70% WDG	broadcast; bare ground	16.2	0.450	1	None

[1] EP = End-use Product.
[2] All applications were made using ground equipment.

Duplicate treated samples and a single control sample of alfalfa forage (>2.5 lbs each) and hay (>1.5 lbs each) were harvested at intervals reflecting normal agricultural practices at each site.

Forage and hay samples were harvested from the first cutting of alfalfa, 41-286 days after planting (DAP). Hay samples from three of the tests were allowed to dry in the field for 2-4 days prior to sampling. Samples from the second and third cutting of alfalfa were not collected. After collection, samples were placed in frozen storage at the test facility within 4 hours of collection, stored frozen for 5-56 days, then shipped frozen by ACDS freezer truck to the analytical laboratory, Bayer Research Park (BRP), Stilwell, KS and stored frozen (<-15° C) prior to analysis. Samples were stored frozen from collection to analysis for up to 23 months.

B.2. Analytical Methodology

Samples of alfalfa forage and hay were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 by using an LC-MS/MS method (Bayer Method 200258), which is similar to the proposed enforcement method for plant commodities (Bayer Method 108340). Only a brief description of the method was included in the submission. For a complete description of the method, refer to 46145302.der (currently under review).

Briefly, homogenized samples were extracted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v). Following filtration and the addition of deuterated internal standards, the residues were cleaned up by solid phase extraction (mixed C_8 and SCX cartridge). Residues were quantified by LC-MS/MS, using deuterated internal reference standards of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586. Residues of metabolites were reported in parent equivalents. The validated LOQ was 0.01 ppm for amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on all matrices. The LODs for the three analytes were calculated based on the recovery data at the LOQ and using the following formula: (SD at the LOQ) x $t_{0.99}$ (for n-1 replicates). The LODs for amicarbazone were 0.002 and 0.004 ppm for alfalfa forage and hay. The LOD for DA MKH 3586 was 0.002 ppm for alfalfa forage and hay The LOD for iPr-2-OH DA MKH 3586 were 0.001 ppm for forage and 0.002 ppm for hay.

In the current submission, the LC-MS/MS method was validated using concurrent method recoveries of alfalfa forage and hay samples fortified separately with amicarbazone, DA MKH 3586 and iPr-2-OH DA MKH 3586 at 0.01 or 0.10 ppm.

C. RESULTS AND DISCUSSION

The number of alfalfa rotational crop field trials and the geographic representation of the residue data on alfalfa forage and hay are adequate according to the latest EPA Guidance.

The total frozen (< -15° C) storage intervals were 11-23 months for alfalfa forage and hay samples (Table C.1). In support of the field rotational crop trials, the petitioner cited storage stability data (45121704.der) submitted in conjunction with a confined rotational crop study. In that review, it was also stated that LC/MS analyses of some metabolites was conducted up to 30 months following sample collection; however, the petitioner noted that these analyses were not quantitative. These data are insufficient to support the current field rotational crop study because quantitative data were not included in MRID 45121704.

TABLE C.1. Summary of Storage Conditions			
Matrix	Storage Temp. (°C)	Actual Storage Duration (months)	Limit of Demonstrated Storage Stability (months) [1]
Alfalfa forage and hay	<-15	11-23	NA

[1] NA = not available. Adequate storage stability data are not available for frozen plant commodities.

The LC-MS/MS method (Bayer Method 200258) used to determine amicarbazone residues in/on alfalfa forage and hay is adequate for data collection. Average concurrent recoveries from alfalfa forage and hay were 97-100% with standard deviations of ±3-6% (Table C.2). Apparent residues of amicarbazone were <LOQ in/on all control samples. The LOQ was 0.01 ppm each for amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on all matrices. LODs were 0.002 and 0.004 ppm for amicarbazone in/on forage and hay, 0.002 ppm for DA MKH 3586 in/on forage and hay, and 0.001 and 0.002 ppm for iPr-2-OH DA MKH 3586 in/on forage and hay. Adequate sample calculations and chromatograms were provided.

TABLE C.2. Summary of Concurrent Recoveries of Amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 from Alfalfa Forage and Hay Using Bayer LC-MS/MS Method 20258.					
Matrix	Analyte	Spike level (mg/kg)	Sample size (n)	Recoveries (%)	Mean ± std dev (%)
Alfalfa forage	Amicarbazone	0.01	7	93-108	99 ± 6
	DA MKH 3586	0.01	7	93-110	100 ± 6
	iPr-2-OH DA MKH 3586	0.01	7	96-105	99 ± 3
Alfalfa hay	Amicarbazone	0.01	6	91-106	100 ± 6
		0.10	3	103-107	
	DA MKH 3586	0.01	6	90-105	97 ± 5
		0.10	3	91-98	
	iPr-2-OH DA MKH 3586	0.01	6	97-107	99 ± 5
		0.10	3	94-99	

At a ~4-month PBI, residues of amicarbazone were <0.01-0.017 ppm in/on alfalfa forage and <0.01-0.054 ppm in/on alfalfa hay samples (Table C.3). Residues of DA MKH 3586 were <0.01 ppm in/on all alfalfa forage and hay samples. Residues of iPr-2OH DA MKH 3586 were <0.01 ppm in/on alfalfa forage and <0.01-0.014 ppm in/on hay. When calculating combined residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 (expressed in parent equivalents), the LOQ (0.01 ppm) was used for individual residues reported at <LOQ and ½ the LOQ (0.005 ppm) was used for calculating the average. Combined residues were <0.030-<0.037 ppm (ave. = 0.017 ppm) in/on alfalfa forage and <0.030-<0.074 ppm (ave. = 0.025 ppm) in/on hay (Table C.4).

Common cultural practices were used to maintain plants, and the weather conditions and the maintenance chemicals and fertilizer used in the study did not have a notable impact on the residue data.

TABLE C.3. **Residues of Amicarbazone in Rotational Alfalfa Planted ~4 months Following a Single Soil Application of Amicarbazone (70% WDG).**

Location (County, State, Year)	EPA Region	Variety	Total Rate (lb ai/A)	PBI[2] (days)	Harvest DAP[1]	Matrix	Residues (ppm)[3]			
							Amicarbazone	DA MKH 3586	i-Pr-2-OH MKH 3586	Combined Residues
North Rose, NY, 2000	1	Excaliber	0.451	120	286	Forage	(0.002)[4], ND[4]	ND, ND	ND, ND	[illegible]
Suffolk, VA, 2000	2	Clean Sweep 1000	0.448	123	277	Forage	ND, (0.003)	ND, ND	ND, ND	<0.03, <0.03
Saginaw, MI, 2000	5	Pioneer 5454	0.451	122	256	Forage	ND, ND	ND, ND	ND, ND	[illegible]
Campbell, MN, 2001	5	Pioneer 54V54	0.451	99	41	Forage	ND, ND	ND, ND	(0.003, 0.002)	[illegible]
Leonard, MO, 2001	5	NK 919	0.453	120	149	Forage	ND, ND	ND, ND	ND, ND	[illegible]
Dow, IL, 2000	5	WL 252 HQ	0.445	113	257	Forage	ND, ND	ND, ND	ND, ND	[illegible]
Carlyle, IL, 2000	5	Vitro	0.445	113	242	Forage	ND, ND	ND, ND	(0.007, 0.003)	<0.03 [illegible]
York, NE, 2001	5	Multi-Queen	0.448	116	61	Forage	0.017, 0.016	ND, ND	ND, ND	<0.037 0.036
Grande Island, NE, 2001	7	Multi-Queen	0.447	116	56	Forage	0.015, 0.015	ND, ND	ND, ND	<0.035 [illegible]
Smithfield, UT, 2000	9	Wintergold	0.454	119	97	Forage	(0.004, 0.006)	ND, ND	ND, (0.001)	<0.03, [illegible]
Fresno, CA, 2000	10	CUF 101	0.434	121	84	Forage	(0.010), 0.015	ND, (0.003)	(0.005, 0.010)	<0.03 [illegible]
Ephrata, WA, 2000	11	WL 252 HQ	0.450	119	52	Forage	(0.002, 0.002)	ND, ND	(0.001), ND	<0.03 [illegible]
North Rose, NY, 2000	1	Excaliber	0.451	120	286	Hay	ND, ND	ND, ND	ND, ND	[illegible]
Suffolk, VA, 2000	2	Clean Sweep 1000	0.448	123	277	Hay	ND, ND	ND, ND	ND, ND	[illegible]
Saginaw, MI, 2000	5	Pioneer 5454	0.451	122	256	Hay	ND, ND	ND, ND	ND, ND	[illegible]
Campbell, MN, 2001	5	Pioneer 54V54	0.451	99	41	Hay	0.017, 0.018	ND, ND	(0.003, 0.003)	[illegible]
Leonard, MO, 2001	5	NK 919	0.453	120	149	Hay	ND, ND	ND, ND	ND, ND	[illegible]
Dow, IL, 2000	5	WL 252 HQ	0.445	113	257	Hay	ND, ND	ND, ND	ND, ND	[illegible]
Carlyle, IL, 2000	5	Vitro	0.445	113	242	Hay	ND, ND	ND, ND	0.012, 0.014	[illegible]
York, NE, 2001	5	Multi-Queen	0.448	116	61	Hay	0.054, 0.041	ND, ND	ND, ND	[illegible]
Grande Island, NE, 2001	7	Multi-Queen	0.447	116	56	Hay	0.029, 0.035	ND, ND	ND, ND	[illegible]
Smithfield, UT, 2000	9	Wintergold	0.454	119	97	Hay	0.022, 0.023	(0.003, 0.003)	(0.005, 0.005)	[illegible]
Fresno, CA, 2000	10	CUF 101	0.434	121	84	Hay	0.013, 0.013	(0.003, 0.003)	0.010, 0.011	[illegible]
Ephrata, WA, 2000	11	WL 252 HQ	0.450	119	52	Hay	(0.005, 0.005)	ND, ND	(0.003), ND	[illegible]

[1] DAP = Days After Planting

[2] PBI = Plant Back Interval.

[3] The LOQ is 0.01 ppm for each analyte in/on each matrix. The LODs were as follows: amicarbazone, 0.002 ppm for forage and 0.004 ppm for hay; DA MKH 3586, 0.002 ppm for forage and hay; iPr-2-OH DA MKH 3586, 0.001 ppm for forage and 0.002 ppm for hay. Total residues are the sum of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 residues in parent equivalents; the LOQ was used for individual residues reported at <LOQ in calculating total residues.

[4] ND = not detected; residues were <LOD. Residues <LOQ but >LOD are reported in parentheses.

TABLE C.4. Summary of Residue Data in Rotational Alfalfa Planted ~4 Months Following a Single Soil Application of Amicarbazone (70% WDG).

Commodity	Total Rate (lb ai/A)	PBI (days)	Residue Levels (ppm) [1]						
			n	Min.	Max.	HAFT[2]	Median (STMdR[3])	Mean (STMR[3])	Std. Dev.
Amicarbazone									
Alfalfa Forage	0.43-0.45	99-123	24	<0.01	0.017	0.017	0.005	0.007	0.005
Alfalfa Hay			24	<0.01	0.054	0.048	0.005	0.014	0.014
DA MKH 3586									
Alfalfa Forage	0.43-0.45	99-123	24	<0.01	<0.01	<0.01	0.005	0.005	0
Alfalfa Hay			24	<0.01	<0.01	<0.01	0.005	0.005	0
iPr-2-OH DA MKH 3586									
Alfalfa Forage	0.43-0.45	99-123	24	<0.01	<0.01	<0.01	0.005	0.005	0.002
Alfalfa Hay			24	<0.01	0.014	0.013	0.005	0.006	0.003
Combined Residues									
Alfalfa Forage	0.43-0.45	99-123	24	<0.03	<0.037	<0.037	0.015	0.017	0.004
Alfalfa Hay			24	<0.03	<0.074	<0.068	0.019	0.025	0.013

[1] The LOQ is 0.01 ppm for each analyte in/on each matrix. The limits of detection (LODs) for amicarbazone were calculated to be 0.002 ppm for alfalfa forage and 0.004 ppm for hay. The LOD for DA MKH 3586 was 0.002 ppm for alfalfa forage and hay. The LODs for iPr-2-OH DA MKH 3586 were 0.001 ppm for forage and 0.002 ppm for hay. Total residues are the sum of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 residues in parent equivalents; the LOQ was used for individual residues reported at <LOQ in calculating total residues.

[2] HAFT = Highest Average Field Trial.

[3] STMdR = Supervised Trial Median Residue; STMR = Supervised Trial Mean Residue. For calculation of the median, mean and standard deviation, ½ the LOQ (0.005 ppm) was used for residues reported at <LOQ.

D. CONCLUSION

Pending submission of adequate supporting storage stability data, the extended field rotational crop data on alfalfa forage and hay are adequate.

E. REFERENCES

None

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
DP Barcode: D309766
PC Code: 114004

Template Version September 2003

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.4.4/OPPTS 860.1900/OECD IIA 6.6.3, 6.8.7 and IIIA 8.6
Field Accumulation in Rotational Crops - Cotton

Primary Evaluator	Manying Xue, Chemist RAB3/HED (7509C)	Date: 03/23/05
Approved by	Leung Cheng, Senior Chemist RAB3/HED (7509C)	Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (1910 Sedwick Rd., Suite B; Durham, NC 27713; submitted 1/07/2005). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46145304 Krolski, M. (2003) MKH 3586 70 WG - Magnitude of the Residues in the Rotational Crop of Cotton. Lab Project Number: 200456: TMN-0246. Unpublished study prepared by Bayer CropScience. 190 p.

EXECUTIVE SUMMARY:

Twelve field rotational crop trials on cotton were conducted at field sites throughout the US during 2000. At each of the test sites, bare soil was treated once with amicarbazone (70% WDG) at 0.40-0.46 lb ai/A using ground equipment. The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. At each site, a rotational crop of cotton was planted 329-367 days (11-12 months) after treatment. Duplicate treated samples and a single control sample of cotton bolls were harvested at intervals reflecting normal agricultural practices at each site, 123-213 days after planting (DAP). Cotton was harvested using a picker at three locations, a stripper at three locations, and by hand at the other six locations. Whole cotton seed samples were ginned and undelinted seed samples were collected from all tests and gin byproducts samples were collected from six tests (picker and stripper only). Samples were stored frozen from collection to analysis for up to 12 months, an interval that is not supported by the available storage stability data. Data demonstrating the stability of residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 in/on representative plant matrices remain outstanding.

Samples were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 using an adequate LC-MS/MS method (Bayer Method 200258) which is similar to the proposed enforcement method for plant commodities (Bayer Method 108340). Briefly, samples were extracted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v), concentrated, and cleaned up by solid phase extraction. Residues were quantified by LC-MS/MS, using deuterated internal standards of each analyte. Residues of metabolites were reported as parent equivalents. The LOQ was 0.01 ppm each for amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on all matrices. The LODs for amicarbazone were 0.005 ppm for undelinted seed and 0.002 ppm for gin byproducts. The LODs

for DA MKH 3586 were 0.006 ppm for seed and 0.005 ppm for gin byproducts. The LODs for iPr-2-OH DA MKH 3586 were 0.002 ppm for seed and 0.004 ppm for gin byproducts.

Residues of amicarbazone were <0.01 ppm in/on all cotton undelinted seed samples and <0.01-0.015 ppm in/on cotton gin byproducts samples from cotton planted at a 12-month PBI. Residues of DA MKH 3586 were <0.01 ppm in/on all cotton seed samples and <0.01-0.026 ppm in/on gin byproducts samples. Residues of iPr-2OH DA MKH 3586 were <0.01-0.033 ppm in/on cotton seed and <0.01-0.169 ppm in/on gin byproducts. Combined residues were <0.030-<0.053 ppm (ave. = 0.017 ppm) in/on cotton undelinted seed and <0.031-0.210 ppm (ave: = 0.084 ppm) in/on gin byproducts.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in this study, the field rotational crop data on cotton are classified as scientifically acceptable, pending submission of acceptable supporting storage stability data. In addition, information pertaining to the weather conditions (including temperatures) over the course of the study, with comparison to historical averages (for both temperature and rainfall), should be submitted for the rotational crop field trials; a discussion of any unusual weather conditions should be included. Because the proposed use pattern of amicarbazone is dependent on soil type, soil pH, and soil organic matter, information pertaining to these characteristics should be submitted for all field trials. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document DP Barcode D309766.

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

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis. There are currently no permanent tolerances established for amicarbazone. The original petitioner was Bayer Corporation; however, Bayer Corporation has since sold amicarbazone to Arvesta Corporation, which is now the petitioner for amicarbazone. In conjunction with the subject tolerance petition, Arvesta has submitted an application for Section 3 registration of a 70% dry flowable (DF) formulation (Amicarbazone DF Herbicide; EPA File Symbol No. 66330-UA). The 70% DF formulation is to be applied as preplant, preemergence, or early postemergence broadcast sprays at 0.22-0.45 lb ai/A for preplant/preemergence application, or 0.10-0.15 lb ai/A for postemergence application. The petitioner has submitted extended field rotational crop trial data on cotton.

The nomenclature and physicochemical properties of amicarbazone are presented below in Tables A.1 and A.2.

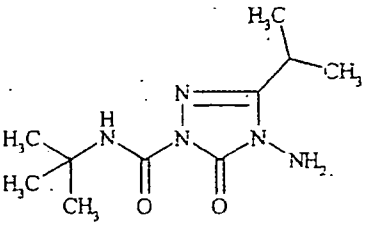
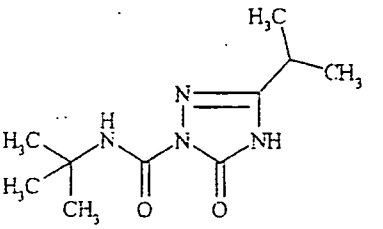
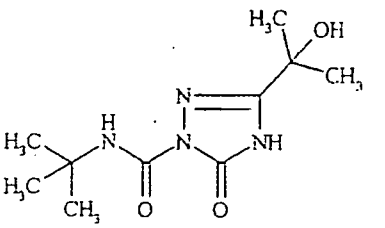
TABLE A.1. Amicarbazone Nomenclature.	
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-*tert*-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS registry number	129909-90-6
End-use formulation (EP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)
DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
*i*Pr-2-OH DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-*H*-1,2,4-triazole-1-carboxamide

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.

Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-4} Pa @ 25°C 1.30×10^{-4} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, $Log(K_{ow})$	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Twelve field rotational crop trials were conducted on cotton at field sites throughout the US during 2000. At each of the test sites, bare soil was treated once with amicarbazone (70% WDG) at 0.396-0.463 lb ai/A using ground equipment. The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. At each site, a rotational crop of cotton was planted 329-367 days (11-12 months) after treatment.

TABLE B.1.1. Trial Site Conditions.

Trial Identification (City, State, Year)	Soil characteristics				Meteorological data	
	Type	%OM	pH	CEC (meq/g)	Total rainfall during study period (inches)	Overall monthly temperature range (°C)
Tifton, GA, 2000	Not reported = NR	NR	NR	NR	65.5	NR
Benoit, MS, 2000	NR	NR	NR	NR	48.8	NR
Greenville, MS, 2000	NR	NR	NR	NR	66.6	NR
Greenville, MS, 2000	NR	NR	NR	NR	66.6	NR
Duncan, OK, 2000	NR	NR	NR	NR	46.8	NR
Littlefield, TX, 2000	NR	NR	NR	NR	21.5	NR
Halfway, TX, 2000	NR	NR	NR	NR	20.5	NR
Eakly, OK, 2000	NR	NR	NR	NR	37.0	NR
Claude, TX, 2000	NR	NR	NR	NR	18.1	NR
Maricopa, AZ, 2000	NR	NR	NR	NR	15.0	NR
Fresno, CA, 2000	NR	NR	NR	NR	10.4	NR
Porterville, CA, 2000	NR	NR	NR	NR	6.6	NR

[1] Detailed meteorological data were not provided.

Detailed soil characteristics data and meteorological data were not provided. Rainfall was supplemented with irrigation as needed.

TABLE B.1.2. Study Use Pattern.

Location (County, State) Year	EP[1]	Application				Tank Mix Adjuvants	Harvest Procedures
		Method; Timing	Vol. (GPA[2])	Single Rate (lb ai/A)	No. of Appl.		
Tifton, GA, 2000	70% WDG	broadcast; bare ground	15.9	0.450	1	None	Picker
Benoit, MS, 2000	70% WDG	broadcast; bare ground	18.9	0.458	1	None	Picker
Greenville, MS, 2000	70% WDG	broadcast; bare ground	12.2	0.453	1	None	Hand
Greenville, MS, 2000	70% WDG	broadcast; bare ground	12.2	0.451	1	None	Hand
Duncan, OK, 2000	70% WDG	broadcast; bare ground	10.1	0.396	1	None	Stripper
Littlefield, TX, 2000	70% WDG	broadcast; bare ground	12.2	0.454	1	None	Stripper
Halfway, TX, 2000	70% WDG	broadcast; bare ground	16.2	0.444	1	None	Stripper
Eakly, OK, 2000	70% WDG	broadcast; bare ground	14.2	0.451	1	None	Hand
Claude, TX, 2000	70% WDG	broadcast; bare ground	15.0	0.452	1	None	Hand
Maricopa, AZ, 2000	70% WDG	broadcast; bare ground	14.8	0.459	1	None	Picker
Fresno, CA, 2000	70% WDG	broadcast; bare ground	14.8	0.463	1	None	Hand
Porterville, CA, 2000	70% WDG	broadcast; bare ground	14.9	0.448	1	None	Hand

[1] EP = End-use Product.
[2] All applications were made using ground equipment.

Duplicate treated samples and a single control sample of cotton bolls were harvested at intervals reflecting normal agricultural practices at each site, 123-213 days after planting (DAP). Cotton was harvested using a picker at three locations, a stripper at three locations, and by hand at the other six locations. After collection, cotton seed samples were placed in frozen storage at the test facility within 5 hours of collection, stored frozen for 0-23 days, then shipped frozen by ACDS freezer truck to ginning facility, Food and Protein Research and Development Center (FPRDC). Bryan, TX and stored frozen (<-5° C) prior to ginning. Following ginning, undelinted seed samples from all tests and gin byproducts samples from six tests (picker and stripper only) were shipped frozen by ACDS freezer truck to the analytical laboratory, Bayer Research Park (BRP), Stilwell, KS and stored frozen (<-5° C) prior to analysis. Samples were stored frozen from collection to analysis for up to 12 months.

B.2. Analytical Methodology

Samples of cotton seed and gin byproducts were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 by using an LC-MS/MS method (Bayer Method 200258), which is similar to the proposed enforcement method for plant commodities (Bayer Method 108340). Only a brief description of the method was included in the submission. For a complete description of the method, refer to 46145302.der (currently under review).

Briefly, homogenized samples were extracted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v). Following filtration and the addition of deuterated internal standards, the residues were cleaned up by solid phase extraction (mixed C_8 and SCX cartridge). Residues were quantified by LC-MS/MS, using deuterated internal reference standards of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586. Residues of metabolites were reported in parent equivalents. The validated LOQ was 0.01 ppm for amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on all matrices. The LODs for the three analytes were calculated based on the recovery data at the LOQ and using the following formula: (SD at the LOQ) x $t_{0.99}$ (for n-1 replicates). The LODs for amicarbazone were 0.005 and 0.002 ppm for cottonseed and gin byproducts. The LODs for DA MKH 3586 were 0.006 ppm for cotton seed and 0.005 ppm for gin byproducts. The LODs for iPr-2-OH DA MKH 3586 were 0.002 ppm for seed and 0.004 ppm for gin byproducts.

In the current submission, the LC-MS/MS method was validated using concurrent method recoveries of cotton seed and gin byproducts samples fortified separately with amicarbazone, DA MKH 3586 and iPr-2-OH DA MKH 3586 each at 0.01 and 0.1 or 0.20 ppm.

C. RESULTS AND DISCUSSION

The number of cotton rotational field trials and the geographic representation of the residue data on cottonseed and cotton gin byproducts are adequate according to the latest EPA Guidance.

The total frozen (< -5° C) storage intervals were 10-12 months for cotton undelinted seed and gin byproducts samples (Table C.1). In support of the field rotational crop trials, the petitioner cited storage stability data (45121704.der) submitted in conjunction with a confined rotational crop study. In that review, it was also stated that LC/MS analyses of some metabolites was conducted up to 30 months following sample collection for certain metabolites; however, the petitioner noted that these analyses were not quantitative. These data are insufficient to support the current field rotational crop study because quantitative data were not included in MRID 45121704.

TABLE C.1. Summary of Storage Conditions

Matrix	Storage Temp. (°C)	Actual Storage Duration (months)	Limit of Demonstrated Storage Stability (months) [1]
Cotton seed and gin byproducts	<-5	10-12	NA

[1] NA = not available. Adequate storage stability data are not available for frozen plant commodities.

The LC-MS/MS method (Bayer Method 200258) used to determine amicarbazone residues in/on cotton undelinted seed and gin byproducts is adequate for data collection. Average concurrent recoveries from seed and gin byproducts were 92-104% with standard deviations of ±4-9% (Table C.2). Apparent residues of amicarbazone were <LOQ in/on all control samples. The LOQ was 0.01 ppm each for amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on all matrices. The LODs were 0.005 and 0.002 ppm for amicarbazone in/on cotton seed and gin byproducts, 0.006 and 0.005 ppm for DA MKH 3586 in/on seeds and gin byproducts, and 0.002 and 0.004 ppm for iPr-2-OH DA MKH 3586 in/on seeds and gin byproducts. Adequate sample calculations and chromatograms were provided.

TABLE C.2. Summary of Concurrent Recoveries of Amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 from Cotton Seed and Gin byproducts using Bayer LC-MS/MS Method 200258.

Cotton Matrix	Analyte	Spike level (mg/kg)	Sample size (n)	Recoveries (%)	Mean ± std dev (%)
Undelinted seed	Amicarbazone	0.01	3	100-113	104 ± 5
		0.10	6	96-106	
	DA MKH 3586	0.01	3	102-119	104 ± 7
		0.10	6	96-104	
	iPr-2-OH DA MKH 3586	0.01	3	100-105	98 ± 6
		0.10	6	86-99	
Gin byproducts	Amicarbazone	0.01	4	95-108	97 ± 9
		0.20	3	84-96	
	DA MKH 3586	0.01	4	81-105	92 ± 8
		0.20	3	93-96	
	iPr-2-OH DA MKH 3586	0.01	4	100-104	99 ± 4
		0.20	3	94-98	

At a ~12-month PBI, residues of amicarbazone were <0.01 ppm in/on all cotton undelinted seed samples and <0.01-0.015 ppm in/on cotton gin byproducts samples (Table C.3). Residues of DA MKH 3586 were <0.01 ppm in/on all cotton seed samples and <0.01-0.026 ppm in/on gin byproducts samples. Residues of iPr-2OH DA MKH 3586 were <0.01-0.033 ppm in/on cotton seed and <0.01-0.169 ppm in/on gin byproducts. When calculating combined residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 (expressed in parent equivalents), the LOQ (0.01 ppm) was used for individual residues reported at <LOQ and ½ the LOQ (0.005 ppm) was used for calculating the average. Combined residues were <0.030-<0.053 ppm (ave. = 0.017 ppm) in/on cotton undelinted seed and <0.031-0.210 ppm (ave. = 0.084 ppm) in/on gin byproducts (Table C.4).

Common cultural practices were used to maintain plants, and the weather conditions and the maintenance chemicals and fertilizer used in the study did not have a notable impact on the residue data.

TABLE C.3. Residues of Amicarbazone in Rotational Cotton Planted ~12 Months Following a Single Soil Application of Amicarbazone (70% WDG).

Location (County, State, Year)	EPA Region	Variety	Total Rate (lb ai/A)	PBI[1] (days)	Harvest DAP[1]	Matrix	Residues (ppm)[2]			
							Amicarbazone	DA MKH 3536	i-Pr-2-OH MKH 3586	Combined Residues
Tifton, GA, 2000.	2	DPL 5415 RR	0.450	364	175 (P)[3]	Undelinted Seed	ND, ND[4]	ND, ND	ND, ND	<0.03, <0.03
Benoit, MS, 2000	4	DPL NuCOTN 33B	0.458	351	147 (P)	Undelinted Seed	ND, ND	ND, ND	(0.003, 0.003)	<0.03, <0.03
Greenville, MS, 2000	4	Suregrow 125 BR	0.453	367	125	Undelinted Seed	ND, ND	ND, ND	ND, ND	<0.03, <0.03
Greenville, MS, 2000	4	DPL NuCOTN 33B	0.451	367	123	Undelinted Seed	ND, ND	ND, ND	ND, ND	<0.03, <0.03
Duncan, OK, 2000	6	Paymaster 2326 BG RR	0.396	351	192 (S)	Undelinted Seed	ND, ND	ND, ND	(0.004, 0.005)	<0.03, <0.03
Brownfield, TX, 2000	8	Paymaster 2326 BG RR	0.454	356	154 (S)	Undelinted Seed	ND, ND	(0.006), ND	0.033, 0.021	<0.053, <0.041
Plainview, TX, 2000	8	Paymaster 2200 RR RR	0.444	361	182 (S)	Undelinted Seed	ND, ND	ND, ND	(0.008, 0.008)	<0.03, <0.03
Elk City, OK, 2000	8	Paymaster 2280	0.451	353	165	Undelinted Seed	ND, ND	ND, ND	(0.003, 0.003)	<0.03, <0.03
Claude, TX, 2000	8	Paymaster 2326 RR	0.452	367	171	Undelinted Seed	ND, ND	ND, ND	(0.002, 0.002)	<0.03, <0.03
Maricopa, AZ, 2000	10	Hybrid 3114-corn	0.459	329	213 (P)	Undelinted Seed	ND, ND	ND, ND	ND, ND	<0.03, <0.03
Fresno, CA, 2000	10	Acala Maxxa	0.463	350	171	Undelinted Seed	ND, ND	ND, ND	ND, ND	<0.03, <0.03
Porterville, CA, 2000	10	DP 6207 Acala	0.448	364	179	Undelinted Seed	ND, ND	ND, ND	ND, (0.002)	<0.03, <0.03
Tifton, GA, 2000	2	DPL 5415 RR	0.450	364	175 (P)	Gin byproducts	(0.005, 0.006)	ND, ND	0.035, 0.041	<0.055, <0.061
Benoit, MS, 2000	4	DPL NuCOTN 33B	0.458	351	147 (P)	Gin byproducts	0.015, (0.009)	0.026, ND	0.169, 0.105	0.210, <0.125
Duncan, OK, 2000	6	Paymaster 2326 BG RR	0.396	351	192 (S)	Gin byproducts	(0.002, 0.003)	ND, ND	0.012, 0.011	<0.042, <0.031
Brownfield, TX, 2000	8	Paymaster 2326 BG RR	0.454	356	154 (S)	Gin byproducts	ND, ND	ND, ND	0.047, 0.047	<0.067, <0.067
Plainview, TX, 2000	8	Paymaster 2200 RR RR	0.444	361	182 (S)	Gin byproducts	0.013, (0.006)	ND, 0.010	0.120, 0.094	<0.143, <0.114
Maricopa, AZ, 2000	10	Hybrid 3114-corn	0.459	329	213 (P)	Gin byproducts	(0.005, 0.007)	ND, (0.005)	0.068, 0.085	<0.088, <0.105

[1] DAP = Days After Planting; PBI = Plant Back Interval.

[2] The LOQ is 0.01 ppm for each analyte in/on each matrix. The calculated LODs were as follows: amicarbazone, 0.005 and 0.002 ppm in/on seeds and gin byproducts; DA MKH 3586, 0.006 and 0.005 ppm in/on seeds and gin byproducts; and iPr-2-OH DA MKH 3586, 0.002 and 0.004 ppm in/on seeds and gin byproducts. Total residues are the sum of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 residues in parent equivalents; the LOQ was used for individual residues reported at <LOQ in calculating total residues.

[3] ND = not detected; residues were <LOD. Residues <LOQ but >LOD are reported in parentheses.

[4] P = harvested using a picker, S = harvested using a stripper.

TABLE C.4. Summary of Residue Data in Rotational Cotton Planted ~12 months Following a Single Soil Application of Amicarbazone (70% WDG).

Commodity	Total Rate (lb ai/A)	PBI (days)	Residue Levels (ppm) [1]						
			n	Min.	Max.	HAFT[2]	Median (STMdR[3])	Mean (STMR[3])	Std. Dev
Amicarbazone									
Cotton Seed	0.40-0.46	329-367	24	<0.01	<0.01	<0.01	0.005	0.005	NA
Cotton Gin byproducts			12	<0.01	0.015	<0.013	0.006	0.006	0.004
DA MKH 3586									
Cotton Seed	0.40-0.46	329-367	24	<0.01	<0.01	<0.01	0.005	0.005	<0.005
Cotton Gin byproducts			12	<0.01	0.026	<0.018	0.005	0.007	0.006
iPr-2-OH DA MKH 3586									
Cotton Seed	0.40-0.46	329-367	24	<0.01	0.033	0.027	0.005	0.006	0.007
Cotton Gin byproducts			12	0.011	0.169	0.137	0.058	0.070	0.047
Combined Residues									
Cotton Seed	0.40-0.46	329-367	24	<0.03	<0.053	<0.047	0.015	0.017	0.006
Cotton Gin byproducts			12	<0.031	0.210	<0.168	0.068	0.084	0.053

1. The LOQ is 0.01 ppm for each analyte in/on each matrix. The calculated LODs were as follows: amicarbazone, 0.005 and 0.002 ppm in/on seeds and gin byproducts; DA MKH 3586, 0.006 and 0.005 ppm for in/on seeds and gin byproducts; and iPr-2-OH DA MKH 3586, 0.002 and 0.004 ppm for in/on seeds and gin byproducts. Total residues are the sum of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 residues in parent equivalents; the LOQ was used for individual residues reported at <LOQ in calculating total residues.
2. HAFT = Highest Average Field Trial.
3. STMdR = Supervised Trial Median Residue; STMR = Supervised Trial Mean Residue. For calculation of the median, mean and standard deviation, ½ the LOQ (0.005 ppm) was used for residues reported at <LOQ.
4. NA=Not Applicable.

D. CONCLUSION

Pending submission of adequate supporting storage stability data, the extended field rotational crop data on cotton are adequate and indicate that tolerances will be required on cotton undelinted seed and gin byproducts.

E. REFERENCES

None

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: 0F6131
DP Barcode: D309766
PC Code: 114004

Template Version September 2003

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.4.1/OPPTS 860.1520/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Processed Food and Feed - Cotton

Primary Evaluator	Manying Xue, Chemist RAB3/HED (7509C)	Date: 03/23/05
Approved by	Leung Cheng, Senior Chemist RAB3/HED (7509C)	Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (1910 Sedwick Rd., Building 100, Suite B; Durham, NC 27713; submitted 1/07/2005). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46145305 Krolski, M. (2003) MKH 3586 70 WG - Magnitude of the Residue in Cottonseed Processed Commodities. Lab Project Number: 200459: TMN-0255. Unpublished study prepared by Bayer CropScience. 255 p.

EXECUTIVE SUMMARY:

In a single test conducted in GA during 2000, bare soil was treated once with amicarbazone (70% WDG) at 2.25 lb ai/A (5x) using ground equipment. The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. A rotational crop of cotton was planted 12 months after treatment (12-month plant-back interval, PBI). Seed cotton was harvested using a mechanical picker at commercial maturity (175 DAP) from the 5x treated plot, then ginned to generate undelinted cottonseed. Triplicate samples of undelinted cottonseed (RAC) were collected and the remaining bulk samples were processed into hulls, meal, and refined oil using simulated commercial procedures. Prior to analysis, cotton undelinted seed samples were stored frozen for a maximum of 10 months and processed cotton matrices were stored frozen for up to 14 days. The 10-month interval is not supported by the available storage stability data. Data demonstrating the stability of residues of amicarbazone, DA MKH 3586, and iPr-2OH DA MKH 3586 in/on representative plant matrices remain outstanding.

Samples were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 using an adequate LC-MS/MS method (Bayer Method 200258) which is similar to the proposed enforcement method for plant commodities (Bayer Method 108340). Briefly, samples were extracted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v), concentrated and cleaned up by solid phase extraction. Oil samples are also initially partitioned with hexane. Residues were quantified by LC-MS/MS, using deuterated internal standards of each analyte. Residues of metabolites were reported as parent equivalents. The LOQ was 0.01 ppm for each of the three analytes (amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586) in/on all matrices. The calculated LODs for amicarbazone were 0.002 ppm for cottonseed refined oil and hulls, 0.003 ppm for meal, and 0.005 ppm for undelinted seed. The

LODs for DA MKH 3586 were 0.006 ppm for undelinted seed, refined oil and hulls, and 0.009 ppm for meal. The LODs for iPr-2-OH DA MKH 3586 were 0.001 ppm for refined oil, 0.002 ppm for undelinted seed, and 0.003 ppm for meal and hulls.

Amicarbazone and DA MKH 3586 residues were each <0.005-<0.006 ppm (each <LOD) and iPr-2-OH DA MKH 3586 residues were 0.004-0.005 ppm (each <LOQ) in/on 3 samples of cotton undelinted seed harvested at maturity from plants grown in soil treated at 5x. Combined residues expressed in parent equivalents were <0.015-<0.016 ppm (<LOQ) in/on undelinted cottonseeds. Amicarbazone and DA MKH 3586 residues were each <LOD in all processed cotton matrices. Residues of iPr-2-OH DA MKH 3586 were 0.004 ppm (<LOQ) ppm in 3 meal samples, <0.001 ppm (<LOD) in 3 refined oil samples, and <0.003-0.003 ppm (<LOQ) in 3 hull samples. Combined residues were each <LOQ in all processed matrices: <0.016 ppm in meal, <0.009 ppm in refined oil, and <0.011 ppm in hull. As residues were <LOQ from all cotton RAC and processed cotton samples, calculation of reliable processing factors was not possible. The maximum theoretical concentration factor for cotton is 6x.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the cotton processing data are classified as scientifically acceptable, pending submission of acceptable supporting storage stability data. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document DP Barcode D309766.

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

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis. There are currently no permanent tolerances established for amicarbazone. The original petitioner was Bayer Corporation; however, Bayer Corporation has since sold amicarbazone to Arvesta Corporation, who is now the petitioner for amicarbazone. In conjunction with the subject tolerance petition, Arvesta has submitted an application for Section 3 registration of a 70% water dispersible granule [WDG, (DF)] formulation (Amicarbazone DF Herbicide; EPA File Symbol No. 66330-UA). The 70% WDG formulation is to be applied as preplant, preemergence, or early postemergence broadcast sprays at 0.22-0.45 lb ai/A for preplant/preemergence application, or 0.10-0.15 lb ai/A for postemergence application. The petitioner has submitted residue data from a processing study on cotton.

The nomenclature and physicochemical properties of amicarbazone are presented below in Tables A.1 and A.2.

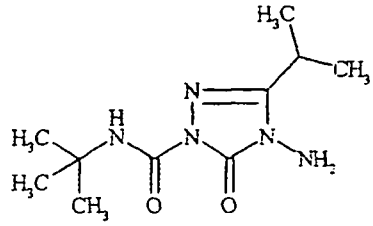
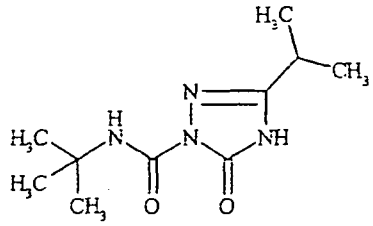
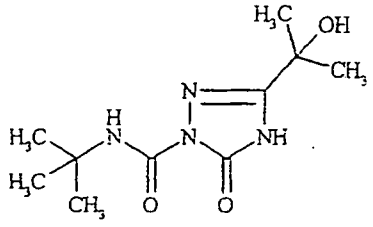
TABLE A.1. Amicarbazone Nomenclature.	
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-*tert*-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS registry number	129909-90-6
End-use formulation (EP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)
DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
*i*Pr-2-OH DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-*H*-1,2,4-triazole-1-carboxamide

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.		
Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-4} Pa @ 25°C 1.30×10^{-4} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, $Log(K_{ow})$	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

In three separate plots, bare soil was treated once with amicarbazone (70% WDG) at 0.450, 2.247, and 2.698 lb ai/A using ground equipment (1x, 5x and 6x rate, Table B.1). The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. A rotational crop of cotton was planted 12 months after treatment (12-month PBI). Seed cotton was harvested using a mechanical picker at commercial maturity (175 DAP) from the 5x treated plot. Cotton did not germinate in the 6x plot and was not collected from the 1x plot, therefore, only data from the 5x test was reported.

TABLE B.1. Study Use Pattern on Cotton						
Location (City, State), Year	Application					
	EP [1]	Method [2]; Timing	Volume (gal/A)	Single Rate [3] (lb a.i./A)	No. of Appl.	Tank Mix Adjuvants
Tifton, GA, 2000	70% WDG	Broadcast soil; bare ground	15.9	0.450 [4]	1	None
			31.9	2.247	1	None
			15.9	2.698 [4]	1	None

1 EP = End-use Product.
2 Applied using ground equipment.
3 These rates represent 1x, 5x, and 6x the seasonal maximum proposed label use rate for any rotated crop (field corn).
4 Cotton did not germinate in the 6x plot and was not collected from the 1x plot, therefore, only data from the 5x test was reported.

B.2. Processing Procedures

After collection, seed cotton samples were placed in frozen storage at the field site within 30 minutes and stored frozen for 15 days, then shipped frozen to FPRDC at Texas A&M University (Bryan, TX) for ginning. Seed cotton samples were ginned to produce undelinted seed samples, which were processed into hulls, meal, and refined oil using simulated commercial procedures. The processed fractions and subsamples of undelinted seed were shipped frozen to the analytical laboratory, Residue Analysis Laboratory at the Bayer Research Park (BRP), Stilwell, KS, (BRP), where they were stored frozen (<-5 °C) prior to analyses. Undelinted seed samples were stored frozen from collection to analysis for up to 10 months. Processed matrices were stored frozen from generation to analysis for up to 14 days.

B.3. Analytical Methodology

Samples of cotton matrices were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 by using an LC-MS/MS method (Bayer Method 200258), which is similar to the proposed enforcement method for plant commodities (Bayer Method 108340). Only a brief description of the method was included in the submission. For a complete description of the method, refer to 46145302.der (currently under review).

Briefly, homogenized samples of each matrix, except oil, were extracted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v). Following filtration and the addition of deuterated internal standards, the residues were cleaned up by solid phase extraction (mixed C_8 and SCX cartridge). For oil samples, residues are extracted by mixing with hexane and then adding an equal volume of 0.1% aqueous acetic acid in ACN:water (4:1, v/v). Residues in the ACN phase are saved, the deuterated internal standards are added, and residues are cleaned up by solid phase extraction. Residues were quantified by LC-MS/MS, using deuterated internal standards of each analyte. Residues of metabolites were reported in parent equivalents. The validated LOQ was 0.01 ppm for each analyte in all cotton matrices. The LODs for the three analytes were calculated based on the recovery data at the LOQ and using the following formula: (SD at the LOQ) x $t_{0.99}$ (for n-1 replicates). The calculated LODs for amicarbazone were 0.002 ppm for cottonseed refined oil and hulls, 0.003 ppm for meal, 0.005 ppm for undelinted seed. The LODs for DA MKH 3586 were 0.006 ppm for undelinted seed, oil and hulls, and 0.009 ppm for meal. The LODs for iPr-2-OH DA MKH 3586 were 0.001 ppm for oil, 0.002 ppm for undelinted seed, and 0.003 ppm for meal and hulls.

In the current submission, the LC-MS/MS method was validated using concurrent method recoveries of cotton undelinted seed and processed cotton matrix samples fortified separately with amicarbazone, DA MKH 3586 and iPr-2-OH DA MKH 3586 at 0.01 ppm.

C. RESULTS AND DISCUSSION

The LC-MS/MS method (Bayer Method 200258) used to determine residues of amicarbazone, DA MKH 3586, and iPr-2OH DA MKH 3586 in/on cotton undelinted seed and processed cotton commodities is adequate for data collection. Average concurrent recoveries from cotton undelinted seed and processed cotton commodities samples fortified separately with amicarbazone, DA MKH 3586 and iPr-2-OH DA MKH 3586 at 0.01 ppm were 88-105% with standard deviations of ± 1-13% (Table C.1). Apparent residues of amicarbazone were <LOQ in/on all control samples. The validated LOQ was 0.01 ppm for amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on all matrices. The LODs for all analytes and matrices ranged from 0.001-0.009 ppm.

TABLE C.1. Summary of Concurrent Recoveries of Amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 from Cotton Matrices.

Matrix	Spiking Level (mg/kg)	Sample size	Amicarbazone		DA MKH 3586		iPr-2-OH DA MKH 3586	
			Recoveries (%)		Recoveries (%)		Recoveries (%)	
			Range	Mean ± sd	Range	Mean ± sd	Range	Mean ± sd
Cotton undelinted seed	0.01	4	95-112	103 ± 7	97-111	104 ± 7	83-100	92 ± 7
Cottonseed meal	0.01	3	96-103	100 ± 4	94-117	102 ± 13	101-107	105 ± 3
Cottonseed refined oil	0.01	3	88-92	90 ± 2	95-109	101 ± 7	87-89	88 ± 1
Cottonseed hulls	0.01	3	103-107	105 ± 2	88-104	96 ± 8	100-106	103 ± 3

The total frozen (< -5° C) storage intervals were 10 months for cotton undelinted seed samples and 9-14 days for processed cotton matrices (Table C.2). In support of the processing study, the petitioner cited storage stability data (45121704.der) submitted in conjunction with a confined rotational crop study. In that review, it was also stated that LC/MS analyses of some metabolites was conducted up to 30 months following sample collection for certain metabolites; however, the petitioner noted that these analyses were not quantitative. These data are insufficient to support the current processing study because quantitative data were not included in MRID 45121704.

TABLE C.2. Summary of Freezer Storage Conditions

Cotton Matrix	Storage Temp. (°C)	Actual Storage Duration (months)[1]	Limit of Demonstrated Storage Stability (months)[2]
Undelinted seed	<-5	10	NA
Hulls, Meal, Refined oil		1	

[1] Extracts were stored frozen for up to 3 days prior to analysis and processed commodities were stored frozen for up to 14 days after processing and prior to analyses.

[2] NA = not available. Adequate storage stability data are not available for frozen plant commodities.

Amicarbazone and DA MKH 3586 residues were each <0.005-<0.006 ppm (each <LOD) and iPr-2-OH DA MKH 3586 residues were 0.004-0.005 ppm (each <LOQ) in/on 3 samples of undelinted seed from cotton planted 12 months following a soil application of amicarbazone at 5x (Table C.3). Combined residues of amicarbazone and its metabolites DA MKH 3586 and iPr-

2-OH DA MKH 3586 (expressed as parent equivalents) in/on undelinted seed were <0.015-<0.016 ppm (<LOQ). Average combined residues were 0.015 ppm in/on undelinted seed samples; the average value was used to calculate the processing factors.

Amicarbazone and DA MKH 3586 residues were each <LOD in all processed cotton matrices. Residues of iPr-2-OH DA MKH 3586 were 0.004 ppm (<LOQ) ppm in 3 meal samples, <0.001 ppm (<LOD) in 3 refined oil samples, and <0.003-0.003 ppm (<LOQ) in 3 hulls samples. Combined residues were each <LOQ in all processed matrices: <0.016 ppm in meal, <0.009 ppm in refined oil, and <0.011 ppm in hulls. As residues were <LOQ from all cotton RAC and processed cotton samples, calculation of reliable processing factors was not possible. The maximum theoretical concentration factor for cotton is 6x.

TABLE C.3. Residue Data from Cotton Processing Study with Amicarbazone (70% WDG).

Trial ID (City, State, Year)	Processed Commodity	Total Rate (lb ai/A)	PBI (days)[1]	Residues (ppm)[2]				Processing Factor[3]
				Amicarbazone	DA MKH 3586	i-Pr-2-OH MKH 3586	Combined Resdiues	
Tifton, GA, 2000	Undelinted seed (RAC)	2.247	364	ND, ND, ND[4]	ND, ND, ND	(0.004, 0.005, 0.004)[4]	<0.015, <0.016, <0.015 **(0.015)**[3]	NA[5]
	Meal			ND, ND, ND	ND, ND, ND	(0.004, 0.004, 0.004)	<0.016, <0.016, <0.016 **(0.016)**	NC[6]
	Refined oil			ND, ND, ND	ND, ND, ND	ND, ND, ND	<0.009, <0.009, <0.009 **(0.009)**	NC[6]
	Hulls			ND, ND, ND	ND, ND, ND	ND, (0.003, 0.003)	<0.011, <0.011, <0.011 **(0.011)**	NC[6]

1 Cotton was planted ~12 months following a single soil application.

2 The LOQ is 0.01 ppm for each analyte in/on each matrix. The LODs for amicarbazone were 0.002 ppm for cottonseed refined oil and hulls, 0.003 ppm for meal, 0.005 ppm for undelinted seed. The LODs for DA MKH 3586 were 0.006 ppm for undelinted seed, refined oil and hulls, and 0.009 ppm for meal. The LODs for iPr-2-OH DA MKH 3586 were 0.001 ppm for refined oil, 0.002 ppm for undelinted seed, and 0.003 ppm for meal and hulls. Total residues are the sum of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 residues in parent equivalents: the LOD was used for individual residues reported at <LOD in calculating total residues.

3 The processing factor was calculated using average total residues (in bold) in the cotton undelinted seed (RAC) and processed fractions.

4 ND = not detected; residues were <LOD. Residues <LOQ, but >LOD are in parentheses.

5 NA= not applicable

6 NC = not calculated. Residues were <LOQ from all cotton RAC samples; therefore, calculation of reliable processing factors was not possible.

D. CONCLUSION

Pending submission of adequate storage stability data, the cotton processing data are adequate. Calculation of reliable processing factors was not possible, since residues were <LOQ from all cottonseed and cotton processed fractions from plant grown in soil treated at 2.25 lbs ai/A (5x the maximum seasonal application rate for plants) at a 12-month PBI.

E. REFERENCES

None

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: 0F6131
DP Barcode: D309766
PC Code: 114004

Template Version September 2003

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.4.1/OPPTS 860.1520/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Processed Food and Feed - Sugarcane

Primary Evaluator	Manying Xue, Chemist RAB3/HED (7509C)	Date: 03/23/05
Approved by	Leung Cheng, Senior Chemist RAB3/HED (7509C)	Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (1910 Sedwick Rd., Building 100, Suite B; Durham, NC 27713; submitted 1/07/2005). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46145306 Krolski, M. (2003) MKH 3586 70 WG - Magnitude of the Residue in Sugarcane Processed Commodities. Lab Project Number: 200460: TMN-0269. Unpublished study prepared by Bayer CropScience. 233 p.

EXECUTIVE SUMMARY:

In a single test conducted in LA during 2000, sugarcane was treated once with amicarbazone (70% WDG) at 1.35, 4.08, and 6.80 lb ai/A in three separate plots using ground equipment. These rates are reported to be 1x, 3x and 5x the maximum use rate proposed for amicarbazone on sugarcane grown outside the U.S.. Sugarcane was harvested using a mechanical chopper at commercial maturity (139 DAT) from the 5x treated plot. Sugarcane samples were not collected from the 1x and 3x tests, therefore, only data from the 5x test were reported. Triplicate samples of sugarcane (RAC) were collected and the remaining bulk samples were processed into bagasse, molasses, and refined sugar using simulated commercial procedures. Prior to analysis, sugarcane samples were stored frozen for a maximum of 23 months, an interval not supported by available stability data, and processed sugarcane matrices were stored frozen for up to 28 days. Data demonstrating the stability of residues of amicarbazone, DA MKH 3586, and iPr-2OH DA MKH 3586 in/on representative plant matrices remain outstanding.

Samples were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 using an adequate LC-MS/MS method (Bayer Method 200258) which is similar to the proposed enforcement method for plant commodities (Bayer Method 108340). Briefly, samples were extracted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v), concentrated and cleaned up by solid phase extraction. Residues were quantified by LC-MS/MS, using deuterated internal standards of each analyte. Residues of metabolites were reported in parent equivalents. The LOQ was 0.01 ppm for each of three analytes in/on all sugarcane matrices. The LODs for amicarbazone were 0.002 ppm for refined sugar, bagasse, molasses, and 0.003 ppm for sugarcane. The LODs for DA MKH 3586 were 0.001 ppm for bagasse, 0.002 ppm for sugarcane and refined sugar, and 0.003 ppm for molasses. The LODs for iPr-2-OH DA

MKH 3586 were 0.001 ppm for refined sugar and bagasse, and 0.002 ppm for sugarcane and molasses.

Amicarbazone residues were each 0.013 ppm, DA MKH 3586 residues were 0.027-0.028 ppm, and iPr-2-OH DA MKH 3586 residues were 0.072-0.075 ppm in/on 3 samples of sugarcane treated at 5x and harvested at maturity. Combined residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 (in parent equivalents) were 0.113-0.114 ppm. Average combined residues were 0.114 ppm in/on sugarcane samples; the average value was used to calculate the processing factors. Average combined residues were <0.008 ppm in refined sugar, 0.782 ppm in molasses, and 0.435 ppm in bagasse. Based on these residues, the processing factors for combined amicarbazone residues were 0.07x for refined sugar, 6.9x for blackstrap molasses, and 3.8x for bagasse. The maximum theoretical concentration factor for sugarcane is >20x.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the sugarcane processing data are classified as scientifically acceptable, pending submission of acceptable supporting storage stability data. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document DP Barcode D309766

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

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis. There are currently no permanent tolerances established for amicarbazone. The original petitioner was Bayer Corporation; however, Bayer Corporation has since sold amicarbazone to Arvesta Corporation, which is now the petitioner for amicarbazone. In conjunction with the subject tolerance petition, Arvesta has submitted an application for Section 3 registration of a 70% water dispersible granule (WDG, DF) formulation (Amicarbazone DF Herbicide; EPA File Symbol No. 66330-UA).

In the U.S., the 70% WDG formulation is being proposed for use on field corn as preplant, preemergence, or early postemergence broadcast sprays at 0.22-0.45 lb ai/A for preplant/preemergence application, or 0.10-0.15 lb ai/A for postemergence application. The petitioner is also proposing a use on sugarcane in other countries; however, detailed information on this use was not available at the time of this review. To support the use on sugarcane, the petitioner has submitted residue data from a processing study on sugarcane.

The nomenclature and physicochemical properties of amicarbazone are presented below in Tables A.1 and A.2.

TABLE A.1. Amicarbazone Nomenclature.

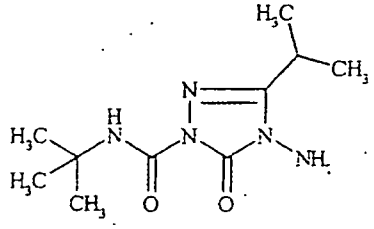
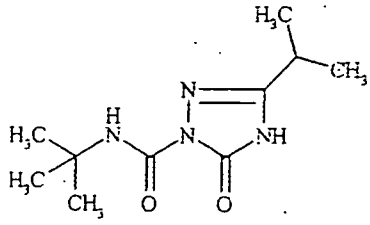
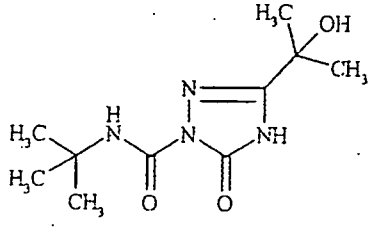
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-*tert*-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS registry number	129909-90-6
End-use formulation (EP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)
DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
*i*Pr-2-OH DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-*H*-1,2,4-triazole-1-carboxamide

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.		
Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-4} Pa @ 25°C 1.30×10^{-4} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, $Log(K_{ow})$	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

In three separate plots, sugarcane was treated once with amicarbazone (70% WDG) at 1.348, 4.083, and 6.804 lb ai/A using ground equipment (Table B.1). Information on the proposed use rate on sugarcane in other countries was not available for this review, but the applied rates were reported to be 1x, 3x, and 5x the proposed use rate on sugarcane. The maximum seasonal use rate for any crop in the U.S. is 0.45 lb ai/A on field corn. Sugarcane was harvested using a mechanical chopper at commercial maturity (139 DAT) from the 5x treated plot. Sugarcane samples were not collected from the 1x and 3x tests; therefore, only data from the 5x test were reported.

TABLE B.1. Study Use Pattern on Sugarcane

Location (City, State), Year	Application					
	EP [1]	Method [2]; Timing	Volume (gal/A)	Single Rate [3] (lb a.i./A)	No. of Appl.	Tank Mix Adjuvants
Rose, LA, 2000	70% WDG	Broadcast foliar, 139 days prior to harvest	18.6	1.348 [4]	1	None
			18.8	4.083 [4]	1	None
			18.8	6.804	1	None

1 EP = End-use Product.
2 Applied using ground equipment.
3 These rates were reported to be 1x, 3x, and 5x the maximum proposed label use rate for sugarcane; however, use directions are not available for sugarcane.
4 Sugarcane was not collected from the 1x and 3x plots, therefore, only data from the 5x test were reported.

B.2. Processing Procedures

After collection, sugarcane samples were placed in frozen storage at the field site within 3.5 hours and stored frozen for 7 days. The samples were then shipped frozen to FPRDC at Texas A&M University (Bryan, TX) for processing. Sugarcane samples were processed into refined sugar, blackstrap molasses, and bagasse using simulated commercial procedures. The processed fractions and subsamples of sugarcane were shipped frozen to the analytical laboratory, Residue Analysis Laboratory at the Bayer Research Park (BRP), Stilwell, KS, where they were stored frozen (<-5 °C) prior to analyses. Sugarcane samples were stored frozen from collection to analysis for up to 23 months. Processed matrices were stored frozen from generation to analysis for up to 28 days.

B.3. Analytical Methodology

Samples of sugarcane matrices were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 by using an LC-MS/MS method (Bayer Method 200258), which is similar to the proposed enforcement method for plant commodities (Bayer Method 108340). Only a brief description of the method was included in the submission. For a complete description of the method, refer to 46145302.der.

Briefly, homogenized samples were extracted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v). Following filtration and the addition of deuterated internal standards, the residues were cleaned up by solid phase extraction (mixed C_8 and SCX cartridge). Residues were quantified by LC-MS/MS, using deuterated internal reference standards of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586. Residues of metabolites were reported in parent equivalents. The validated LOQ was 0.01 ppm for amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on all sugarcane matrices. The LODs for the three analytes were calculated based on the recovery data at the LOQ and using the following formula: (SD at the LOQ) x $t_{0.99}$ (for n-1 replicates). The LODs for amicarbazone were calculated to be 0.002 ppm for refined sugar, bagasse, molasses, and 0.003 ppm for sugarcane (RAC). The LODs for DA MKH 3586 were 0.001 ppm for bagasse, 0.002 ppm for sugarcane and refined sugar, and 0.003 ppm for molasses. The LODs for iPr-2-OH DA MKH 3586 were 0.001 ppm for refined

sugar and bagasse, and 0.002 ppm for sugarcane and molasses. Combined LODs were 0.007 ppm for sugarcane, 0.005 ppm for sugar, 0.007 ppm for molasses, and 0.004 ppm for bagasse

In the current submission, the LC-MS/MS method was validated using concurrent method recoveries of sugarcane and processed sugarcane matrix samples fortified separately with amicarbazone, DA MKH 3586 and iPr-2-OH DA MKH 3586 at 0.01-0.70 ppm.

C. RESULTS AND DISCUSSION

The LC-MS/MS method (Bayer Method 200258) used to determine residues of amicarbazone, DA MKH 3586, and iPr-2OH DA MKH 3586 in/on sugarcane and processed sugarcane commodities is adequate for data collection. Average concurrent recoveries from sugarcane and processed sugarcane commodities samples fortified separately with amicarbazone, DA MKH 3586 and iPr-2-OH DA MKH 3586 at 0.01-0.70 ppm were 90-114% with standard deviations of ± 1-8% (Table C.1). Apparent residues of amicarbazone were <LOQ in/on all control samples. The validated LOQ was 0.01 ppm for amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on all matrices. The LODs for each analyte in each matrix ranged from 0.001-0.003 ppm, and combined LODs were 0.004-0.007 ppm.

TABLE C.1. **Summary of Concurrent Recoveries of Amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 from Sugarcane Matrices.**

Matrix	Spiking Level (mg/kg)	Sample size	Amicarbazone		DA MKH 3586		iPr-2-OH DA MKH 3586	
			Recoveries (%)		Recoveries (%)		Recoveries (%)	
			Range	Mean ± std dev	Range	Mean ± std dev	Range	Mean ± std dev
Sugarcane	0.01	3	91-98	96 ± 4	97-100	98 ± 3	94-98	97 ± 3
	0.10	3	96-100		94-101		94-101	
Refined sugar	0.01	3	100-103	102 ± 2	105-118	114 ± 8	98-99	98 ± 1
Blackstrap molasses	0.01	3	99-104	97 ± 5	96-103	96 ± 5	97-100	97 ± 2
	0.70	3	92-95		91-94		93-96	
Bagasse	0.01	3	88-93	93 ± 4	98-101	94 ± 6	89-92	90 ± 2
	0.20	3	92-98		85-91		87-91	

The total frozen (< -5° C) storage intervals were 23 months for sugarcane samples and 17-28 days for processed sugarcane matrices (Table C.2). In support of the processing study, the petitioner cited storage stability data (45121704.der) submitted in conjunction with a confined rotational crop study. In that review, it was also stated that LC/MS analyses of some metabolites were conducted up to 30 months following sample collection for certain metabolites; however, the petitioner noted that these analyses were not quantitative. These data are insufficient to support the current processing study because quantitative data were not included in MRID 45121704.

TABLE C.2. Summary of Freezer Storage Conditions			
Sugarcane Matrix	Storage Temp. (°C)	Actual Storage Duration (months)[1]	Limit of Demonstrated Storage Stability (months)[2]
Sugarcane	<-5	23	NA
Bagasse, Molasses, Refined sugar		<1	

[1] Extracts were stored frozen for up to 4 days prior to analysis and processed commodities were stored frozen for 17-28 days after processing and prior to analyses.
[2] NA = not available. Adequate storage stability data are not available for frozen plant commodities.

Amicarbazone residues were each 0.013 ppm, DA MKH 3586 residues were 0.027-0.028 ppm, and iPr-2-OH DA MKH 3586 residues were 0.072-0.075 ppm in/on 3 samples of sugarcane treated at 5x and harvested at maturity (Table C.3). Combined residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 (expressed in parent equivalents) were 0.113-0.114 ppm. Average combined residues were 0.114 ppm in/on sugarcane samples; the average value was used to calculate the processing factors.

Amicarbazone residues were <0.002 ppm (<LOD) in 3 refined sugar samples, 0.089-0.090 ppm in 3 molasses samples, and 0.089-0.092 ppm in 3 bagasse samples. DA MKH 3586 residues were <0.002 ppm (<LOD) in 3 refined sugar samples, 0.173-0.189 ppm in 3 molasses samples, and 0.154-0.157 ppm in 3 bagasse samples. Residues of iPr-2-OH DA MKH 3586 were 0.004 ppm (<LOQ) in 3 refined sugar samples, 0.510-0.512 ppm in 3 molasses samples, and 0.187-0.192 ppm in 3 bagasse samples. Average combined residues were <0.008 ppm in refined sugar, 0.782 ppm in molasses, and 0.435 ppm in bagasse. Based on these residues, the processing factors for combined amicarbazone residues were 0.07x for refined sugar, 6.9x for molasses, and 3.8x for bagasse. The maximum theoretical concentration factor for sugarcane is >20x.

TABLE C.3. Residue Data from Sugarcane Processing Study with Amicarbazone (70%-WDG).

Trial ID (City, State, Year)	Processed Commodity	Total Rate (lb ai/A)	PHI (days)[1]	Residues (ppm)[2]				Processing Factor[3]
				Amicarbazone	DA MKH 3586	i-Pr-2-OH MKH 3586	Total	
Rose, LA, 2000	Sugarcane (RAC)	0.802	139	0.013, 0.013, 0.013	0.028, 0.027, 0.027	0.072, 0.074, 0.075	0.113, 0.114, 0.114 **(0.114)**[3]	NA[5]
	Refined sugar			ND, ND, ND[4]	ND, ND, ND	(0.004, 0.004, 0.004)[4]	<0.008, <0.008, <0.008 **(0.008)**	0.07x
	Molasses			0.090, 0.089, 0.089	0.173, 0.189, 0.183	0.512, 0.510, 0.512	0.775, 0.787, 0.784 **(0.782)**	6.86x
	Bagasse			0.092, 0.089, 0.091	0.154, 0.157, 0.156	0.187, 0.188, 0.192	0.433, 0.434, 0.439 **(0.435)**	3.82x

1 PHI = pre-harvest interval; the number of days between application and harvest.
2 The LOQ is 0.01 ppm for each analyte in/on each matrix. The LODs for amicarbazone were 0.002 ppm for refined sugar, bagasse, molasses, and 0.003 ppm for sugarcane. The LODs for DA MKH 3586 were 0.001 ppm for bagasse, 0.002 ppm for sugarcane and refined sugar, and 0.003 ppm for molasses. The LODs for iPr-2-OH DA MKH 3586 were 0.001 ppm for refined sugar and bagasse, and 0.002 ppm for sugarcane and molasses. Total residues are the sum of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 residues in parent equivalents; the LOD was used for individual residues reported at <LOD in calculating total residues.
3 The processing factor was calculated using average total residues (in **bold**) in the sugarcane (RAC) and processed fractions.
4 ND = not detected; residues were <LOD. Residues <LOQ but >LOD are in parentheses.
5 NA= not applicable

D. CONCLUSION

Pending submission of adequate storage stability data, The sugarcane processing data are adequate. The processing factors for combined amicarbazone residues were 0.07x for refined sugar, 6.9x for blackstrap molasses, and 3.8x for bagasse.

E. REFERENCES

None

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: 0F6131
DP Barcode: D309766
PC Code: 114004

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.4.4/OPPTS 860.1900/OECD IIA 6.6.3, 6.8.7 and IIIA 8.6
Field Accumulation in Rotational Crops - Soybean

Primary Evaluator	Manying Xue, Chemist RAB3/HED (7509C)	Date: 03/23/05
Approved by	Leung Cheng, Senior Chemist RAB3/HED (7509C)	Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (1910 Sedwick Rd., Suite B; Durham, NC 27713; submitted 1/07/2005). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46145307 Krolski, M. (2003) MKH 3586 70 WG - Magnitude of the Residues in the Rotational Crop of Soybeans. Lab Project Number: 200461: TMN-0264. Unpublished study prepared by Bayer CropScience. 302 p.

EXECUTIVE SUMMARY:

Twenty field rotational crop trials on soybeans were conducted at field sites throughout the US during 2000. At each of the test sites, bare soil was treated once with amicarbazone (70% WDG) at 0.44-0.47 lb ai/A using ground equipment. The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. A rotational crop of soybeans was planted 22-57 days after treatment at 19 of the 20 test sites. Due to phytotoxicity, soybeans from one test site were replanted the following season (366-day PBI). Duplicate treated samples and a single control sample of soybean forage, hay, and seed were harvested at intervals reflecting normal agricultural practices at each site. Hay samples were allowed to dry in the field for 1-12 days prior to sampling. Samples were stored frozen from collection to analysis for up to 26 months, an interval that is not supported by the available storage stability data. Data demonstrating the stability of residues of amicarbazone, DA MKH 3586, and *i*Pr-2OH DA MKH 3586 in/on representative plant matrices remain outstanding.

Samples were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 using an adequate LC-MS/MS method (Bayer Method 200258) which is similar to the proposed enforcement method for plant commodities (Bayer Method 108340). Briefly, samples were extracted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v), concentrated, and cleaned up by solid phase extraction. Residues were quantified by LC-MS/MS, using deuterated internal standards of each analyte. Residues of metabolites were reported in parent equivalents. The LOQ was 0.01 ppm each for amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 in/on all matrices. The LOD for amicarbazone was 0.003 ppm for soybean forage, hay, and seed. The LOD for DA MKH 3586 was 0.002 ppm for forage, 0.004 ppm for hay, and 0.003 ppm for seed. The LOD for *i*Pr-2-OH DA MKH 3586 was 0.003 ppm for forage, 0.001 ppm for hay, and 0.002 ppm for seed.

For soybeans planted ~1 month post-treatment, residues of amicarbazone were <0.010-0.430 ppm in/on soybean forage, 0.015-1.326 ppm in/on soybean hay, and <0.01 (<LOQ) in/on soybean seed. Residues of DA MKH 3586 were <0.01-0.089 ppm in/on forage, <0.01-0.270 ppm in/on hay, and <0.01 ppm in/on seed samples. Residues of iPr-2OH DA MKH 3586 were 0.143-1.132 ppm in/on forage, 0.562-4.236 ppm in/on hay, and 0.031-0.582 ppm in/on seed. Combined residues were <0.268-1.264 ppm (ave. = 0.750 ppm) in/on soybean forage, <0.629-4.569 ppm (ave. = 2.569 ppm) in/on hay, and <0.051-<0.602 ppm (ave. = 0.225 ppm) in/on seed.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in this study, the field rotational crop data on soybeans are classified as scientifically acceptable, pending submission of acceptable supporting storage stability data. In addition, information pertaining to the weather conditions (including temperatures) over the course of the study, with comparison to historical averages (for both temperature and rainfall), should be submitted for the rotational crop field trials; a discussion of any unusual weather conditions should be included. Because the proposed use pattern of amicarbazone is dependent on soil type, soil pH, and soil organic matter, information pertaining to these characteristics should be submitted for all field trials. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document DP Barcode D309766.

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

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis. There are currently no permanent tolerances established for amicarbazone. The original petitioner was Bayer Corporation; however, Bayer Corporation has since sold amicarbazone to Arvesta Corporation, which is now the petitioner for amicarbazone. In conjunction with the subject tolerance petition, Arvesta has submitted an application for Section 3 registration of a 70% water dispersible granule (WDG, DF) formulation (Amicarbazone DF Herbicide; EPA File Symbol No. 66330-UA). The 70% WDG formulation is to be applied as preplant, preemergence, or early postemergence broadcast sprays at 0.22-0.45 lb ai/A for preplant/preemergence application, or 0.10-0.15 lb ai/A for postemergence application. The petitioner has submitted extended field rotational crop trial data on soybean.

The nomenclature and physicochemical properties of amicarbazone are presented below in Tables A.1 and A.2.

TABLE A.1. Amicarbazone Nomenclature.

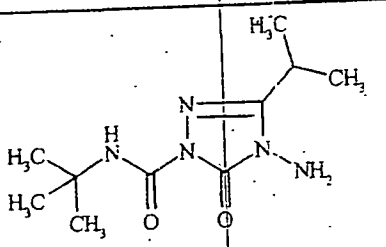
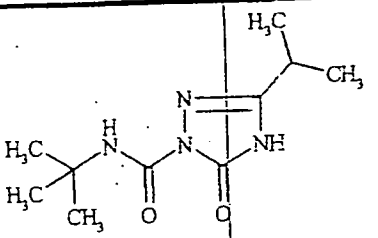
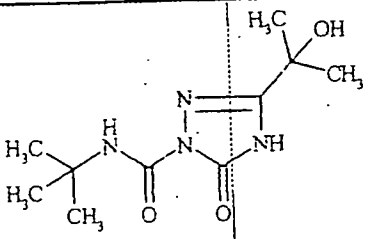
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-*tert*-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS registry number	129909-90-6
End-use formulation (EP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)
DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
*i*Pr-2-OH DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-*H*-1,2,4-triazole-1-carboxamide

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.

Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.		
Parameter	Value	Reference*
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, Xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-6} Pa @ 25°C 1.30×10^{-6} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, Log(K_{ow})	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @ 221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Twenty soybean field rotational crop trials were conducted at field sites throughout the US during 2000. At each of the test sites, bare soil was treated once with amicarbazone (70% WDG) at 0.442-0.467 lb ai/A using ground equipment. The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. A rotational crop of soybeans was planted 22-57 days after treatment at 19 of the 20 test sites, the majority 17 test having PBIs around 30 days. Due to phytotoxicity, soybeans from one test site were replanted the following season (366-day PBI).

TABLE B.1.1. Trial Site Conditions.

Trial Identification (City, State, Year)	Soil characteristics				Meteorological data	
	Type	%OM	pH	CEC (meq/g)	Total rainfall during study period (inches)	Overall monthly temperature range (°C)
Tifton, GA, 2000	Not reported = NR	NR	NR	NR	61.8	NR
Athens, GA, 2000	NR	NR	NR	NR	11.4	NR
Benoit, MS, 2000	NR	NR	NR	NR	10.1	NR
Greenville, MS, 2000	NR	NR	NR	NR	16.6	NR
Washington, LA, 2000	NR	NR	NR	NR	18.1	NR
Richwood, OH, 2000	NR	NR	NR	NR	19.9	NR
Campbell, MN, 2000	NR	NR	NR	NR	10.4	NR
Leonard, MO, 2000	NR	NR	NR	NR	23.9	NR
Dow, IL, 2000	NR	NR	NR	NR	31.5	NR
Carlyle, IL, 2000	NR	NR	NR	NR	31.6	NR
York, NE, 2000	NR	NR	NR	NR	10.7	NR
Kirklin, IN, 2000	NR	NR	NR	NR	21.9	NR
Keysport, IL, 2000	NR	NR	NR	NR	31.6	NR
Dalton, MN, 2000	NR	NR	NR	NR	14.4	NR
Grafton, IL, 2000	NR	NR	NR	NR	30.5	NR
York, NE, 2000	NR	NR	NR	NR	11.6	NR
Williamston, MI, 2000	NR	NR	NR	NR	18.6	NR
New Holland, OH, 2000	NR	NR	NR	NR	16.3	NR
Bagley, IA, 2000	NR	NR	NR	NR	13.5	NR
Richland, IA, 2000	NR	NR	NR	NR	21.8	NR

[1] Detailed meteorological data were not provided.

Detailed meteorological data were not provided. Rainfall was supplemented with irrigation as needed.

TABLE B.1.2. Study Use Pattern.

Location (County, State; Year)	EP[1]	Application				Tank Mix Adjuvants
		Method; Timing	Vol. (GPA[2])	Single Rate (lb ai/A)	No. of Appl.	
Tifton, GA, 2000	70% WDG	broadcast; bare ground	15.9	0.450	1	None
Athens, GA, 2000	70% WDG	broadcast; bare ground	17.0	0.455	1	None
Benoit, MS, 2000	70% WDG	broadcast; bare ground	18.7	0.456	1	None
Greenville, MS, 2000	70% WDG	broadcast; bare ground	12.2	0.449	1	None
Washington, LA, 2000	70% WDG	broadcast; bare ground	13.5	0.456	1	None
Richwood, OH, 2000	70% WDG	broadcast; bare ground	17.0	0.453	1	None
Campbell, MN, 2000	70% WDG	broadcast; bare ground	15.1	0.451	1	None
Leonard, MO, 2000	70% WDG	broadcast; bare ground	17.9	0.453	1	None
Dow, IL, 2000	70% WDG	broadcast; bare ground	14.8	0.446	1	None
Carlyle, IL, 2000	70% WDG	broadcast; bare ground	16.7	0.452	1	None
York, NE, 2000	70% WDG	broadcast; bare ground	19.4	0.446	1	None
Kirklin, IN, 2000	70% WDG	broadcast; bare ground	17.9	0.455	1	None
Keysport, IL, 2000	70% WDG	broadcast; bare ground	16.5	0.446	1	None
Dalton, MN, 2000	70% WDG	broadcast; bare ground	15.0	0.448	1	None
Grafton, IL, 2000	70% WDG	broadcast; bare ground	14.7	0.442	1	None
York, NE, 2000	70% WDG	broadcast; bare ground	19.3	0.445		None
Williamston, MI, 2000	70% WDG	broadcast; bare ground	14.2	0.453	1	None
New Holland, OH, 2000	70% WDG	broadcast; bare ground	15.2	0.461	1	None
Bagley, IA, 2000	70% WDG	broadcast; bare ground	20.2	0.452	1	None
Richland, IA, 2000	70% WDG	broadcast; bare ground	18.6	0.467	1	None

1 EP = End-use Product.
2 All applications were made using ground equipment.

Duplicate treated samples and a single control sample of soybean forage and hay (>2.5 lbs each) were harvested at intervals reflecting normal agricultural practices at each site. 21-66 days after planting (DAP). Hay samples were allowed to dry in the field for 1-12 days prior to sampling. Seed samples were harvested 107-174 DAP. After collection, samples were placed in frozen storage at the test facility within 3 hours of collection, stored frozen for 1-123 days, then shipped frozen by ACDS freezer truck to the analytical laboratory, Bayer Research Park (BRP), Stilwell, KS and stored frozen (<-15° C) prior to analysis. Samples were stored frozen from collection to analysis for up to 26 months.

B.2. Analytical Methodology

Samples of soybean seed, forage and hay were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 by using an LC-MS/MS method (Bayer Method 200258), which is similar to the proposed enforcement method for plant commodities (Bayer Method 108340). Only a brief description of the method was included in the submission. For a complete description of the method, refer to 46145302.der (currently under review).

Briefly, homogenized samples were extracted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v). Following filtration and the addition of deuterated internal standards, the residues were cleaned up by solid phase extraction (mixed C_8 and SCX cartridge). Residues were quantified by LC-MS/MS, using deuterated internal reference standards of amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586. Residues of metabolites were reported in parent equivalents. The validated LOQ was 0.01 ppm for amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 in/on all matrices. The LODs for the three analytes were calculated based on the recovery data at the LOQ and using the following formula: (SD at the LOQ) x $t_{0.99}$ (for n-1 replicates). The calculated LOD was 0.003 ppm for amicarbazone in/on soybean forage, hay, and seed. The LODs for DA MKH 3586 were 0.002 ppm for forage, 0.004 ppm for hay, and 0.003 ppm for seed. The LODs for *i*Pr-2-OH DA MKH 3586 were 0.003 ppm for forage, 0.001 ppm for hay, and 0.002 ppm for seed.

In the current submission, the LC-MS/MS method was validated using concurrent method recoveries of soybean forage, hay, and seed samples fortified separately with amicarbazone, DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 at the method LOQ (0.01 ppm) and at 2.0 ppm (forage), 5.0 ppm (hay) or 0.6 ppm (seed).

C. RESULTS AND DISCUSSION

The number of soybean rotational field trials and the geographic representation of the residue data on soybean forage, hay and seeds are adequate according to the requirements described in OPPTS 860.1500.

The total frozen (< -15° C) storage intervals were 13-26 months for soybean forage, hay, and seed samples (Table C.1). In support of the field rotational crop trials, the petitioner cited storage stability data (45121704.der) submitted in conjunction with a confined rotational crop study. In that review, it was also stated that LC/MS analyses of some metabolites were conducted up to 30 months following sample collection for certain metabolites; however, the petitioner noted that these analyses were not quantitative. These data are insufficient to support the current field rotational crop study because quantitative data were not included in MRID 45121704.

The LC-MS/MS method (Bayer Method 200258) used to determine residues of amicarbazone in/on soybean forage and hay is adequate for data collection. Average concurrent recoveries from soybean forage, hay, and seed were 96-104% with standard deviations of ±4-13% (Table

C.2). Apparent residues of amicarbazone were <LOQ in/on all control samples. The LOQ was 0.01 ppm each for amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 in/on all matrices. Calculated LODs were 0.003 ppm for amicarbazone in/on soybean forage, hay, and seed. The LODs for DA MKH 3586 were 0.002 ppm for forage, 0.004 ppm for hay, and 0.003 ppm for seed. The LODs for *i*Pr-2-OH DA MKH 3586 were 0.003 ppm for forage, 0.001 ppm for hay, and 0.002 ppm for seed. Adequate sample calculations and chromatograms were provided.

TABLE C.1. Summary of Storage Conditions

Matrix	Storage Temp. (°C)	Actual Storage Duration (months)[1]	Limit of Demonstrated Storage Stability (months)[2]
Soybean forage and hay	<-15	13-26	NA

1 Extracts were stored frozen for up to 5 days prior to analysis.
2 NA = not available. Adequate storage stability data are not available for frozen plant commodities.

TABLE C.2. Summary of Concurrent Recoveries of Amicarbazone, DA MKH 3586, and *i*Pr-2-OH DA MKH 3586 from Soybean Forage, Hay, and Seed using Bayer LC-MS/MS Method 200258.

Matrix	Analyte	Spike level (mg/kg)	Sample size (n)	Recoveries (%)	Mean ± std dev (%)
Soybean forage	Amicarbazone	0.01	9	87-107	96 ± 5
		2.0	3	90-98	
	DA MKH 3586	0.01	9	91-105	98 ± 5
		2.0	3	93-102	
	*i*Pr-2-OH DA MKH 3586	0.01	9	88-112	100 ± 7
		2.0	3	94-97	
Soybean hay	Amicarbazone	0.01	8	93-116	104 ± 10
		5.0	3	84-115	
	DA MKH 3586	0.01	8	84-116	98 ± 9
		5.0	3	92-104	
	*i*Pr-2-OH DA MKH 3586	0.01	8	84-107	98 ± 7
		5.0	3	92-96	
Soybean seed	Amicarbazone	0.01	8	86-118	100 ± 11
		0.60	3	90-109	
	DA MKH 3586	0.01	8	96-116	99 ± 13
		0.60	3	75-85	
	*i*Pr-2-OH DA MKH 3586	0.01	8	91-105	98 ± 4
		0.60	3	93-99	

Due to phytotoxicity, soybeans from one trial were replanted the following season (366 day PBI); the PBIs for all remaining tests were 22-57 days, with most tests (17 out of 20) having PBIs of ~1 month. Residue data from the 366-day PBI test are reported (Table C.3), but are not included in the summary data or conclusions.

For soybeans planted ~1 month post-treatment, residues of amicarbazone were <0.010-0.430 ppm in/on 37 soybean forage samples, 0.015-1.326 ppm in/on 37 soybean hay samples, and <0.01 (<LOQ) in/on 39 soybean seed samples (Table C.3). Residues of DA MKH 3586 were <0.01-0.089 ppm in/on forage, <0.01-0.270 ppm in/on hay, and <0.01 ppm in/on seed samples. Residues of *i*Pr-2OH DA MKH 3586 were 0.143-1.132 ppm in/on forage, 0.562-4.236 ppm in/on hay, and 0.031-0.582 ppm in/on seed.

When calculating combined residues of amicarbazone, DA MKH 3586, and *i*Pr-2-OH DA MKH 3586 (expressed in parent equivalents), the LOQ (0.01 ppm) was used for individual residues reported at <LOQ and ½ the LOQ (0.005 ppm) was used for calculating the average. Combined residues were <0.268-1.264 ppm (ave. = 0.750 ppm) in/on soybean forage, <0.629-4.569 ppm (ave. = 2.569 ppm) in/on hay, and <0.051-<0.602 ppm (ave. = 0.225 ppm) in/on seed (Table. C.4).

Common cultural practices were used to maintain plants, and the weather conditions and the maintenance chemicals and fertilizer used in the study did not have a notable impact on the residue data.

TABLE C.3. Amicarbazone Residues in Rotational Soybeans Planted ~1 Month Following a Single Soil Application of Amicarbazone (70% WDG).

Location (County, State, Year)	EPA Region	Variety	Total Rate (lb ai/A)	PBI[1] (days)	Harvest DAP[1]	Matrix	Residues (ppm)[1]			
							Amicarbazone	DA MKH 3586	1-Pr-2-OH MKH 3586	Combined Residues
Tifton, GA, 2000	2	DR62699RR	0.450	366[3]	55	Forage	0.018, 0.016	ND, ND[4]	0.194, 0.176	<0.222, 0.202
Athens, GA, 2000	2	S73-Z5	0.455	30	61	Forage	(0.006, 0.006)[4]	(0.006, 0.009)	0.612, 0.814	0.632, <0.834
Benoit, MS, 2000	4	Pioneer 9492 RR	0.456	22	21	Forage	0.192, 0.182	0.086, 0.089	0.533, 0.615	0.811, 0.885
Greenville, MS; 2000	4	DP 5960 RR	0.449	30	66	Forage	0.042, 0.054	0.030, 0.037	0.421, 0.420	0.494, 0.510
Washington, LA, 2000	4	DP 5806 RR	0.456	29	46	Forage	0.071, 0.062	0.067, 0.038	1.004, 0.834	1.142, 0.933
Richwood, OH, 2000	5	GH 3467 STS	0.453	37	61	Forage	0.048, 0.039	ND, ND	0.449, 0.220	<0.507, <0.268
Campbell, MN, 2000	5	Garst D085	0.451	30	41	Forage	0.183, 0.232	(0.007, 0.008)	0.144, 0.163	<0.337, 0.403
Leonard, MO, 2000	5	NK 3911	0.453	56	40	Forage	0.193, 0.058	0.081, 0.055	0.991, 0.845	1.264, 0.958
Dow, IL, 2000	5	Asgrow 3701	0.446	29	58	Forage	0.032, 0.056	(0.006), 0.012	0.260, 0.545	<0.302, 0.61
Carlyle, IL, 2000	5	William 82	0.452	29	47	Forage	0.306, 0.237	(0.008, 0.008)	0.143, 0.165	<0.459, 0.413
York, NE, 2000	5	Mycogen 5289	0.446	25	57	Forage	0.044, 0.058	0.031, 0.051	0.530, 0.811	0.604, 0.919
Kirklin, IN, 2000	5	Pioneer 93B01	0.455	29	66	Forage	0.098, 0.077	0.014, (0.007)	0.532, 0.490	0.654, 0.575
Keysport, IL, 2000	5	H3429R	0.446	28	45	Forage	0.022, 0.030	0.062, 0.036	0.988, 0.895	1.072, 0.961
Dalton, MN, 2000	5	Vision	0.448	28	37	Forage	0.430, 0.288	0.012, 0.011	0.342, 0.322	0.783, 0.620
Grafton, IL, 2000	5	Asgrow 3701	0.442	29	58	Forage	0.038, 0.055	0.021, 0.022	1.089, 1.132	1.148, 1.209
York, NE, 2000	5	Mycogen 5269	0.445	25	55	Forage	0.153, 0.148	0.036, 0.034	0.968, 0.947	1.157, 1.130
Williamston, MI, 2000	5	Agri-pro 1995	0.453	29	63	Forage	0.114	0.018	0.562	0.693
New Holland, OH, 2000	5	Resnik	0.461	37	49	Forage	0.053, 0.050	(0.005, 0.006)	0.520, 0.577	0.583, <0.633
Bagley, IA, 2000	5	9294	0.452	28	42	Forage	0.100, 0.075	(0.008, 0.007)	0.468, 0.365	<0.577, <0.450
Richland, IA, 2000	5	Pioneer 93B45	0.467	57	56	Forage	0.085, 0.071	0.072, 0.062	1.004, 0.908	1.160, 1.041

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.4.4/OPPTS 860.1900/OECD IIA 6.6.3, 6.8.7 and IIIA 8.6
Field Accumulation in Rotational Crops - Soybean

TABLE C.3. Amicarbazone Residues in Rotational Soybeans Planted ~1 Month Following a Single Soil Application of Amicarbazone (70% WDG).

Location (County, State, Year)	EPA Region	Variety	Total Rate (lb ai/A)	PBI[1] (days)	Harvest DAP[1]	Matrix	Residues (ppm)[3]			
							Amicarbazone	DA MKH 3586	i-Pr-2-OH MKH 3586	Combined Residues
Tifton, GA, 2000	2	DR62699RR	0.450	366[4]	55	Hay	0.042, 0.068	ND, ND	0.777, 0.835	<0.829, <0.913
Athens, GA, 2000	2	S73-Z5	0.455	30	61	Hay	0.015, 0.020	0.023, 0.018	2.451, 2.624	2.490, 2.662
Benoit, MS, 2000	4	Pioneer 9492 RR	0.456	22	34	Hay	0.272, 0.235	0.270, 0.092	3.389, 2.417	3.931, 2.744
Greenville, MS, 2000	4	DP 5960 RR	0.449	30	66	Hay	0.209, 0.237	0.019, 0.022	2.690, 2.652	2.918, 2.910
Washington, LA, 2000	4	DP 5806 RR	0.456	29	46	Hay	0.183, 0.160	0.230, 0.119	2.854, 2.889	3.167, 3.167
Richwood, OH, 2000	5	GH 3467 STS	0.453	37	61	Hay	0.133, 0.096	ND, ND	0.783, 0.755	<0.926, <0.861
Campbell, MN, 2000	5	Garst D085	0.451	30	41	Hay	0.681, 0.647	0.040, 0.039	0.792, 0.647	1.513, 1.332
Leonard, MO, 2000	5	NK 3911	0.453	56	40	Hay	0.143, 0.123	0.070, 0.074	1.804, 2.006	2.016, 2.202
Dow, IL, 2000	5	Asgrow 3701	0.446	29	69	Hay	0.241, 0.239	0.021, 0.020	1.681, 1.926	1.943, 2.184
Carlyle, IL, 2000	5	William 82	0.452	29	47	Hay	0.150, 0.114	0.183, 0.158	4.236, 3.867	4.569, 4.138
York, NE, 2000	5	Mycogen 5289	0.446	25	57	Hay	0.501, 0.504	0.169, 0.195	3.054, 2.896	3.724, 3.595
Kirklin, IN, 2000	5	Pioneer 93B01	0.455	29	66	Hay	0.057, 0.063	(0.005, 0.009)	0.562, 0.567	<0.629, <0.641
Keysport, IL, 2000	5	H3429R	0.446	28	45	Hay	0.061, 0.081	0.076, 0.085	2.643, 3.158	2.780, 3.324
Dalton, MN, 2000	5	Vision	0.448	28	37	Hay	1.158, 1.325	0.039, 0.048	1.588, 1.638	2.785, 3.011
Grafton, IL, 2000	5	Asgrow 3701	0.442	29	69	Hay	0.102, 0.097	0.045, 0.031	3.260, 2.540	3.406, 2.667
York, NE, 2000	5	Mycogen 5269	0.445	25	55	Hay	0.406, 0.408	0.100, 0.107	3.402, 3.387	3.908, 4.036
Williamston, MI, 2000	5	Agri-pro 1995	0.453	29	63	Hay	0.242	0.034	1.408	1.683
New Holland, OH, 2000	5	Resnik	0.461	37	49	Hay	0.180, 0.158	0.013, 0.012	2.089, 1.996	2.281, 2.166
Bagley, IA, 2000	5	9294	0.452	28	42	Hay	0.232, 0.173	0.014, (0.007)	1.232, 0.934	1.478, <1.115
Richland, IA, 2000	5	Pioneer 93B45	0.467	57	57	Hay	0.135, 0.146	0.040, 0.046	2.880, 2.905	3.055, 3.097

TABLE C.3. Amicarbazone Residues in Rotational Soybeans Planted ~1 Month Following a Single Soil Application of Amicarbazone (70% WDG).

Location (County, State, Year)	EPA Region	Variety	Total Rate (lb ai/A)	PBI[1] (days)	Harvest DAP[1]	Matrix	Residues (ppm)[2]			
							Amicarbazone	DA MKH 3586	iPr-2-OH MKH 3586	Combined Residues
Tifton, GA, 2000	2	DR62699RR	0.450	366[4]	174	Seed	ND, ND	ND, ND	0.045, 0.049	<0.065, <0.069
Athens, GA, 2000	2	S73-Z5	0.455	30	138	Seed	ND, ND	ND, ND	0.268, 0.224	<0.288, <0.244
Benoit, MS, 2000	4	Pioneer 9492 RR	0.456	22	107	Seed	ND, ND	ND, ND	0.184, 0.225	<0.204, <0.244
Greenville, MS, 2000	4	DP 5960 RR	0.449	30	151	Seed	ND, ND	ND, ND	0.071, 0.087	<0.091, <0.107
Washington, LA, 2000	4	DP 5806 RR	0.456	29	119	Seed	ND, ND	ND, ND	0.088, 0.072	<0.108, <0.092
Richwood, OH, 2000	5	GH 3467 STS	0.453	37	120	Seed	ND, ND	ND, ND	0.051, 0.068	0.071, 0.088
Campbell, MN, 2000	5	Garst D085	0.451	30	115	Seed	ND, ND	ND, ND	0.582, 0.556	0.602, 0.576
Leonard, MO, 2000	5	NK 3911	0.453	56	116	Seed	ND, ND	ND, ND	0.293, 0.242	<0.313, <0.262
Dow, IL, 2000	5	Asgrow 3701	0.446	29	124	Seed	ND, ND	ND, ND	0.046, 0.042	<0.066, <0.062
Carlyle, IL, 2000	5	William 82	0.452	29	103	Seed	ND, ND	ND, ND	0.033, 0.037	0.052, 0.057
York, NE, 2000	5	Mycogen 5289	0.446	25	109	Seed	ND, ND	ND, ND	0.291, 0.244	<0.311, <0.264
Kirklin, IN, 2000	5	Pioneer 93B01	0.455	29	129	Seed	ND, ND	ND, ND	0.344, 0.357	0.364, <0.377
Keysport, IL, 2000	5	H3429R	0.446	28	136	Seed	ND, ND	ND, ND	0.150, 0.218	0.170, <0.238
Dalton, MN, 2000	5	Vision	0.448	28	108	Seed	ND, ND	ND, ND	0.137, 0.142	0.157, 0.162
Grafton, IL, 2000	5	Asgrow 3701	0.442	29	124	Seed	ND, ND	ND, ND	0.366, 0.389	<0.386, <0.409
York, NE, 2000	5	Mycogen 5269	0.445	25	112	Seed	ND, ND	ND, ND	0.286, 0.289	<0.303, <0.305
Williamston, MI, 2000	5	Agri-pro 1995	0.455	29	135	Seed	ND, (0.003)	ND, ND	0.509, 0.539	<0.529, <0.559
New Holland, OH, 2000	5	Resnik	0.461	37	124	Seed	ND, ND	ND, ND	0.088, 0.097	0.108, 0.117
Bagley, IA, 2000	5	9294	0.452	28	114	Seed	ND, ND	ND, ND	0.031, 0.034	<0.051, <0.054
Richland, IA, 2000	5	Pioneer 93B45	0.467	57	120	Seed	ND, ND	ND, ND	0.060, 0.053	0.080, 0.073

[1] DAP = Days After Planting; PBI = Plant Back Interval.
[2] The LOQ is 0.01 ppm for each analyte in/on each matrix. The limit of detection (LOD) for amicarbazone was calculated to be 0.003 ppm for soybean forage, hay, and seed. The LODs for DA MKH 3586 were 0.002 ppm for forage, 0.004 ppm for hay, and 0.003 ppm for seed. The LOD for iPr-2-OH DA MKH 3586 were 0.003 ppm for forage, 0.001 ppm for hay, and 0.002 ppm for seed. Total residues are the sum of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 residues in parent equivalents; the LOQ was used for individual residues reported at LOQ in calculating total residues.

[3] Due to phytotoxicity, soybeans from this trial were replanted the following season.
[4] ND = not detected; residues were <LOD. Residues <LOQ but >LOD are reported in parentheses.

TABLE C.4. Summary of Residue Data in Rotational Soybeans Planted ~1 Month Following a Single Soil Application of Amicarbazone (70% WDG). [1]

Soybean Matrix	Total Rate (lb ai/A)	PBI (days)	Residue Levels (ppm) [2]						
			n	Min.	Max.	HAFT [3]	Median (STMdR[4])	Mean (STMR[4])	Std. Dev.
Amicarbazone									
Forage	0.44-0.47	22-57 [5]	37	<0.01	0.430	0.359	0.071	0.108	0.095
Hay			37	0.015	1.326	1.242	0.173	0.268	0.285
Seed			38	<0.01	<0.01	<0.01	0.005	0.005	0
DA MKH 3586									
Forage	0.44-0.47	22-57	37	<0.01	0.089	0.088	0.018	0.028	0.027
Hay			37	<0.01	0.270	0.182	0.040	0.067	0.069
Seed			38	<0.01	<0.01	<0.01	0.005	0.005	0
iPr-2-OH DA MKH 3586									
Forage	0.44-0.47	22-57	37	0.143	1.132	1.111	0.545	0.612	0.298
Hay			37	0.562	4.236	4.052	2.451	2.232	0.995
Seed			38	0.031	0.582	0.569	0.167	0.205	0.161
Combined Residues									
Forage	0.44-0.47	22-57	37	<0.268	1.264	1.179	0.654	0.750	0.289
Hay			37	<0.629	4.569	4.354	2.744	2.569	1.038
Seed			38	<0.051	<0.602	<0.589	0.187	0.225	0.161

[1] Due to phytotoxicity, soybeans from one trial were replanted the following season (366 day PBI); data from this trial were not included in the residue calculations.
[2] The LOQ is 0.01 ppm for each analyte in/on each matrix. The LOD for amicarbazone was calculated to be 0.003 ppm for soybean forage, hay, and seed. The LODs for DA MKH 3586 were 0.002 ppm for forage, 0.004 ppm for hay, and 0.003 ppm for seed. The LODs for iPr-2-OH DA MKH 3586 were 0.003 ppm for forage, 0.001 ppm for hay, and 0.002 ppm for seed. Total residues are the sum of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 residues in parent equivalents; the LOQ was used for individual residues reported at <LOQ in calculating total residues.
[3] HAFT = Highest Average Field Trial.
[4] STMdR = Supervised Trial Median Residue; STMR = Supervised Trial Mean Residue. For calculation of the median, mean and standard deviation, ½ the LOQ (0.005 ppm) was used for residues reported at <LOQ.
[5] With the exception of two tests with PBIs of 56 and 57 days, the PBIs were generally around 30 days.

D. CONCLUSION

Pending submission of adequate supporting storage stability data, the extended soybean field rotational crop data are adequate.

E. REFERENCES

None

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: 0F6131
DP Barcode: D309766
PC Code: 114004

Template Version September 2003

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.4.1/OPPTS 860.1520/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Processed Food and Feed - Soybeans

Primary Evaluator	Manying Xue, Chemist RAB3/HED (7509C)	Date: 03/23/05
Approved by	Leung Cheng, Senior Chemist RAB3/HED (7509C)	Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (1910 Sedwick Rd., Building 100, Suite B; Durham, NC 27713; submitted 1/07/2005). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46145308 Krolski, M. (2003) MKH 3586 70 WG - Magnitude of the Residue in Processed Commodities from Rotational Crop Soybeans and Request for Waiver of the Study on Magnitude of the Residue in Soybean Aspirated Grain Fractions. Lab Project Number: 200462: TMN-0263. Unpublished study prepared by Bayer CropScience. 236 p.

EXECUTIVE SUMMARY:

In a single test conducted in KS during 2000, bare soil was treated once with amicarbazone (70% WDG) at 0.45, 0.135, or 2.25 lb ai/A using ground equipment. The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. A rotational crop of soybeans was planted immediately following treatment (0-day PBI). Soybean seed were harvested at commercial maturity (144 DAT) from the 1x treated plot. Soybeans did not germinate in two plots treated at 3x and 5x, therefore, only data from the 1x test was reported. Triplicate samples of soybean seed (RAC) were collected and the remaining bulk samples were processed into hulls, meal, and deodorized oil using simulated commercial procedures. Prior to analysis, soybean seed samples were stored frozen for a maximum of 24 months, an interval not supported by available stability data, and processed soybean matrices were stored frozen for up to 14 days. Data demonstrating the stability of residues of amicarbazone, DA MKH 3586, and iPr-2OH DA MKH 3586 in/on representative plant matrices remain outstanding.

Samples were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 using an adequate LC-MS/MS method (Bayer Method 200258), which is similar to the proposed enforcement method for plant commodities (Bayer Method 108340). Briefly, samples were extracted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v), concentrated and cleaned up by solid phase extraction. Oil samples are also initially partitioned with hexane. Residues were quantified by LC-MS/MS, using deuterated internal standards of each analyte. Residues of metabolites were reported in parent equivalents. The LOQ was 0.01 ppm each for analyte in/on all matrices. The LODs for amicarbazone were 0.003 ppm for soybean seed and oil, 0.004 ppm for meal, and 0.009 ppm for hulls. The LODs for DA MKH 3586 were 0.002 ppm for meal, 0.003 ppm for seed, 0.005 ppm for oil, and 0.010 ppm for hulls.

The LODs for iPr-2-OH DA MKH 3586 were 0.002 ppm for seed and meal, 0.003 ppm for oil, and 0.009 ppm for hulls.

Combined residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 (expressed in parent equivalents) were <0.172-<0.181 ppm in/on 3 soybean seed samples Average combined residues were 0.175 ppm in/on seed samples.

Amicarbazone and DA MKH 3586 residues were each <LOD in all processed soybean matrices Residues of iPr-2-OH DA MKH 3586 were 0.147-0.157 ppm in 3 hulls samples, 0.210-0.218 ppm in 3 meal samples, and <0.003 ppm (<LOD) in 3 deodorized oil samples. Combined residues were <0.166-<0 176 ppm in hulls, <0.216-<0.224 ppm in meal, and <0.011 ppm in deodorized oil Average combined residues were 0.171 ppm in hulls, 0.220 ppm in meal, and 0.011 ppm in oil Based on average residues, the processing factors for combined amicarbazone residues were 1.0x for hulls, 1.3x for meal, and <0.1x for oil. The maximum theoretical concentration factor for soybeans is 12x.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

Under the conditions and parameters used in the study, the soybean processing data except for AGF are classified as scientifically acceptable, pending submission of acceptable supporting storage stability data. Aspirated grain fractions (AGF) were not generated and the petitioner requested a waiver for the requirement to generate AGF residue data. The request for a waiver for AGF data and the acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D309766].

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

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis. There are currently no permanent tolerances established for amicarbazone. The original petitioner was Bayer Corporation; however, Bayer Corporation has since sold amicarbazone to Arvesta Corporation, who is now the petitioner for amicarbazone. In conjunction with the subject tolerance petition, Arvesta has submitted an application for Section 3 registration of a 70% water dispersible granule (WDG, DF) formulation (Amicarbazone DF Herbicide; EPA File Symbol No. 66330-UA). The 70% WDG formulation is to be applied as preplant, preemergence, or early postemergence broadcast sprays at 0.22-0.45 lb ai/A for preplant/preemergence application, or 0.10-0.15 lb ai/A for

postemergence application. The petitioner has submitted residue data from a processing study on soybeans.

The nomenclature and physicochemical properties of amicarbazone are presented below in Tables A.1 and A.2.

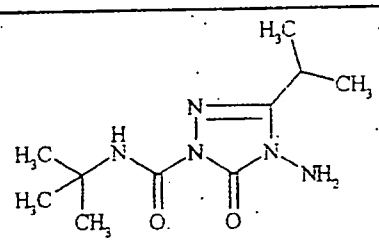
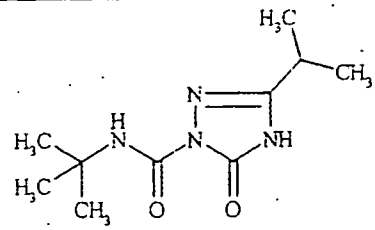
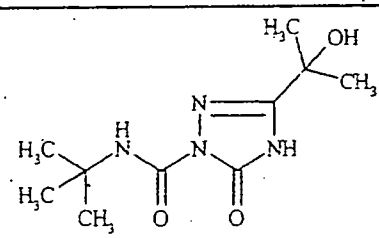
TABLE A.1. Amicarbazone Nomenclature.	
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-tert-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS registry number	129909-90-6
End-use formulation (EP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)
DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
*i*Pr-2-OH DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-*H*-1,2,4-triazole-1-carboxamide

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.		
Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-6} Pa @ 25°C 1.30×10^{-6} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, Log(K_{ow})	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

In three separate plots, bare soil was treated once with amicarbazone (70% WDG) at 0.451, 1.352, and 2.253 lb ai/A using ground equipment (1x, 3x and 5x rate, Table B.1). The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. A rotational crop of soybeans was planted immediately following treatment (0-day PBI). Soybean seed was harvested at commercial maturity (144 DAT) from the 1x treated plot. Soybeans did not germinate in the 3x and 5x plots, therefore, only data from the 1x test was reported.

TABLE B.1. Study Use Pattern on Soybeans						
Location (City, State), Year	Application					
	EP [1]	Method [2]; Timing	Volume (gal/A)	Single Rate [3] (lb a.i./A)	No. of Appl.	Tank Mix Adjuvants
Stilwel, KS, 2000	70% WDG	Broadcast soil; bare ground	10.1	0.451	1	None
			10.1	1.352 [4]	1	None
			10.1	2.253 [4]	1	None

1 EP = End-use Product.
2 Applied using ground equipment
3 These rates represent 1x, 3x, and 5x the seasonal maximum proposed label use rate for any rotated crop (field corn)
4 Soybeans did not germinate in the 3x and 5x plots, therefore, only data from the 1x test was reported

B.2. Processing Procedures

After collection, soybean seed samples were placed in frozen storage at the field site within 30 minutes and stored frozen for 8 days, then shipped frozen to FPRDC at Texas A&M University (Bryan, TX). Seed samples were processed into hulls, meal, and deodorized oil using simulated commercial procedures. Aspirated grain fractions (AGF) were not generated and the petitioner requested a waiver for the requirement to generated AGF residue data. The processed fractions and subsamples of seed were shipped frozen to the analytical laboratory, Residue Analysis Laboratory at the Bayer Research Park (BRP), Stilwell, KS, (BRP), where they were stored frozen (<-5 °C) prior to analyses. Seed samples were stored frozen from collection to analysis for up to 24 months. Processed matrices were stored frozen from generation to analysis for up to 14 days.

B.3. Analytical Methodology

Samples of soybean matrices were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 using an LC-MS/MS method (Bayer Method 200258), which is similar to the proposed enforcement method for plant commodities (Bayer Method 108340). Only a brief description of the method was included in the submission. For a complete description of the method, refer to 46145302.der (currently under review).

Briefly, homogenized samples of each matrix, except oil, were extracted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v). Following filtration and the addition of deuterated internal standards, the residues were cleaned up by solid phase extraction (mixed C_8 and SCX cartridge). For oil samples, residues are extracted by mixing with hexane and then adding an equal volume of 0.1% aqueous acetic acid in ACN:water (4:1, v/v). Residues in the ACN phase are saved, the deuterated internal standards are added, and residues are cleaned up by solid phase extraction. Residues were quantified by LC-MS/MS, using deuterated internal standards of each analyte. Residues of metabolites were reported in parent equivalents. The validated LOQ was 0.01 ppm for each analyte in all soybean matrices. The LODs for the three analytes were calculated based on the recovery data at the LOQ and using the following formula: (S.D at the LOQ) x $t_{0.99}$ (for n-1 replicates). The calculated LODs for amicarbazone were 0.003 ppm for soybean seed and oil, 0.004 ppm for meal, and 0.009 ppm for hulls. The LODs for DA MKH 3586 were 0.002 ppm for meal, 0.003 ppm for seed, 0.005 ppm for oil, and 0.010 ppm for hulls. The LODs for iPr-2-OH DA MKH 3586 were 0.002 ppm for seed and meal, 0.003 ppm for oil, and 0.009 ppm for hulls. The combined LODs were 0.008 ppm for seed and meal, 0.011 ppm for oil, and 0.028 ppm for hulls.

In the current submission, the LC-MS/MS method was validated using concurrent method recoveries of soybean seed and processed soybean matrix samples fortified separately with amicarbazone, DA MKH 3586 and iPr-2-OH DA MKH 3586 at 0.01-0.30 ppm.

C. RESULTS AND DISCUSSION

The LC-MS/MS method (Bayer Method 200258) used to determine residues of amicarbazone, DA MKH 3586, and iPr-2OH DA MKH 3586 in/on soybean seed and processed soybean commodities is adequate for data collection. Average concurrent recoveries from soybean seed and processed soybean commodities samples fortified separately with amicarbazone, DA MKH 3586 and iPr-2-OH DA MKH 3586 at 0.01-0.30 ppm were 89-106% with standard deviations of ± 2-13% (Table C.1). Apparent residues of amicarbazone were <LOQ in/on all control samples. The validated LOQ was 0.01 ppm for amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on all matrices. The LODs for all analytes and matrices ranged from 0.002-0.010 ppm.

TABLE C.1. Summary of Concurrent Recoveries of Amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 from Soybean Matrices.

Matrix	Spiking Level (mg/kg)	Sample size	Amicarbazone		DA MKH 3586.		iPr-2-OH DA MKH 3586	
			Recoveries (%)		Recoveries (%)		Recoveries (%)	
			Range	Mean ± std dev	Range	Mean ± std dev	Range	Mean ± std dev
Soybean seed	0.01	3	101-109	106 ± 4	98-108	100 ± 6	86-96	95 ± 5
	0.20	3	100-111		94-99		97-100	
Soybean hulls	0.01	3	80-103	98 ± 9	81-111	93 ± 13	79-104	95 ± 9
	0.20	3	97-105		82-94		95-103	
Soybean meal	0.01	3	92-101	101 ± 5	106-110	101 ± 7	97-100	98 ± 2
	0.30	3	100-105		92-96		95-98	
Deodorized oil	0.01	3	94-100	97 ± 3	93-104	97 ± 6	87-93	89 ± 3

The total frozen (< -5° C) storage intervals was 24 months for soybean seed samples and 9-14 days for processed soybean matrices (Table C.2). In support of the processing study, the petitioner cited storage stability data (45121704.der) submitted in conjunction with a confined rotational crop study. In that review, it was also stated that LC/MS analyses of some metabolites was conducted up to 30 months following sample collection for certain metabolites; however, the petitioner noted that these analyses were not quantitative. These data are insufficient to support the current processing study because quantitative data were not included in MRID 45121704.

TABLE C.2. Summary of Freezer Storage Conditions

Soybean Matrix	Storage Temp (°C)	Actual Storage Duration (months)[1]	Limit of Demonstrated Storage Stability (months)
Seed	-5	24	NA[2]
Hulls, Meal, Deodorized oil		<1	

1 Extracts were stored frozen for up to 3 days prior to analysis and processed commodities were stored frozen for up to 14 days after processing and prior to analyses

2 NA = not available. Adequate storage stability data are not available for frozen plant commodities

Amicarbazone and DA MKH 3586 residues were each <0.003 ppm (<LOD) and iPr-2-OH DA MKH 3586 residues were 0.166-0.175 ppm in/on 3 samples of soybean seed treated at 1x and harvested at maturity (Table C.3). Combined residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 (expressed in parent equivalents) were <0.172-<0.181 ppm. Average combined residues were 0.175 ppm in/on seed samples; the average value was used to calculate the processing factors.

Amicarbazone and DA MKH 3586 residues were each <LOD in all processed soybean matrices. Residues of iPr-2-OH DA MKH 3586 were 0.147-0.157 ppm in 3 hulls samples, 0.210-0.218 ppm in 3 meal samples, and <0.003 ppm (<LOD) in 3 deodorized oil samples. Combined residues were <0.166-<0.176 ppm in hulls, <0.216-<0.224 ppm in meal, and <0.011 ppm in deodorized oil. Average combined residues were 0.171 ppm in hulls, 0.220 ppm in meal, and 0.011 ppm in deodorized oil. Based on these residues, the processing factors for combined amicarbazone residues were 0.98x for hulls, 1.26x for meal, and 0.06x for deodorized oil. The maximum theoretical concentration factor for soybeans is 12x. Aspirated grain fractions (AGF) were not generated and the petitioner requested a waiver for the requirement to generate AGF residue data.

TABLE C.3. Residue Data from Soybean Processing Study with Amicarbazone (70% WDG).

Trial ID (City, State, Year)	Processed Commodity	Total Rate (lb ai/A)	PHI (days)	Residues (ppm)[2]				Processing Factor[3]
				Amicarbazone	DA MKH 3586	iPr-2-OH MKH 3586	Combined Residues	
Stillwell, KS, 2000	Seed (RAC)	0.451	144	ND, ND, ND[4]	ND, ND, ND	0.166, 0.166, 0.175	<0.172, <0.172, <0.181 **(0.175)**	NA[5]
	Hulls			ND, ND, ND	ND, ND, ND	0.147, 0.151, 0.157	<0.166, <0.170, <0.176 **(0.171)**	0.98x
	Meal			ND, ND, ND	ND, ND, ND	0.218, 0.213, 0.210	<0.224, <0.219, <0.216 **(0.220)**	1.26x
	Deodorized oil			ND, ND, ND	ND, ND, ND	ND, ND, ND	<0.011, <0.011, <0.011 **(0.011)**	0.06x

1 A single soil application was made at 0.45 lb ai/A (1x) immediately prior to planting soybeans.

2 The LOQ is 0.01 ppm for each analyte in/on each matrix. The LODs for amicarbazone were 0.003 ppm for soybean seed and oil, 0.004 ppm for meal, and 0.009 ppm for hulls. The LODs for DA MKH 3586 were 0.002 ppm for meal, 0.003 ppm for seed, 0.005 ppm for oil, and 0.010 ppm for hulls. The LODs for iPr-2-OH DA MKH 3586 were 0.002 ppm for seed and meal, 0.003 ppm for oil, and 0.009 ppm for hulls. Total residues are the sum of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 residues in parent equivalents; the LOD was used for individual residues reported at <LOD in calculating total residues.

3 The processing factor was calculated using average total residues (in bold) in the soybean seed (RAC) and processed fractions.

4 ND = not detected; residues were <LOD.

5 NA= not applicable

D. CONCLUSION

The processing factors for amicarbazone residues were 1.0x for hulls, 1.3x for meal, and <0.1x for deodorized oil, pending submission of adequate storage stability data. Data for AGF were not provided.

E. REFERENCES

None

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: 0F6131
DP Barcode: D309766
PC Code: 114004

Template Version September 2003

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.4.4/OPPTS 860.1900/OECD IIA 6.6.3, 6.8.7 and IIIA 8.6
Field Accumulation in Rotational Crops - Wheat

Primary Evaluator	Manying Xue, Chemist RAB3/HED (7509C)	Date: 03/23/05
Approved by	Leung Cheng, Senior Chemist RAB3/HED (7509C)	Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (1910 Sedwick Rd., Suite B; Durham, NC 27713; submitted 1/07/2005). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46145309 Krolski, M. (2003) MKH 3586 70 WG - Magnitude of the Residues in the Rotational Crop of Wheat. Lab Project Number: 200463: TMN-0286. Unpublished study prepared by Bayer CropScience. 395 p.

EXECUTIVE SUMMARY:

Twenty rotational crop field trials on winter wheat were conducted at sites throughout the US during 2000-2001. At each of the test sites, bare soil was treated once with amicarbazone (70% WDG) at 0.40-0.46 lb ai/A using ground equipment. The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. At each site, a rotational crop of wheat was planted 115-130 days after treatment (~4-month plant-back interval, PBI). Duplicate treated samples and a single control sample of wheat forage, hay, grain, and straw were harvested at intervals reflecting normal agricultural practices at each site. Hay samples were allowed to dry in the field for 2-19 days prior to sampling. Samples were stored frozen from collection to analysis for up to 19 months, an interval that is not supported by the available storage stability data. Data demonstrating the stability of residues of amicarbazone, DA MKH 3586, and iPr-2OH DA MKH 3586 in/on representative plant matrices remain outstanding.

Samples were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 using an adequate LC-MS/MS method (Bayer Method 200258) which is similar to the proposed enforcement method for plant commodities (Bayer Method 108340). Briefly, samples were extracted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v), concentrated and cleaned up by solid phase extraction. Residues were quantified by LC-MS/MS, using deuterated internal standards of each analyte. Residues of metabolites were reported in parent equivalents. The LOQ was 0.01 ppm each analyte in/on all matrices. The calculated LOD for amicarbazone was 0.001 ppm for wheat grain, 0.002 ppm for forage and hay, and 0.005 ppm for straw. The LOD for DA MKH 3586 was 0.002 ppm for forage, hay, and grain, and 0.003 ppm for straw. The LOD for iPr-2-OH DA MKH 3586 was 0.001 ppm for hay and grain, 0.002 ppm for forage, and 0.004 ppm for straw.

Residues of amicarbazone were <0.010-0.249 ppm in/on 40 wheat forage samples, <0.010-0.566 ppm in/on 40 wheat hay samples, and <0.010 (all <LOQ) in/on 40 wheat grain samples, and <0.010-0.280 ppm in/on 40 wheat straw samples. Residues of DA MKH 3586 were <0.01-0.184 ppm in/on forage, <0.01-0.029 ppm in/on hay, <0.01 ppm in/on grain, and <0.01-0.045 ppm in/on straw. Residues of iPr-2OH DA MKH 3586 were <0.10-0.166 ppm in/on forage, 0.027-0.308 ppm in/on hay, <0.010-0.0.075 ppm in/on grain, and <0.01-0.236 ppm in/on straw. Combined residues were <0.030-0.485 ppm (ave. = 0.136 ppm) in/on wheat forage, <0.040-0.878 ppm (ave. = 0.159 ppm) in/on hay, <0.030-<0.095 ppm (ave. = 0.033 ppm) in/on grain, and <0.030-0.450 ppm (ave. = 0.109 ppm) in/on straw.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in this study, the field rotational crop data on wheat are classified as scientifically acceptable, pending submission of acceptable supporting storage stability data. In addition, information pertaining to the weather conditions (including temperatures) over the course of the study, with comparison to historical averages (for both temperature and rainfall), should be submitted for the rotational crop field trials; a discussion of any unusual weather conditions should be included. Because the proposed use pattern of amicarbazone is dependent on soil type, soil pH, and soil organic matter, information pertaining to these characteristics should be submitted for all field trials. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document DP Barcode D309766.

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

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis. There are currently no permanent tolerances established for amicarbazone The original petitioner was Bayer Corporation; however, Bayer Corporation has since sold amicarbazone to Arvesta Corporation, which is now the petitioner for amicarbazone. In conjunction with the subject tolerance petition, Arvesta has submitted an application for Section 3 registration of a 70% water dispersible granule (WDG, DF) formulation (Amicarbazone DF Herbicide; EPA File Symbol No. 66330-UA). The 70% WDG formulation is to be applied as preplant, preemergence, or early postemergence broadcast sprays at 0.22-0.45 lb ai/A for preplant/preemergence application, or 0.10-0.15 lb ai/A for postemergence application. The petitioner has submitted rotational crop field trial data on wheat.

The nomenclature and physicochemical properties of amicarbazone are presented below in Tables A.1 and A.2.

TABLE A.1. Amicarbazone Nomenclature.

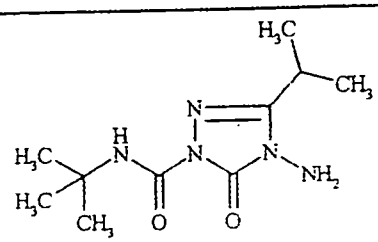
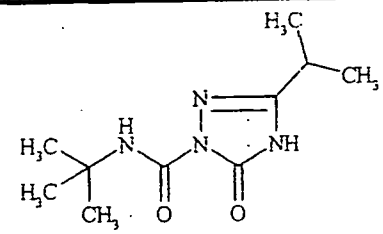
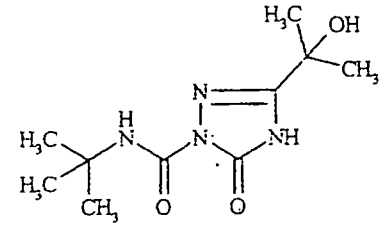
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N-tert*-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS registry number	129909-90-6
End-use formulation (EP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)
DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
*i*Pr-2-OH DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-*H*-1,2,4-triazole-1-carboxamide

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.		
Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-6} Pa @ 25°C 1.30×10^{-6} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, Log(K_{ow})	pKa = 17(log P_{ow}=1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Twenty winter wheat rotational crop field trials were conducted at sites throughout the US during 2000-2001. At each of the test sites, bare soil was treated once with amicarbazone (70% WDG) at 0.400-0.458 lb ai/A using ground equipment. The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. A rotational crop of winter wheat was planted 115-130 days after treatment (~4-month PBI).

TABLE B.1.1. Trial Site Conditions.

Trial Identification (City, State, Year)	Soil characteristics				Meteorological data	
	Type	%OM	pH	CEC (meq/g)	Total rainfall during study period (inches)	Overall monthly temperature range (°C)
Tifton, GA, 2000	Not reported = NR	NR	NR	NR	37.0	NR
Benoit, MS, 2000	NR	NR	NR	NR	48.5	NR
Saginaw, MI, 2000	NR	NR	NR	NR	33.5	NR
Campbell, MN, 2000	NR	NR	NR	NR	23.6	NR
Stafford, KS, 2000	NR	NR	NR	NR	29.8	NR
Pratt, KS, 2000	NR	NR	NR	NR	21.3	NR
Geneva, MN, 2000	NR	NR	NR	NR	35.9	NR
Eakly, OK, 2000	NR	NR	NR	NR	34.2	NR
Velva, ND, 2000	NR	NR	NR	NR	20.6	NR
Ellendale, ND, 2001	NR	NR	NR	NR	20.1	NR
Lake Andes, SD, 2001	NR	NR	NR	NR	20.8	NR
Velva, ND, 2000	NR	NR	NR	NR	26.2	NR
Banard, SD, 2000	NR	NR	NR	NR	22.9	NR
Hart, TX, 2000	NR	NR	NR	NR	14.7	NR
Cordell, OK, 2000	NR	NR	NR	NR	20.7	NR
Claude, TX, 2000	NR	NR	NR	NR	26.3	NR
Frederick, OK, 2000	NR	NR	NR	NR	39.0	NR
Littlefield, TX, 2000	NR	NR	NR	NR	11.4	NR
Uvalde, TX, 2000	NR	NR	NR	NR	19.1	NR
Parkdale, OR, 2000	NR	NR	NR	NR	21.3	NR

[1] Detailed meteorological data were not provided.

Detailed meteorological data were not provided. Rainfall was supplemented with irrigation as needed.

TABLE B.1.2. Study Use Pattern.

Location (County, State; Year)	EP[1]	Application				Tank Mix Adjuvants
		Method; Timing	Vol. (GPA[2])	Single Rate (lb ai/A)	No. of Appl.	
Tifton, GA, 2000	70% WDG	broadcast; bare ground	14.2	0.450	1	None
Benoit, MS, 2000	70% WDG	broadcast; bare ground	17.4	0.457	1	None
Saginaw, MI, 2000	70% WDG	broadcast; bare ground	16.2	0.450	1	None
Campbell, MN, 2000	70% WDG	broadcast; bare ground	15.0	0.450	1	None
Stafford, KS, 2000	70% WDG	broadcast; bare ground	15.2	0.443	1	None
Pratt, KS, 2000	70% WDG	broadcast; bare ground	15.2	0.458	1	None
Geneva, MN, 2000	70% WDG	broadcast; bare ground	18.0	0.452	1	None
Eakly, OK, 2000	70% WDG	broadcast; bare ground	14.3	0.454	1	None
Velva, ND, 2000	70% WDG	broadcast; bare ground	15.1	0.449	1	None
Ellendale, ND, 2001	70% WDG	broadcast; bare ground	14.9	0.445	1	None
Lake Andes, SD, 2001	70% WDG	broadcast; bare ground	19.1	0.442	1	None
Velva, ND, 2000	70% WDG	broadcast; bare ground	15.0	0.446	1	None
Banard, SD, 2000	70% WDG	broadcast; bare ground	14.7	0.442	1	None
Hart, TX, 2000	70% WDG	broadcast; bare ground	16.2	0.449	1	None
Cordell, OK, 2000	70% WDG	broadcast; bare ground	10.6	0.441	1	None
Claude, TX, 2000	70% WDG	broadcast; bare ground	15.2	0.458	1	None
Frederick, OK, 2000	70% WDG	broadcast; bare ground	12.[illegible]	0.400	1	None
Littlefield, TX, 2000	70% WDG	broadcast; bare ground	20.1	0.453	1	None
Uvalde, TX, 2000	70% WDG	broadcast; bare ground	15.3	0.455	1	None
Parkdale, OR, 2000	70% WDG	broadcast; bare ground	20.7	0.455	1	None

1 EP = End-use Product
2 All applications were made using ground equipment

Duplicate treated samples and a single control sample of wheat forage, hay, grain, and straw (>2.5 lbs each) were harvested at intervals reflecting normal agricultural practices at each site. Forage was collected at the tillering stage, 105-259 days after planting (DAP). Hay was collected at the flag leaf stage, 134-293 DAP, and allowed to dry in the field for 2-19 days prior to sampling. Grain and straw samples were harvested at crop maturity, 177-331 DAP. After collection, samples were placed in frozen storage at the test facility within 6 hours of collection, stored frozen for 0-91 days, then shipped frozen by ACDS freezer truck to the analytical laboratory, Bayer Research Park (BRP), Stilwell, KS and stored frozen (<-15° C) prior to analysis. Samples were stored frozen from collection to analysis for up to 19 months

B.2. Analytical Methodology

Samples of wheat matrices were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 by using an LC-MS/MS method (Bayer Method 200258), which is similar to the proposed enforcement method for plant commodities (Bayer Method 108340). Only a brief description of the method was included in the submission. For a complete description of the method, refer to 46145302.der (currently under review).

Briefly, homogenized samples were extracted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v). Following filtration and the addition of deuterated internal standards, the residues were cleaned up by solid phase extraction (mixed C_8 and SCX cartridge). Residues were quantified by LC-MS/MS, using deuterated internal standards of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586. Residues of metabolites were reported in parent equivalents. The validated LOQ was 0.01 ppm for amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on all matrices. The LODs for the three analytes were calculated based on the recovery data at the LOQ and using the following formula: (SD at the LOQ) x $t_{0.99}$ (for n-1 replicates). The LODs for amicarbazone were calculated to be 0.001 ppm for wheat grain, 0.002 ppm for forage and hay, and 0.005 ppm for straw. The LODs for DA MKH 3586 were 0.002 ppm for forage, hay, and grain, and 0.003 ppm for straw. The LOD for iPr-2-OH DA MKH 3586 were 0.001 ppm for hay and grain, 0.002 ppm for forage, and 0.004 ppm for straw.

In the current submission, the LC-MS/MS method was validated using concurrent method recoveries of wheat forage and hay samples fortified separately with amicarbazone, DA MKH 3586 and iPr-2-OH DA MKH 3586 at 0.01-0.70 ppm.

C. RESULTS AND DISCUSSION

The number of winter wheat rotational field trials and the geographic representation of the residue data on wheat forage, hay, straw and grain are adequate according to the latest EPA Guidance.

The total frozen (< -15° C) storage intervals were 3-19 months for wheat forage, hay, and grain samples (Table C.1). In support of the field rotational crop trials, the petitioner cited storage stability data (45121704.der) submitted in conjunction with a confined rotational crop study. In that review, it was also stated that LC/MS analyses of some metabolites were conducted up to 30 months following sample collection; however, the petitioner noted that these analyses were not quantitative. These data are insufficient to support the current field rotational crop study because quantitative data were not included in MRID 45121704.

TABLE C.1. Summary of Storage Conditions

Matrix	Storage Temp. (°C)	Actual Storage Duration (months)[1]	Limit of Demonstrated Storage Stability (months)[2]
Wheat forage, hay, grain, and straw	<-15	3-19	NA

1 Extracts were stored frozen for up to 3 days prior to analysis.
2 NA = not available. Adequate storage stability data are not available for frozen plant commodities.

The LC-MS/MS method (Bayer Method 200258) used to determine residues of amicarbazone, DA MKH 3586, and iPr-2OH DA MKH 3586 in/on wheat RACs is adequate for data collection. Average concurrent recoveries for all analytes from wheat forage, hay, grain, and straw were 91-101% with standard deviations of ±3-7% (Table C.2). Apparent residues of amicarbazone were <LOQ in/on all control samples. The LOQ was 0.01 ppm each analyte in/on all matrices. The LOD for amicarbazone was 0.001 ppm for wheat grain, 0.002 ppm for forage and hay, and 0.005 ppm for straw. The LODs for DA MKH 3586 was 0.002 ppm for forage, hay, and grain, and 0.003 ppm for straw. The LOD for iPr-2-OH DA MKH 3586 were 0.001 ppm for hay and grain, 0.002 ppm for forage, and 0.004 ppm for straw. Adequate sample calculations and chromatograms were provided.

For winter wheat planted ~4 months post-treatment, residues of amicarbazone were <0.010-0.249 ppm in/on 40 wheat forage samples, <0.010-0.566 ppm in/on 40 wheat hay samples, and <0.010 (all <LOQ) in/on 40 wheat grain samples, and <0.010-0.280 ppm in/on 40 wheat straw samples (Table C.3). Residues of DA MKH 3586 were <0.01-0.184 ppm in/on forage, <0.01-0.029 ppm in/on hay, <0.01 ppm in/on grain, and <0.01-0.045 ppm in/on straw. Residues of iPr-2OH DA MKH 3586 were <0.10-0.166 ppm in/on forage, 0.027-0.308 ppm in/on hay, <0.010-0.0.075 ppm in/on grain, and <0.01-0.236 ppm in/on straw.

When calculating combined residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 (expressed in parent equivalents), the LOQ (0.01 ppm) was used for individual residues reported at <LOQ and ½ the LOQ (0.005 ppm) was used for calculating the average. Combined residues were <0.030-0.485 ppm (ave. = 0.136 ppm) in/on wheat forage, <0.040-0.878 ppm (ave. = 0.159 ppm) in/on hay, <0.030-<0.095 ppm (ave. = 0.033 ppm) in/on grain, and <0.030-0.450 ppm (ave. = 0.109 ppm) in/on straw (Table C.4).

Common cultural practices were used to maintain plants, and the weather conditions and the maintenance chemicals and fertilizer used in the study did not have a notable impact on the residue data.

TABLE C.2. Summary of Concurrent Recoveries of Amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 from Wheat Matrices Using Bayer LC-MS/MS Method 200258.

Matrix	Analyte	Spike level (mg/kg)	Sample size (n)	Recoveries (%)	Mean ± std dev (%)
Wheat forage	Amicarbazone	0.01	8	87-104	97 ± 6
		0.20	3	96-102	
	DA MKH 3586	0.01	8	94-112	100 ± 5
		0.20	3	97-101	
	iPr-2-OH DA MKH 3586	0.01	8	93-103	98 ± 4
		0.20	3	99-102	
Wheat hay	Amicarbazone	0.01	9	85-101	95 ± 4
		0.70	3	92-96	
	DA MKH 3586	0.01	9	88-103	94 ± 5
		0.70	3	89-96	
	iPr-2-OH DA MKH 3586	0.01	9	89-99	95 ± 3
		0.70	3	90-94	
Wheat grain	Amicarbazone	0.01	8	97-105	101 ± 3
		0.10	3	100-107	
	DA MKH 3586	0.01	8	92-104	100 ± 5
		0.10	3	103-105	
	iPr-2-OH DA MKH 3586	0.01	8	92-105	100 ± 4
		0.10	3	99-103	
Wheat straw	Amicarbazone	0.01	8	31-97	91 ± 5
		0.30	3	90-98	
	DA MKH 3586	0.01	8	83-104	93 ± 7
		0.30	3	91-92	
	iPr-2-OH DA MKH 3586	0.01	8	86-99	93 ± 4
		0.30	3	90-93	

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.4.4/OPPTS 860.1900/OECD IIA 6.6.3, 6.8.7 and IIIA 8.6
Field Accumulation in Rotational Crops - Wheat

TABLE C.3. **Amicarbazone Residues in Rotational Winter Wheat Planted ~4 Months Following a Single Soil Application of Amicarbazone (70% WDG) at ~0.45 lb ai/A.**

Location (County, State, Year)	EPA Region	Winter Wheat Variety	Total Rate (lb ai/A)	PBI[2] (days)	Harvest DAP[1]	Matrix	Residues (ppm)[3]			
							Amicarbazone	DA MKH 3586	i-Pr-2-OH MKH 3586	Total
Tifton, GA, 2000	2	Coker 9663	0.450	118	105	Forage	0.035, 0.031	0.012, 0.017	0.115, 0.132	0.162, 0.179
Benoit, MS, 2000	4	Pioneer 2684	0.457	122	155	Forage	(0.006, 0.005)[4]	ND, ND[4]	0.047, 0.050	<0.072, <0.075
Saginaw, MI, 2000	5	Pioneer 25W33	0.450	124	204	Forage	0.031, 0.032	0.025, 0.026	0.057, 0.061	0.113, 0.118
Campbell, MN, 2000	5	Roughrider	0.450	120	243	Forage	0.014, 0.014	(0.006, 0.006)	0.039, 0.040	<0.069, 0.069
Stafford, KS, 2000	5	2174	0.443	115	193	Forage	(0.003, 0.002)	(0.010, 0.009)	0.074, 0.071	<0.107, 0.103
Pratt, KS, 2000	5	Thunderbolt	0.458	123	188	Forage	(0.004, 0.005)	(0.007, 0.006)	0.046, 0.051	<0.077, 0.082
Geneva, MN, 2000	5	Crimsom	0.452	121	218	Forage	0.017, 0.019	(0.003, 0.003)	0.042, 0.042	<0.071, <0.074
Eakly, OK, 2000	6	Tonkawa	0.454	118	179	Forage	ND, ND	ND, ND	(0.008, 0.007)	<0.030, <0.030
Velva, ND, 2000	7	Elkhorn	0.449	120	253	Forage	(0.009, 0.010)	0.012, 0.014	0.076, 0.076	<0.107, <0.109
Ellendale, ND, 2001	7	Harding HRWW	0.445	117	245	Forage	0.136, 0.107	0.184, 0.173	0.166, 0.166	0.485, 0.446
Lake Andes, SD, 2001	7	Harding 2020	0.442	119	221	Forage	0.061, 0.093	0.040, 0.049	0.046, 0.047	0.147, 0.189
Velva, ND, 2000	7	Roughrider	0.446	120	259	Forage	0.043, 0.044	0.032, 0.033	0.055, 0.066	0.130, 0.143
Banard, SD, 2000	7	Arapohoe	0.442	123	248	Forage	0.013, 0.046	0.031, 0.034	0.086, 0.066	0.131, 0.145
Hart, TX, 2000	8	Tam 200	0.449	120	145	Forage	0.084, 0.073	0.024, 0.017	0.044, 0.047	0.152, 0.136
Cordell, OK, 2000	8	Jagger	0.441	130	143	Forage	(0.009), 0.023	0.021, 0.040	0.097, 0.094	<0.147, 0.156
Claude, TX, 2000	8	Jagger	0.458	123	232	Forage	(0.003, 0.003)	ND, ND	(0.007, 0.006)	<0.030, <0.030
Frederick, OK, 2000	8	Custer	0.400	124	206	Forage	ND, ND	(0.005), ND	0.076, 0.055	<0.151, 0.075
Littlefield, TX, 2000	8	Tam 202	0.453	125	171	Forage	0.214, 0.249	0.068, 0.060	0.115, 0.111	0.397, 0.419
Uvalde, TX, 2000	8	Ogallala	0.455	119	105	Forage	0.087, 0.088	(0.003), ND	0.032, 0.029	<0.132, <0.126
Parkdale, OR, 2000	11	Stephens	0.455	120	246	Forage	0.088, 0.095	ND, ND	0.038, 0.039	<0.137, 0.144

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.4.4/OPPTS 860.1900/OECD IIA 6.6.3, 6.8.7 and IIIA 8.6
Field Accumulation in Rotational Crops - Wheat

TABLE C.3. Amicarbazone Residues in Rotational Winter Wheat Planted ~4 Months Following a Single Soil Application of Amicarbazone (70% WDG) at ~0.45 lb ai/A.

Location (County, State, Year)	EPA Region	Winter Wheat Variety	Total Rate (lb ai/A)	PBI (days)	Harvest DAP[1]	Matrix	Residues (ppm)[3]			
							Amicarbazone	DA MKH 3586	i-Pr-2-OH MKH 3586	Total
Tifton, GA, 2000	2	Coker 9663	0.450	118	134	Hay	0.024, 0.018	ND, ND	0.167, 0.166	<0.201, <0.194
Benoit, MS, 2000	4	Pioneer 2684	0.457	122	184	Hay	(0.004, 0.005)	ND, ND	0.031, 0.050	<0.054, <0.076
Saginaw, MI, 2000	5	Pioneer 25W33	0.450	124	239	Hay	0.020, 0.013	ND, ND	0.031, 0.027	<0.061, <0.050
Campbell, MN, 2000	5	Roughrider	0.450	120	282	Hay	0.020, .019	ND, ND	0.055, 0.050	<0.084, <0.079
Stafford, KS, 2000	5	2174	0.443	115	228	Hay	(0.007, 0.006)	ND, ND	0.168, 0.133	<0.194, <0.158
Pratt, KS, 2000	5	Thunderbolt	0.458	123	221	Hay	(0.010, 0.009)	ND, ND	0.116, 0.097	<0.146, <0.126
Geneva, MN, 2000	5	Crimsom	0.452	121	268	Hay	0.019, 0.019	ND, ND	0.060, 0.049	<0.088, <0.077
Eakly, OK, 2000	6	Tonkawa	0.454	118	206	Hay	(0.008, 0.006)	ND, ND	0.050, 0.062	<0.077, <0.090
Velva, ND, 2000	7	Elkhorn	0.449	120	293	Hay	0.012, (0.010)	ND, ND	0.067, 0.053	<0.089, <0.083
Ellendale, ND, 2001	7	Harding HRWW	0.445	117	269	Hay	0.028, 0.030	(0.003), ND	0.053, 0.054	<0.094, <0.093
Lake Andes, SD, 2001	7	Harding 2020	0.442	119	262	Hay	0.059, 0.043	(0.004, 0.002)	0.065, 0.052	<0.138, <0.106
Velva, ND, 2000	7	Roughrider	0.446	120	294	Hay	0.095, 0.109	ND, ND	0.090, 0.110	<0.195, <0.229
Banard, SD, 2000	7	Arapohoe	0.442	123	278	Hay	0.022, 0.022	0.011, (0.009)	0.195, 0.171	0.228, <0.212
Hart, TX, 2000	8	Tam 200	0.449	120	194	Hay	0.128, 0.167	(0.002, 0.004)	0.091, 0.118	<0.231, <0.299
Cordell, OK, 2000	8	Jagger	0.441	130	167	Hay	(0.010, 0.008)	ND, ND	0.059, 0.058	<0.088, <0.086
Claude, TX, 2000	8	Jagger	0.458	123	255	Hay	(0.002), ND	(0.002, 0.004)	0.086, 0.071	<0.110, <0.095
Frederick, OK, 2000	8	Custer	0.400	124	236	Hay	ND, ND	ND, ND	0.045, 0.043	<0.065, <0.063
Littlefield, TX, 2000	8	Tam 202	0.453	125	196	Hay	0.566, 0.549	0.029, 0.021	0.272, 0.308	0.866, 0.878
Uvalde, TX, 2000	8	Ogallala	0.455	119	141	Hay	0.115, 0.094	(0.010, 0.008)	0.100, 0.075	<0.234, <0.186
Parkdale, OR, 2000	11	Stephens	0.455	120	263	Hay	0.079, 0.111	ND, ND	0.032, 0.037	<0.121, <0.158

TABLE C.3. Amicarbazone Residues in Rotational Winter Wheat Planted ~4 Months Following a Single Soil Application of Amicarbazone (70% WDG) at ~0.45 lb ai/A.

Location (County, State, Year)	EPA Region	Winter Wheat Variety	Total Rate (lb ai/A)	PBI[2] (days)	Harvest DAP[1]	Matrix	Residues (ppm)[3]			
							Amicarbazone	DA MKH 3586	i-Pr-2-OH MKH 3586	Total
Tifton, GA, 2000	2	Coker 9663	0.450	118	188	Grain	ND, ND	ND, ND	0.066, 0.075	<0.086, <0.095
Benoit, MS, 2000	4	Pioneer 2684	0.457	122	226	Grain	ND, ND	ND, ND	(0.009, 0.009)	<0.030, <0.030
Saginaw, MI, 2000	5	Pioneer 25W33	0.450	124	285	Grain	ND, ND	ND, ND	0.018, 0.018	<0.038, <0.038
Campbell, MN, 2000	5	Roughrider	0.450	120	313	Grain	ND, ND	ND, ND	(0.005, 0.005)	<0.030, <0.03
Stafford, KS, 2000	5	2174	0.443	115	267	Grain	ND, ND	ND, ND	0.022, 0.035	<0.042, <0.055
Pratt, KS, 2000	5	Thunderbolt	0.458	123	264	Grain	ND, ND	ND, ND	0.021, 0.029	<0.041, <0.049
Geneva, MN, 2000	5	Crimsom	0.452	121	303	Grain	ND, ND	ND, ND	(0.008, 0.007)	<0.030, <0.030
Eakly, OK, 2000	6	Tonkawa	0.454	118	252	Grain	ND, ND	ND, ND	0.012, (0.007)	<0.032, <0.030
Velva, ND, 2000	7	Elkhorn	0.449	120	323	Grain	ND, ND	ND, ND	0.020, 0.019	<0.040, <0.039
Ellendale, ND, 2001	7	Harding HRWW	0.445	117	302	Grain	(0.001, 0.001)	ND, ND	0.021, 0.022	<0.042, <0.043
Lake Andes, SD, 2001	7	Harding 2020	0.442	119	298	Grain	ND, ND	ND, ND	(0.004, 0.004)	<0.030, <0.030
Velva, ND, 2000	7	Roughrider	0.446	120	331	Grain	(0.001, 0.001)	ND, ND	0.027, 0.029	<0.049, <0.050
Banard, SD, 2000	7	Arapohoe	0.442	123	320	Grain	ND, ND	ND, ND	0.024, 0.025	<0.044, <0.044
Hart, TX, 2000	8	Tam 200	0.449	120	238	Grain	(0.001, 0.001)	ND, ND	0.023, 0.024	<0.044, <0.046
Cordell, OK, 2000	8	Jagger	0.441	130	210	Grain	ND, ND	ND, ND	0.066, 0.066	<0.086, <0.086
Claude, TX, 2000	8	Jagger	0.458	123	286	Grain	ND, ND	ND, ND	0.016, 0.013	<0.036, <0.03
Frederick, OK, 2000	8	Custer	0.400	124	261	Grain	ND, ND	ND, ND	0.027, 0.029	<0.046, <0.049
Littlefield, TX, 2000	8	Tam 202	0.453	125	232	Grain	(0.002, 0.002)	ND, ND	0.039, 0.039	<0.060, <0.06
Uvalde, TX, 2000	8	Ogallala	0.455	119	177	Grain	(0.001), ND	ND, ND	0.017, 0.017	<0.038, <0.037
Parkdale, OR, 2000	11	Stephens	0.455	120	329	Grain	(0.001, 0.002)	ND, ND	0.020, 0.021	<0.042, <0.043

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.4.4/OPPTS 860.1900/OECD IIA 6.6.3, 6.8.7 and IIIA 8.6
Field Accumulation in Rotational Crops - Wheat

TABLE C.3. Amicarbazone Residues in Rotational Winter Wheat Planted ~4 Months Following a Single Soil Application of Amicarbazone (70% WDG) at ~0.45 lb ai/A.

Location (County, State, Year)	EPA Region	Winter Wheat Variety	Total Rate (lb ai/A)	PBI[2] (days)	Harvest DAP[1]	Matrix	Residues (ppm)[3]			
							Amicarbazone	DA MKH 3586	i-Pr-2-OH MKH 3586	Total
Tifton, GA, 2000	2	Coker 9663	0.450	118	188	Straw	0.012, 0.013	(0.009, 0.007)	0.098, 0.087	<0.129, <0.118
Benoit, MS, 2000	4	Pioneer 2684	0.457	122	226	Straw	ND, ND	ND, ND	0.074, 0.049	<0.093, <0.069
Saginaw, MI, 2000	5	Pioneer 25W33	0.450	124	285	Straw	(0.008), ND	ND, ND	0.033, (0.009)	<0.061, <0.030
Campbell, MN, 2000	5	Roughrider	0.450	120	313	Straw	ND, ND	ND, ND	0.025, 0.021	<0.045, <0.041
Stafford, KS, 2000	5	2174	0.443	115	267	Straw	ND, (0.005)	0.012, 0.017	0.157, 0.236	<0.178, <0.278
Pratt, KS, 2000	5	Thunderbolt	0.458	123	264	Straw	ND, ND	(0.004, 0.008)	0.026, 0.040	<0.050, <0.068
Geneva, MN, 2000	5	Crimsom	0.452	121	303	Straw	(0.006, 0.005)	ND, ND	0.056, 0.044	<0.082, <0.069
Eakly, OK, 2000	6	Tonkawa	0.454	118	252	Straw	ND, ND	ND, ND	0.014, 0.010	<0.044, <0.030
Velva, ND, 2000	7	Elkhorn	0.449	120	323	Straw	ND, ND	(0.004, 0.004)	0.043, 0.045	<0.066, <0.069
Ellendale, ND, 2001	7	Harding HRWW	0.445	117	302	Straw	0.023, 0.027	(0.006, 0.008)	0.107, 0.112	<0.145, <0.157
Lake Andes, SD, 2001	7	Harding 2020	0.442	119	298	Straw	0.019, 0.016	(0.004), ND	0.046, 0.039	<0.078, <0.065
Velva, ND, 2000	7	Roughrider	0.446	120	331	Straw	0.035, 0.040	ND, (0.003)	0.066, 0.059	<0.111, <0.112
Banard, SD, 2000	7	Arapohoe	0.442	123	320	Straw	(0.005), ND	(0.009), 0.014	0.062, 0.081	<0.096, <0.104
Hart, TX, 2000	8	Tam 200	0.449	120	238	Straw	0.108, 0.118	(0.009, 0.009)	0.093, 0.085	<0.220, <0.223
Cordell, OK, 2000	8	Jagger	0.441	130	210	Straw	0.010, 0.012	0.021, 0.019	0.143, 0.137	0.174, 0.168
Claude, TX, 2000	8	Jagger	0.458	123	286	Straw	ND, ND	(0.003), ND	0.037, 0.040	<0.061, <0.060
Frederick, OK, 000	8	Custer	0.400	124	261	Straw	ND, ND	ND, ND	0.030, 0.028	<0.050, <0.048
Littlefield, TX, 2000	8	Tam 202	0.453	125	232	Straw	0.197, 0.280	0.041, 0.045	0.094, 0.125	0.332, 0.450
Uvalde, TX, 2000	8	Ogallala	0.455	119	177	Straw	0.102, 0.086	0.012, (0.010)	0.087, 0.068	0.200, <0.174
Parkdale, OR, 2000	11	Stephens	0.455	120	329	Straw	0.046, 0.038	(0.004, 0.004)	0.050, 0.049	<0 110, <0.102

[1] DAP = Days After Planting
[2] PBI = Plant Back Interval.
[3] The LOQ is 0.01 ppm for each analyte in/on each matrix. The LOD for amicarbazone was 0.001 ppm for wheat grain, 0.002 ppm for forage and hay, and 0.005 ppm for straw. The LODs for DA MKH 3586 were 0.002 ppm for forage, hay, and grain, and 0.003 ppm for straw. The LOD for iPr-2-OH DA MKH 3586 were 0.001 ppm for hay and grain, 0.002 ppm for forage, and 0.004 ppm for straw. Total residues are the sum of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 residues in parent equivalents; the LOQ was used for individual residues reported at <LOQ in calculating total residues.
[4] ND = not detected; residues were <LOD. Residues <LOQ but >LOD are reported in parentheses.

TABLE C.4. Summary of Residue Data in Rotational Winter Wheat Planted ~4 Months Following a Soil Application of Amicarbazone (70% WDG).

Wheat Matrix	Total Rate (lb ai/A)	PBI (days)	Residue Levels (ppm) [1]						
			n	Min.	Max.	HAFT[2]	Median (STMdR[3])	Mean (STMR[4])	Std. Dev.
Amicarbazone									
Forage	0.40-0.46	115-130	40	<0.01	0.249	0.232	0.021	0.045	0.056
Hay			40	<0.01	0.566	0.558	0.019	0.062	0.123
Grain			40	<0.01	<0.01	<0.01	0.005	0.005	0.002
Straw			40	<0.01	0.280	0.239	0.006	0.032	0.057
DA MKH 3586									
Forage	0.40-0.46	115-130	40	<0.01	0.184	0.179	0.011	0.026	0.039
Hay			40	<0.01	0.029	0.025	0.005	0.006	0.005
Grain			40	<0.01	<0.01	<0.01	0.005	0.005	0
Straw			40	<0.01	0.045	0.043	0.005	0.009	0.009
iPr-2-OH DA MKH 3586									
Forage	0.40-0.46	115-130	40	<0.01	0.166	0.166	0.053	0.063	0.038
Hay			40	0.027	0.308	0.290	0.064	0.090	0.064
Grain			40	<0.01	0.075	0.071	0.021	0.024	0.018
Straw			40	<0.01	0.236	0.197	0.053	0.068	0.046
Combined Residues									
Forage	0.40-0.46	115-130	40	<0.03	0.485	0.466	0.120	0.136	0.112
Hay			40	<0.04	0.878	0.872	0.094	0.159	0.178
Grain			40	<0.03	<0.095	<0.090	0.029	0.033	0.018
Straw			40	<0.03	0.450	0.391	0.080	0.109	0.090

[1] The LOQ is 0.01 ppm for each analyte in/on each matrix. The LOD for amicarbazone was 0.001 ppm for wheat grain, 0.002 ppm for forage and hay, and 0.005 ppm for straw. The LOD for DA MKH 3586 were 0.002 ppm for forage, hay, and grain, and 0.003 ppm for straw. The LOD for iPr-2-OH DA MKH 3586 were 0.001 ppm for hay and grain, 0.002 ppm for forage, and 0.004 ppm for straw. Total residues are the sum of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 residues in parent equivalents; the LOQ was used for individual residues reported at <LOQ in calculating total residues.

[2] HAFT = Highest Average Field Trial.

[3] STMdR = Supervised Trial Median Residue. STMR = Supervised Trial Mean Residue. For calculation of the median, mean and standard deviation, ½ the LOQ (0.005 ppm) was used for residues reported at <LOQ

D. CONCLUSION

Pending submission of adequate supporting storage stability data, the extended field rotational winter wheat data are adequate.

E. REFERENCES

None

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: 0F6131
DP Barcode: D309766
PC Code: 114004

Template Version September 2003

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.4.1/OPPTS 860.1520/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Processed Food and Feed - Wheat

Primary Evaluator	Manying Xue, Chemist RAB3/HED (7509C)	Date: 03/23/05
Approved by	Leung Cheng, Senior Chemist RAB3/HED (7509C)	Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (1910 Sedwick Rd., Building 100, Suite B; Durham, NC 27713; submitted 1/07/2005). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46145310 Krolski, M. (2003) MKH 3586 70 WG - Magnitude of the Residues in Processed Commodities from Rotational Crop Wheat and Request for Waiver of the Study on Magnitude of the Residue in Wheat Aspirated Grain Fractions. Lab Project Number: 200465: TMN-0285. Unpublished study prepared by Bayer CropScience. 480 p.

EXECUTIVE SUMMARY:

In a single test conducted in KS during 2000, bare soil was treated once with amicarbazone (70% WDG) at 0.45 lb ai/A using ground equipment. The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. A rotational crop of wheat was planted immediately following treatment (0-day PBI). Wheat grain was harvested at commercial maturity (273 DAT) from the 1x treated plot. Wheat did not germinate in two plots treated at 1.35 and 2.26 lb ai/A (3x and 5x), therefore, only data from the 1x test was reported. Triplicate samples of wheat grain (RAC) were collected and the remaining bulk samples were processed into bran, middlings, shorts, flour, and germ using simulated commercial procedures. Prior to analysis, wheat grain samples were stored frozen for a maximum of 15 months, an interval not supported by available stability data, and processed wheat matrices were stored frozen for up to 18 days. Data demonstrating the stability of residues of amicarbazone, DA MKH 3586, and *i*Pr-2OH DA MKH 3586 in/on representative plant matrices remain outstanding.

Samples were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 using an adequate LC-MS/MS method (Bayer Method 200258) which is similar to the proposed enforcement method for plant commodities (Bayer Method 108340). Briefly, samples were extracted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v), concentrated and cleaned up by solid phase extraction. Residues were quantified by LC-MS/MS, using deuterated internal standards of each analyte. Residues of metabolites were reported in parent equivalents. The LOQ was 0.01 ppm each analyte in/on all wheat matrices, for combined LOQs of 0.03 ppm. The LODs for amicarbazone were 0.001 ppm for grain, 0.002 ppm for germ, 0.003 ppm for bran and shorts, 0.004 ppm for flour, and 0.006 ppm for middlings. The LODs for

DA MKH 3586 were 0.001 ppm for bran, 0.002 ppm for grain and shorts, 0.003 ppm for middlings and germ, and 0.005 ppm for flour. The LODs for iPr-2-OH DA MKH 3586 were 0.001 ppm for grain, shorts, and germ, and 0.002 ppm for bran, flour, and middlings. The combined LODs were 0.004 ppm for grain, 0.006 ppm for bran, shorts and germ, and 0.011 ppm for flour and middlings.

Combined residues of amicarbazone and its metabolites in/on wheat grain were <0.042-<0.046 ppm and averaged 0.044 ppm. Combined residues were <0.057-<0.065 ppm in bran, <0.047-<0.051 ppm in flour, <0.058-<0.061 ppm in shorts, <0.046-<0.048 ppm in middlings, and <0.039-<0.043 ppm in germ. Average combined residues were 0.062 ppm in bran, 0.049 ppm in flour, 0.059 ppm in shorts, 0.047 ppm in middlings, and 0.040 ppm in germ. Based on average residues, the processing factors for combined amicarbazone residues were 1.4x for bran, 1.1x for flour, 1.3x for shorts, 1.1x for middlings, and 0.9x for germ. The maximum theoretical concentration factor for wheat is 8x.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

Under the conditions and parameters used in the study, the wheat processing data except for AGF are classified as scientifically acceptablepending submission of acceptable supporting storage stability data. Aspirated grain fractions (AGF) were not generated; and the petitioner requested a waiver of the requirement to generate AGF residue data. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document DP Barcode D309766.

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

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis. There are currently no permanent tolerances established for amicarbazone. The original petitioner was Bayer Corporation; however, Bayer Corporation has since sold amicarbazone to Arvesta Corporation, who is now the petitioner for amicarbazone. In conjunction with the subject tolerance petition, Arvesta has submitted an application for Section 3 registration of a 70% water dispersible granule (WDG, DF) formulation (Amicarbazone DF Herbicide; EPA File Symbol No. 66330-UA). The 70% WDG formulation is to be applied as preplant, preemergence, or early postemergence broadcast sprays at 0.22-0.45 lb ai/A for preplant/preemergence application, or 0.10-0.15 lb ai/A for

postemergence application. The petitioner has submitted residue data from a processing study on wheat.

The nomenclature and physicochemical properties of amicarbazone are presented below in Tables A.1 and A.2.

TABLE A.1. Amicarbazone Nomenclature.

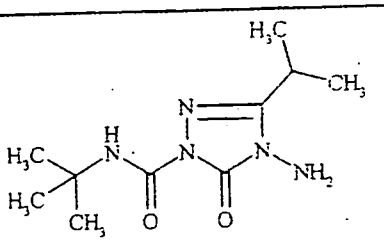
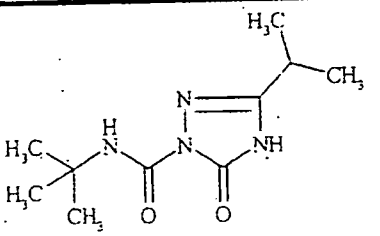
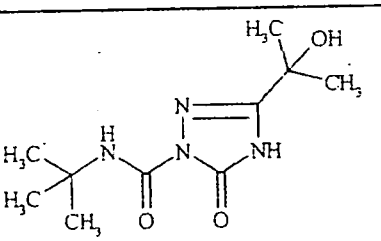
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-tert-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS registry number	129909-90-6
End-use formulation (EP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)
DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
*i*Pr-2-OH DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-*H*-1,2,4-triazole-1-carboxamide

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.		
Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-4} Pa @ 25°C 1.30×10^{-4} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, Log(K_{OW})	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

In three separate plots, bare soil was treated once with amicarbazone (70% WDG) at 0.451, 1.352, and 2.255 lb ai/A using ground equipment (1x, 3x and 5x rate, Table B.1). The maximum seasonal use rate for any rotated crop is 0.45 lb ai/A on field corn. A rotational crop of winter wheat was planted immediately following treatment (0-day PBI). Wheat grain was harvested at commercial maturity (273 DAT) from the 1x treated plot. Wheat did not germinate in the 3x and 5x plots, therefore, only data from the 1x test was reported.

TABLE B.1. Study Use Pattern on Wheat						
Location (City, State), Year	Application					
	EP[1]	Method[2]; Timing	Volume (gal/A)	Single Rate[3] (lb a.i./A)	No. of Appl.	Tank Mix Adjuvants
Stilwel, KS, 2000	70% WDG	Broadcast soil; bare ground	20.3	0.451	1	None
			20.3	1.352[4]	1	None
			20.3	2.255[4]	1	None

1 EP = End-use Product
2 Applied using ground equipment.
3 These rates represent 1x, 3x, and 5x the seasonal maximum proposed label use rate for any rotated crop (field corn).
4 Wheat did not germinate in the 3x and 5x plots, therefore, only data from the 1x test was reported.

B.2. Processing Procedures

After collection, wheat grain samples were stored frozen at the field site for 14 days, then shipped frozen to FPRDC at Texas A&M University (Bryan, TX). Grain samples were processed into bran, middlings, shorts, flour, and germ using simulated commercial procedures. Aspirated grain fractions (AGF) were not generated and the petitioner requested a waiver for the requirement to generated AGF residue data. The processed fractions and subsamples of grain were shipped frozen to the analytical laboratory, Residue Analysis Laboratory at the Bayer Research Park (BRP), Stilwell, KS, (BRP), where they were stored frozen (<-5 °C) prior to analyses. Grain samples were stored frozen from collection to analysis for up to 15 months. Processed matrices were stored frozen from generation to analysis for up to 18 days.

B.3. Analytical Methodology

Samples of wheat matrices were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 by using an LC-MS/MS method (Bayer Method 200258), which is similar to the proposed as the enforcement method for plant commodities (Bayer Method 108340). Only a brief description of the method was included in the submission. For a complete description of the method, refer to 46145302.der (currently under review).

Briefly, homogenized samples were extracted with 0.1% aqueous acetic acid in ACN:water (4:1, v/v). Following filtration and the addition of deuterated internal standards, the residues were cleaned up by solid phase extraction (mixed C_8 and SCX cartridge). Residues were quantified by LC-MS/MS, using deuterated internal standards of amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586. Residues of metabolites were reported in parent equivalents. The validated LOQ was 0.01 ppm for amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on all wheat matrices. The LODs for the three analytes were calculated based on the recovery data at the LOQ and using the following formula: (S.D at the LOQ) x $t_{0.99}$ (for n-1 replicates). The calculated LODs for amicarbazone were 0.001 ppm for wheat grain, 0.002 ppm for germ, 0.003 ppm for bran and shorts, 0.004 ppm for flour, and 0.006 ppm for middlings. The LODs for DA MKH 3586 were 0.001 ppm for bran, 0.002 ppm for grain and shorts, 0.003 ppm for middlings and germ, and 0.005 ppm for flour. The LODs for iPr-2-OH DA MKH 3586 were 0.001 ppm for grain, shorts, and germ, and 0.002 ppm for bran, flour, and middlings. The LODs for combined residues were 0.004 ppm for grain, 0.006 ppm for bran, shorts and germ, and 0.011 ppm for flour and middlings.

In the current submission, the LC-MS/MS method was validated using concurrent method recoveries of wheat grain and processed wheat matrix samples fortified separately with amicarbazone, DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 at 0.01-0.10 ppm.

C. RESULTS AND DISCUSSION

The LC-MS/MS method (Bayer Method 200258) used to determine residues of amicarbazone, DA MKH 3586, and *i*Pr-2OH DA MKH 3586 in/on wheat grain and processed wheat

commodities is adequate for data collection. Average concurrent recoveries from wheat grain and processed wheat commodities samples fortified separately with amicarbazone, DA MKH 3586 and iPr-2-OH DA MKH 3586 at 0.01-0.10 ppm were 95-105% with standard deviations of ± 1-9% (Table C.1). Apparent residues of amicarbazone were <LOQ in/on all control samples. The validated LOQ was 0.01 ppm for amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 in/on all matrices. The LODs for each analyte in each matrix ranged from 0.001-0.006 ppm.

TABLE C.1. Summary of Concurrent Recoveries of Amicarbazone, DA MKH 3586, and *i*Pr-2-OH DA MKH 3586 from Wheat Matrices.

Matrix	Spiking Level (mg/kg)	Sample size	Amicarbazone		DA MKH 3586		iPr-2-OH DA MKH 3586	
			Recoveries (%)		Recoveries (%)		Recoveries (%)	
			Range	Mean ± std dev	Range	Mean ± std dev	Range	Mean ± std dev
Wheat grain	0.01	3	99-102	95 ± 7	96-97	97 ± 1	102-105	100 ± 5
	0.05	3	84-93		95-99		93-97	
Wheat bran	0.01	3	95-102	97 ± 4	99-101	99 ± 2	97-100	98 ± 2
	0.10	3	92-101		94-99		95-99	
Wheat flour	0.01	3	103-111	101 ± 9	98-109	97 ± 7	103-108	101 ± 6
	0.10	3	85-110		89-94		94-97	
Wheat shorts	0.01	3	105-113	105 ± 7	95-99	98 ± 2	104-105	99 ± 6
	0.10	3	95-104		96-99		92-96	
Wheat middlings	0.01	3	93-108	100 ± 5	99-105	99 ± 5	97-100	98 ± 1
	0.10	3	99-102		94-99		97-98	

The total frozen (< -5° C) storage intervals were 13-15 months for wheat grain samples and 12-18 days for processed wheat matrices (Table C.2). In support of the processing study, the petitioner cited storage stability data (45121704.der) submitted in conjunction with a confined rotational crop study. In that review, it was also stated that LC/MS analyses of some metabolites was conducted up to 30 months following sample collection for certain metabolites; however, the petitioner noted that these analyses were not quantitative. These data are insufficient to support the current processing study because quantitative data were not included in MRID 45121704.

TABLE C.2. Summary of Freezer Storage Conditions

Wheat Matrix	Storage Temp. (°C)	Actual Storage Duration (months)[1]	Limit of Demonstrated Storage Stability (months)
Grain	<-5	13-15	NA[2]
Bran, Germ, Flour, Middlings, Shorts			

[1] Extracts were stored frozen for up to 3 days prior to analysis and processed commodities were stored frozen for up to 18 days after processing and prior to analyses.

[2] NA = not available. Adequate storage stability data are not available for frozen plant commodities

Amicarbazone residues were <0.002 ppm (<LOD), DA MKH 3586 residues were <0.001 ppm (<LOD), and *i*Pr-2-OH DA MKH 3586 residues were 0.040-0.043 ppm in/on 3 samples of wheat grain treated at 1x and harvested at maturity (Table C.3). Combined residues of amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 (expressed as parent equivalents) were <0.042-<0.046 ppm. Average combined residues were 0.044 ppm in/on grain samples; the average value was used to calculate the processing factors.

Amicarbazone and DA MKH 3586 residues were each <LOD in all processed wheat matrices. Residues of *i*Pr-2-OH DA MKH 3586 were 0.053-0.061 ppm in 3 bran samples, 0.038-0.042 ppm in 3 flour samples, 0.053-0.056 ppm in 3 shorts samples, 0.037-0.039 ppm in 3 middlings samples, and 0.034-0.038 ppm in 3 germ samples. Combined residues were <0.057-<0.065 ppm in bran, <0.047-<0.051 ppm in flour, <0.058-<0.061 ppm in shorts, <0.046-<0.048 ppm in middlings, and <0.039-<0.043 ppm in germ. Average combined residues were 0.062 ppm in bran, 0.049 ppm in flour, 0.059 ppm in shorts, 0.047 ppm in middlings, and 0.040 ppm in germ. Based on these residues, the processing factors for combined amicarbazone residues were 1.4x for bran, 1.1x for flour, 1.3x for shorts, 1.1x for middlings, and 0.9x for germ. The maximum theoretical concentration factor for wheat is 8x. Aspirated grain fractions (AGF) were not generated and the petitioner requested a waiver for the requirement to generate AGF residue data.

TABLE C.3. **Residue Data from Wheat Processing Study with Amicarbazone (70% WDG).**

Trial ID (City, State, Year)	Processed Commodity	Total Rate (lb ai/A)	PHI [1] (days)	Residues (ppm) [2]				Processing Factor [3]
				Amicarbazone	DA MKH 3586	*i*-Pr-2-OH MKH 3586	Combined Residues	
Stillwell, KS, 2000	Grain (RAC)	0.451	273	ND, ND, ND [4]	ND, ND, ND	0.043, 0.040, 0.042	<0.046, <0.042, <0.045 **(0.044)** [3]	NA
	Bran			ND, ND, ND	ND, ND, ND	0.060, 0.061, 0.053	<0.064, <0.065, <0.057 **(0.062)**	1.41x
	Flour			ND, ND, ND	ND, ND, ND	0.041, 0.038, 0.042	<0.050, <0.047, <0.051 **(0.049)**	1.11x
	Shorts			ND, ND, ND	ND, ND, ND	0.053, 0.056, 0.053	<0.058, <0.061, <0.058 **(0.059)**	1.34x
	Middlings			ND, ND, ND	ND, ND, ND	0.037, 0.038, 0.039	<0.046, <0.047, <0.048 **(0.047)**	1.07x
	Germ			ND, ND, ND	ND, ND, ND	0.034, 0.038, 0.034	<0.039, <0.043, <0.039 **(0.040)**	0.91x

[1] A single soil application was made immediately prior to planting wheat.

[2] The LOQ is 0.01 ppm for each analyte in/on each matrix. The LOD for amicarbazone were 0.001 ppm for wheat grain, 0.002 ppm for germ, 0.003 ppm for bran and shorts, 0.004 ppm for flour, and 0.006 ppm for middlings. The LODs for DA MKH 3586 were 0.001 ppm for bran, 0.002 ppm for grain and shorts, 0.003 ppm for middlings and germ, and 0.005 ppm for flour. The LODs for iPr-2-OH DA MKH 3586 were 0.001 ppm for grain, shorts, and germ, and 0.002 ppm for bran, flour, and middlings. Total residues are the sum of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 residues in parent equivalents; the LOD was used for individual residues reported at <LOD in calculating total residues.

[3] The processing factor was calculated using average total residues (**bold**) in the wheat grain and processed fractions.

[4] ND = not detected; residues were <LOD.

NA= not applicable

D. CONCLUSION

The processing factors for amicarbazone residues were 1.4x for bran, 1.1x for flour, 1.3x for shorts, 1.1x for middlings, and 0.9x for germ, pending submission of adequate stability data. Data for AGF were not provided.

E. REFERENCES

None

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: 0F6131
DP Barcode: D309766
PC Code: 114004

Template Version September 2003

12

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.5.1/OPPTS 860.1480/OECD IIA 6.4.1, 6.4.2 and IIIA 8.2, 8.4.1, 8.4.2
Livestock Feeding Study - Dairy Cattle

Primary Evaluator: Manying Xue, Chemist
RAB3/HED (7509C) Date: 03/23/05

Approved by: Leung Cheng, Senior Chemist
RAB3/HED (7509C) Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; updated draft submitted 09/21/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

45121707 Haan, R. (2000) MKH 3586--A 30-Day Dairy Cattle Feeding Study: Lab Project Number: M6060401: 108709. Unpublished study prepared by Battelle. 161 p.

EXECUTIVE SUMMARY:

Arvesta Corporation has submitted a ruminant feeding study with amicarbazone. Encapsulated amicarbazone was administered orally to three groups of three lactating Holstein cows for 30 days. Cows were dosed at 0.374, 1.238, or 4.538 ppm in the diet. Milk was collected twice daily, and samples were composited daily for each cow. Milk samples were collected on study days 0, 4, 8, 12, 16, 18, 20, 22, 24, 26, and 28. Cows were sacrificed within 6 hours of the final dose, and samples of muscle, liver, kidney and fat were collected.

Amicarbazone-related residues were below the method limit of quantitation (LOQ; <0.010 ppm) in whole milk samples collected over the course of the dosing period from the high-dose group. Because no quantifiable residues were observed in milk from the high-dose group, milk samples from the low- and mid-dose group were not analyzed.

Amicarbazone-related residues were 1.165-1.193 ppm in liver, 0.080-0.127 ppm in kidney, 0.014-0.021 ppm in muscle, and <0.010-0.012 ppm in fat from the high-dose group. Residues from the mid- and low-dose groups were 0.305-0.440 ppm and 0.191-0.235 ppm in liver, and 0.033-0.039 ppm and 0.017-0.018 ppm in kidney, respectively. Residues were below the method LOQ (<0.010 ppm) in muscle and fat samples from the mid-dose group; therefore, samples of muscle and fat from the low-dose group were not analyzed. The relationship between dosing level and amicarbazone-related residues appears to be linear in liver and kidney.

Cow tissues and milk were analyzed for amicarbazone-related residues using the proposed LC/MS/MS enforcement method for livestock commodities. Amicarbazone-related residues, including amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586, are oxidized to iPr-2-OH DA MKH 3586 and quantitated by LC/MS/MS; residues are reported as amicarbazone equivalents. The concurrent method recovery data included in the submission

reflected fortification at the method LOQ (0.01 ppm) only. These data are adequate for milk, muscle, and fat but are not adequate for kidney and liver. Residues greater than 10x the LOQ were observed in kidney, and residues greater than 100x the LOQ were observed in liver. Additional validation data reflecting 10x the LOQ were provided for liver and kidney in conjunction with the method submission (refer to the DER for MRID 45121705); these data are adequate to bracket the observed residue levels for kidney but not for liver.

The maximum storage intervals from collection to analysis were 27 days for whole milk and 237 days for tissues. In support of the feeding study, the petitioner referenced data from an extraction efficiency study (refer to the DER for MRID 45121713), which indicated that residue levels of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 in radiolabeled liver from a goat metabolism study were not significantly changed following frozen storage for up to ~45 months. These data are adequate to support the storage intervals and conditions of tissue samples from the cattle feeding study. Because milk samples were analyzed within one month of collection, storage stability data for milk will not be required.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the livestock feeding study residue data are classified as scientifically acceptable, pending submission of adequate method validation data for liver reflecting the range of residue levels observed in the study.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document DP Barcode D288216.

COMPLIANCE:

Signed and dated 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

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis.

TABLE A.1. Amicarbazone Nomenclature.

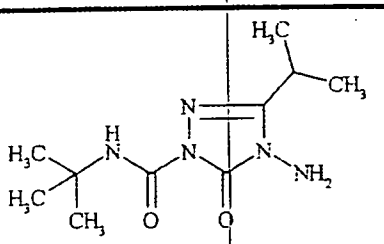
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-*tert*-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS #	129909-90-6
End-use formulation (EUP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.

Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-6} Pa @ 25°C 1.30×10^{-6} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, $Log(K_{ow})$	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. EXPERIMENTAL DESIGN

B.1. Livestock

TABLE B.1.1. Description of Livestock Used in the Feeding Study.

Species	Breed	Age	Weight at study initiation (kg)	Health status	Description of housing/holding area
Dairy cow	Holstein	4-5 years	550-662	Healthy and normal throughout study	Outdoor sand floor pens with overhead shelters at Southwest Bio-Labs, Inc. (Las Cruces, NM); average minimum and maximum temperatures were 0 and 16 °C, and average relative humidity was 64%.

TABLE B.1.2. Dietary Regime.

Treatment group	Composition of Diet	Feed consumption (kg/day)	Water	Acclimation period	Predosing
Group 1 (control, 1 cow)	Commercial total mixed ration *ad libitum*	20-39	Tap water *ad libitum*	Upon receipt until study initiation; 10 days	None
Group 2 (low dose, 3 cows)		20-34			
Group 3 (mid dose, 3 cows)		15-37			
Group 4 (high dose, 3 cows)		12-39			

TABLE B.1.3. Dosing Regime.

Treatment group	Treatment Type	Level of administered dose (mg/day)	Residue intake in diet (ppm) [a]	Vehicle	Timing/ Duration
Group 1 (control)	Oral via balling gun	0; cellulose placebo	0	Gelatin capsule	Once per day after the morning milking for 30 consecutive days
Group 2 (low dose)		6.552-6.888	0.374		
Group 3 (mid dose)		21.420-22.356	1.238		
Group 4 (high dose)		75.960-80.160	4.538		

[a] Average dose rate calculated on the basis of feed consumption over the course of the study, as reported by the petitioner.

TABLE B.1.4 Sample Collection.

Milk collected	Amount of milk produced during normal production	Urine, feces and cage wash collected	Interval from last dose to sacrifice (days)	Tissues harvested and analyzed
Milk was collected twice daily (a.m. and p.m.). Average daily production during dosing was 16.5-28.6 kg per cow.	Average daily production during acclimation period was 16.1-26.2 kg per cow.	Not collected	Within 6 hours	Liver, kidneys, fat (composite of omental, renal, and subcutaneous), and muscle (composite of loin, round, and flank)

B.2. Sample Handling and Preparation

Daily milk samples (p.m. and a.m. prior to next dose) were composited for each cow and composited milk samples were stored frozen (≤-10 °C) on site prior to shipment to the analytical facility. A subsample of Day-28 milk from the high-dose group was separated into cream and whey by centrifugation. Tissue samples were chopped into small pieces and frozen (≤-10 °C)

within 30 minutes of sacrifice, after which they were homogenized to a powder in the presence of liquid nitrogen. After collection and processing, all samples were stored frozen prior to shipment to Bayer by express mail on dry ice.

B.3. Analytical Methodology

Samples of tissues and milk from each individual cow were analyzed by Bayer Corporation (Agricultural Division, Stilwell, KS) for amicarbazone-related residues using the proposed LC/MS/MS enforcement method (refer to the DER for MRID 45121705 for details of the method).

Briefly, homogenized tissue samples were extracted with 0.05% aqueous phosphoric acid using accelerated solvent extraction (ASE) at 150 °C and 1500 psi. Milk, cream, and whey samples were not subjected to ASE extraction, but were diluted with water. Amicarbazone-related residues in diluted milk samples and tissue extracts were then oxidized to iPr-2-OH DA MKH 3586 with potassium permanganate at 70 °C for 1 hour. Oxidized residues were cleaned up on a solid phase extraction cartridge; residues of iPr-2-OH DA MKH 3586 were eluted with methanol. The eluate was concentrated to dryness, and residues were redissolved in methanol:water (3:7, v:v) for analysis by LC/MS/MS using electrospray ionization in the positive ion mode. An internal standard of d_6-labeled iPr-2-OH DA MKH 3586 was added to each sample. A single external standard bracketed each analytical run, and residues were quantified using the internal and external standards and were reported as amicarbazone equivalents. The estimated limit of detection (LOD) was 0.005 ppm in milk and tissues. The validated LOQ was 0.010 ppm for milk and tissues.

C. RESULTS AND DISCUSSION

Lactating dairy cows (3 cows per dose group) were orally dosed with amicarbazone at levels equivalent to 0.374, 1.238, and 4.538 ppm in the diet for 30 consecutive days. Cows were milked twice daily, and samples were composited daily for each cow. Milk samples were collected on study days 0, 4, 8, 12, 16, 18, 20, 22, 24, 26, and 28, and a subsample of Day-28 milk from the high-dose group was centrifuged to produce whey and cream for analysis. Cows were sacrificed within 6 hours of the final dose, and samples of muscle, liver, kidney and fat were collected.

Cow tissues and milk were analyzed for amicarbazone-related residues using the proposed LC/MS/MS enforcement method for livestock commodities. Amicarbazone-related residues, including amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586, are oxidized to iPr-2-OH DA MKH 3586 and quantitated by LC/MS/MS; residues are reported as amicarbazone equivalents. The validated method LOQ for amicarbazone-related residues is 0.010 ppm for milk, whey, cream, and tissues. Concurrent method recovery data are presented in Table C.1; fortifications were only conducted at the method LOQ. The concurrent method recovery data included in the submission are adequate for milk, muscle, and fat but are not adequate for kidney and liver. Residues greater than 10x the LOQ were observed in kidney, and

residues greater than 100x the LOQ were observed in liver. Method validation data be submitted for liver reflecting the range of residue levels observed in this feeding study.

The results of the feeding study are presented in Table C.3 and summarized in Table C.4. Amicarbazone-related residues were below the method LOQ (<0.010 ppm) in whole milk samples collected over the course of the dosing period from the high-dose group; residues were also below the LOQ in whey and cream processed from 28-day high-dose milk. Because no quantifiable residues were observed in milk from the high-dose group, milk samples from the low- and mid-dose group were not analyzed.

Amicarbazone-related residues were 1.165-1.193 ppm in liver, 0.080-0.127 ppm in kidney, 0.014-0.021 ppm in muscle, and <0.010-0.012 ppm in fat from the high-dose group. Residues were 0.305-0.440 ppm and 0.191-0.235 ppm in liver, and 0.033-0.039 ppm and 0.017-0.018 ppm in kidney, respectively, from the mid- and low-dose groups. Residues were below the method LOQ (<0.010 ppm) in muscle and fat samples from the mid-dose group; therefore, samples of muscle and fat from the low-dose group were not analyzed. The relationship between dosing level and amicarbazone-related residues appears to be linear in liver and kidney; see Figure C.1.

Apparent amicarbazone-related residues were nondetectable (<0.005 ppm) in/on 12 samples of milk and one sample each of whey, cream, liver, kidney, muscle, and fat from an undosed cow.

Sample storage conditions and intervals are summarized in Table C.2. The maximum storage intervals from collection to analysis were 27 days for whole milk, 148 days for whey and cream, and 237 days for tissues; dates of sample extraction were not included. In support of the feeding study, the petitioner included data from an extraction efficiency study (refer to the DER for MRID 45121713) using a radiolabeled goat liver sample from the ruminant metabolism study. Analysis of the radiolabeled goat liver sample indicated that residue levels of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 were not significantly changed following frozen storage for up to ~45 months. These data are adequate to support the storage intervals and conditions of tissue samples from the cattle feeding study. Because milk samples were analyzed within one month of collection, storage stability data for milk will not be required. No storage stability data are available for whey and cream, therefore, the reported results for whey and cream are not supported. No additional data will be required at this time. Residue data for whey and cream are not required.

TABLE C.1. Summary of Concurrent Recoveries of Amicarbazone and Its Metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 from Lactating Cattle Matrices.

Matrix	Analyte[a]	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev (%)
Milk	Amicarbazone-related residues	0.010	12	79, 80, 80, 81, 83, 83, 86, 87, 87, 88, 90, 91	85 ± 4
Whey	Amicarbazone-related residues	0.010	1	105	105
Cream	Amicarbazone-related residues	0.010	1	91	91
Liver	Amicarbazone-related residues	0.010	4	78, 80, 84, 85	82 ± 3
Kidney	Amicarbazone-related residues	0.010	4	74, 78, 79, 93	81 ± 8
Muscle	Amicarbazone-related residues	0.010	3	74, 78, 81	78 ± 4
Fat	Amicarbazone-related residues	0.010	3	73, 74, 82	76 ± 5

[a] Fortified with a 1:1:1 mixture of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586; residues determined as iPr-2-OH DA MKH 3586, expressed in parent equivalents.

TABLE C.2. Summary of Storage Conditions.

Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Storage Duration from Collection to Analysis	Interval of Demonstrated Storage Stability
Cow, whole milk	<-10	3-27 days (<1 month)	None required
Cow, whey and cream		148 days (4.9 months)	None available
Cow, tissues		86-237 days (2.8-7.8 months)	No significant change in the metabolic profile in [^{14}C]amicarbazone-treated goat liver stored for up to 45 months [a]

[a] Data from an extraction efficiency study (refer to the DER for MRID 45121713).

TABLE C.3. Residue Data from Ruminant Feeding Study with Amicarbazone.

Matrix	Collection Time	Feeding Level (ppm)	Pre-Slaughter Interval (days)	Residues, Amicarbazone Equivalents (ppm) [a]		
				Cow 1	Cow 2	Cow 3
Whole Milk	Day 0	4.538	Not Applicable (N/A)	*0.006*	ND	*0.007*
	Day 4		N/A	ND	ND	ND
	Day 8		N/A	*0.007*	ND	*0.008*
	Day 12		N/A	*0.006*	ND	*0.006*
	Day 16		N/A	*0.005*	ND	*0.005*
	Day 18		N/A	*0.007*	ND	*0 005*
	Day 20		N/A	*0.006*	ND	*0 006*
	Day 22		N/A	*0.008*	ND	*0.005*
	Day 24		N/A	*0.006*	ND	ND
	Day 26		N/A	*0.006*	ND	*0.005*
	Day 28		N/A	*0 005*	ND	*0.005*
Whey	Day 28		N/A	–	–	*0.006* [b]
Cream	Day 28		N/A	–	–	*0.005* [b]

TABLE C.3. Residue Data from Ruminant Feeding Study with Amicarbazone.

Matrix	Collection Time	Feeding Level (ppm)	Pre-Slaughter Interval (days)	Residues, Amicarbazone Equivalents (ppm) [a]		
				Cow 1	Cow 2	Cow 3
Liver	Sacrifice	4.538	0	1.193	1.165	1.184
		1.238	0	0.383	0.305	0.440
		0.374	0	0.197 [b]	0.191 [b]	0.235 [b]
Kidney	Sacrifice	4.538	0	0.080	0.107	0.127
		1.238	0	0.033	0.036	0.039
		0.374	0	0.018 [b]	0.018 [b]	0.017 [b]
Muscle	Sacrifice	4.538	0	0.021	0.014	0.019
		1.238	0	*0.006* [b]	*0.005* [b]	*0.007* [b]
Fat	Sacrifice	4.538	0	*0.009*	*0.008*	0.012
		1.238	0	ND	ND	*0.005* [b]

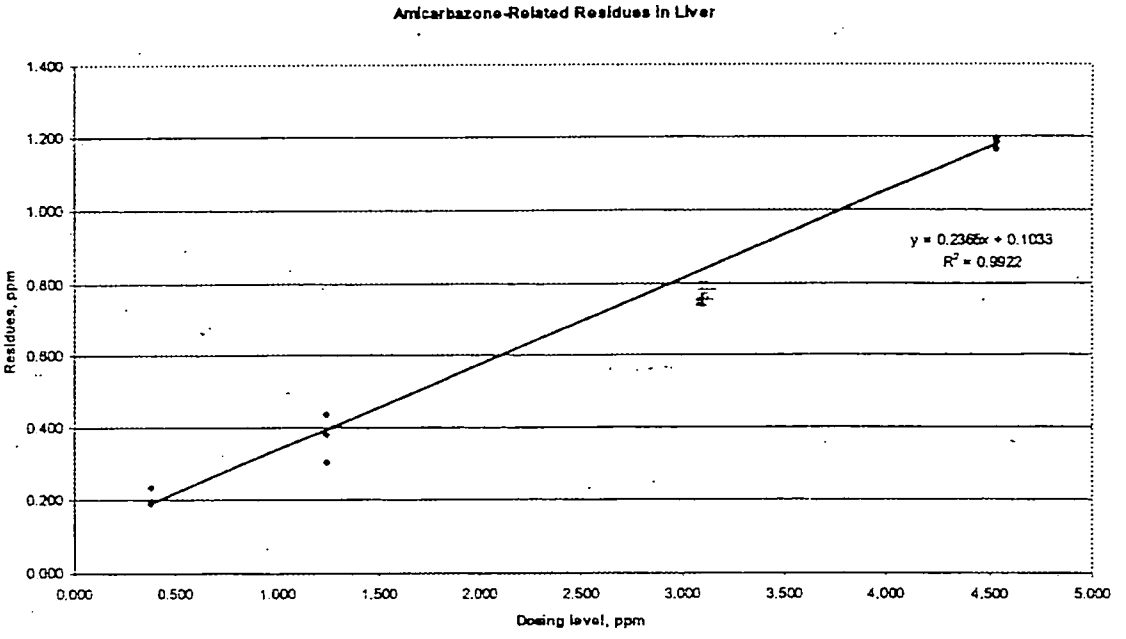
[a] ND = Not detectable. Residues reported between the LOD and LOQ are italicized. The LOD and LOQ, respectively, are 0.005 and 0.010 ppm for milk and tissues.
[b] The highest residues of replicate analyses are reported.

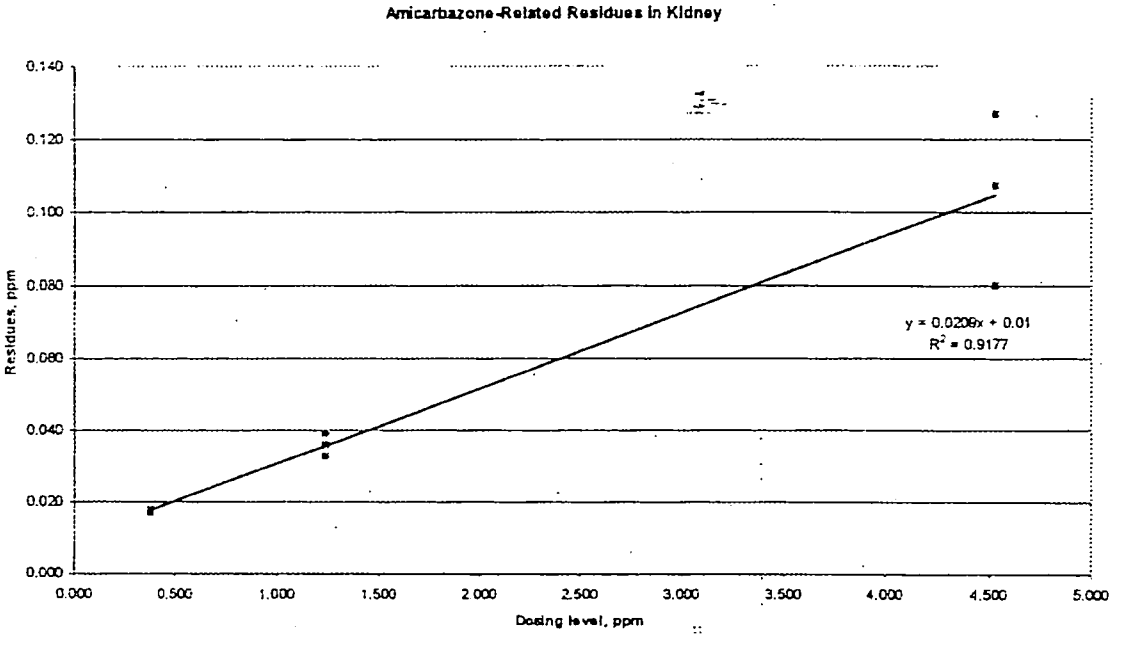
TABLE C.4. Summary of Residue Data from Ruminant Feeding Study with Amicarbazone.

Matrix	Feeding Level (ppm)	Pre-Slaughter Interval (days)	Residue Levels (ppm)[a]					
			n	Min.	Max.	Median	Mean	Std. Dev.
Whole milk	4.538	Not applicable (N/A)	30	<0.010	<0.010	0.005	0.006	0.001
Whey	4.538	N/A	1	<0.010	<0.010	0.006	0.006	N/A
Cream	4.538	N/A	1	<0.010	<0.010	0.005	0.005	N/A
Liver	4.538	0	3	1.165	1.193	1.184	1.181	0.014
	1.238	0	3	0.305	0.440	0.383	0.376	0.068
	0.374	0	3	0.191	0.235	0.197	0.208	0.024
Kidney	4.538	0	3	0.080	0.127	0.107	0.105	0.024
	1.238	0	3	0.033	0.039	0.036	0.036	0.003
	0.374	0	3	0.017	0.018	0.018	0.018	0.001
Muscle	4.538	0	3	0.014	0.021	0.019	0.018	0.004
	1.238	0	3	<0.010	<0.010	0.006	0.006	0.001
Fat	4.538	0	3	<0.010	0.012	0.009	0.010	0.002
	1.238	0		<0.010	<0.010	0.005	0.005	0

[a] For the calculation/reporting of minimum and maximum, the LOQ value (<0.010 ppm for milk and tissues) was used for residues reported between the LOQ and LOD and as nondetectable (ND) in Table C.3. For calculation of the median, mean, and standard deviation, the LOD (0.005 ppm, which corresponds to half the LOQ) was used for residues reported as ND and the actual value was used for residues reported between the LOD and the LOQ.

FIGURE C.1. Linear Regression of Residues on Feeding Level in Liver and Kidney.





D. CONCLUSION

The submitted dairy cattle feeding study is adequate to demonstrate the magnitude of amicarbazone-related residues in ruminant commodities, pending submission of adequate method validation data for liver reflecting the range of residue levels observed in the feeding study. The ruminant feeding study reflects dietary levels of amicarbazone at 0.374, 1.238, and 4.538 ppm.

In samples from the high-dose group, amicarbazone-related residues in whole milk were less than the method LOQ (<0.010 ppm). Because no quantifiable residues were observed in milk from the high-dose group, milk samples from the low- and mid-dose group were not analyzed. Amicarbazone-related residues in liver and kidney were found to have a linear relationship with the dosing level. For muscle and fat, residues were above the LOQ (<0.010 ppm) in samples from the high-dose group but were below the LOQ in samples from the mid-dose group; samples from the low-dose group were not analyzed.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: PP#0F6131
DP Barcode: D288216
PC Code: 114004

Template Version September 2003

13

Primary Evaluator	Manying Xue, Chemist RAB3/HED (7509C)	Date: 03/23/05
Approved by	Leung Cheng, Senior Chemist RAB3/HED (7509C)	Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; updated draft submitted 09/21/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

45121631 De Haan, R. and Brown, F. (1998) The Distribution and Metabolism of (Triazolinone-3-(carbon 14) MKH 3586 in Laying Hens: Lab Project Number: M6040501: 107543. Unpublished study prepared by Bayer Corp. 155 p.

EXECUTIVE SUMMARY:

Arvesta Corporation has submitted a study investigating the metabolism of [triazilinone-3-^{14}C]amicarbazone (specific activity 100,000 dpm/µg) in hens. Radiolabeled amicarbazone was administered orally to ten laying hens at an average of 15.3 ppm in the diet. The hens were dosed once per day for three consecutive days. Eggs were collected twice daily throughout the study, and tissues (muscle, fat, and liver) were collected at sacrifice; eggs and tissues were pooled by matrix (and study day in the case of eggs). The in-life phase of the study was conducted by Southwest Bio-Labs, Inc. (Las Cruces, NM), and the analytical phase of the study was conducted by Bayer Corporation (Stilwell, KS).

Total radioactive residues (TRR) were 0.009 ppm in Day-1 eggs, 0.023 ppm in Day-2 eggs, 0.034 ppm in Day-3 eggs, 0.061 ppm in muscle, 0.032 ppm in fat, and 0.476 ppm in liver from ten hens dosed orally with [^{14}C]amicarbazone. A large portion of the TRR (~58-91% TRR) was extracted from hen commodities, except fat, using ACN/water (muscle and liver) or ACN (eggs); extraction of fat with ACN yielded 40% TRR. Hydrolysis using 1.5% trifluoroacetic acid released additional radioactivity from fat (44% TRR) and eggs (5-22% TRR). Protease hydrolysis released additional radioactivity from liver and muscle (34-39% TRR). These methods adequately extracted residues from hen matrices. Nonextractable residues accounted for 3-14% TRR (0.0004-0.019 ppm) in hen matrices. The petitioner normalized the extraction results, yielding material balances of 99-101%; however, reported accountability prior to normalization was 84-105%. No storage stability data were submitted to support the study, and the actual storage intervals prior to final analysis were not reported.

Parent amicarbazone was a minor residue in the extracts of all matrices, at 1-7% TRR (0.0006-0.010 ppm); however, amicarbazone was identified in the protease hydrolysate of liver at 30% TRR (0.143 ppm). The major residue identified in eggs and tissues was iPr-2-OH DA MKH

3586, at 24% TRR (0.015 ppm) in muscle, 9% TRR (0.043 ppm) in liver, 10% TRR (0.0032 ppm) in fat, and 20-35% TRR (0.0032-0.0068 ppm) in eggs. Metabolite DA MKH 3586 was a significant residue in eggs (19-26% TRR, 0.0023-0.0065 ppm) but was found in lesser amounts in tissues (4-9% TRR, 0.0029-0.019 ppm). Metabolite iPr-1-OH DA MKH 3586 was a significant residue in liver (24% TRR, 0.114 ppm) and was also found in muscle (2% TRR, 0.001 ppm). Metabolite iPr-1-Ene DA MKH 3586 was a significant residue in fat and eggs (11-15% TRR, 0.0010-0.0048 ppm) but was found in lesser amounts in muscle and liver (≤8% TRR, ≤0.010 ppm). Metabolite iPr-Acid DA MKH 3586 was identified in liver only (11% TRR, 0.052 ppm). Other identified metabolites, each ≤7% TRR (≤0.005 ppm) were triazolinone MKH 3586, triazolinone DA MKH 3586, tBu-OH MKH 3586, and iPr-1,2-diOH MKH 3586 (muscle and liver only). The remainder of the radioactivity consisted of unknowns, which were generally polar in nature.

The petitioner proposed that amicarbazone is metabolized in hens via loss of the triazole amino group (deamination), yielding DA MKH 3586, followed by hydroxylation of the isopropyl methine carbon to form iPr-2-OH DA MKH 3586. Dehydration would then result in iPr-Ene DA MKH 3586, which may undergo epoxidation and hydrolysis to form iPr-1,2-diOH DA MKH 3586. Hydroxylation of DA MKH 3586 at an isopropyl methyl carbon would yield iPr-1-OH DA MKH 3586, which may then undergo oxidation to form iPr-Acid DA MKH 3586. Minor pathways involve hydroxylation of the tert-butyl group of amicarbazone to form tBu-OH MKH 3586, and hydrolytic cleavage of the carboxamide side chain of amicarbazone or DA MKH 3586 to form triazolinone MKH 3586 or triazolinone DA MKH 3586.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Additional information pertaining to sample storage intervals should be submitted before the hen metabolism data may be classified as scientifically acceptable. The dates of sample extraction and analysis should be provided for all samples before HED can determine whether storage stability data are required to support this study.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D288216.

COMPLIANCE:

Signed and dated 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

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis.

TABLE A.1. Amicarbazone Nomenclature.

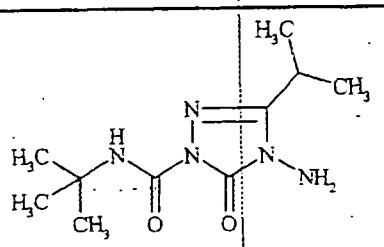
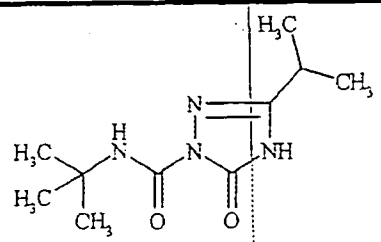
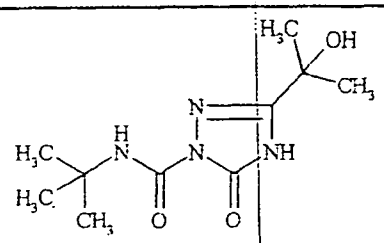
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-*tert*-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS registry number	129909-90-6
End-use formulation (EP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)
DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
*i*Pr-2-OH DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-*H*-1,2,4-triazole-1-carboxamide

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.

Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-6} Pa @ 25°C 1.30×10^{-6} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, Log(K_{ow})	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. EXPERIMENTAL DESIGN

B.1. Livestock

TABLE B.1.1. General Test Animal Information.

Species	Breed	Age	Weight at study initiation (kg)	Health Status	Description of housing/holding area
Laying hen	Leg Horn	Approximately 103 weeks	1.50-1.83; average of 1.65	Appeared healthy throughout study	Metabolism cages housed at Southwest Bio-Labs, Inc. (Las Cruces, NM), with average temperatures ranging 19-23 °C, relative humidity of 41%, and photoperiod of 14 hours light and 10 hours dark

A total of ten hens were dosed with the test substance.

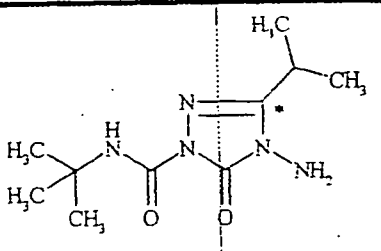
TABLE B.1.2. Test Animal Dietary Regime.

Composition of Diet	Feed consumption (kg/day)	Water	Acclimation period	Predosing
Commercial hen feed, *ad libitum*	0.063-0.112; average of 0.088	Potable water, *ad libitum*	19 days	None

TABLE B.1.3. Test Animal Dosing Regime.			
Treatment Type	Feeding Level (ppm test material in food)	Vehicle	Timing/Duration
Oral	15.3	Gelatine capsules administered using a balling gun	Once per day for three consecutive days

The petitioner reported the feeding level to be 12.7 ppm in the diet. Instead of using the actual feed consumption of hens from this study, the petitioner calculated the feeding level based on the assumption that a hen consumes 6.4% of its body weight in feed per day.

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics.	
Chemical structure	
Radiolabel position	Triazolinone-3-^{14}C
Lot No.	Not specified; the petitioner stated that the test material was obtained from extra dosing capsules from the goat metabolism study (see DER for MRID 45121630)
Purity	99.4%
Specific activity	100,000 dpm/µg

B.3. Sampling Information

TABLE B.3.1. Sample Collection Information.			
Eggs collected	Excreta collected	Interval from last dose to sacrifice	Tissues harvested and analyzed
Eggs were collected twice daily. During the dosing period, seven hens produced a total of 2 eggs each, and three hens produced three eggs each (average of 0.8 eggs per day). During the week prior to dosing, the hens produced 2-8 eggs each (average of 0.8 eggs per day).	Excreta collected daily	4-5 hours	Liver, fat (composite of omental and subcutaneous), and muscle (composite of leg and breast) were collected from each hen and pooled by tissue.

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Eggs were collected twice daily. The afternoon eggs were combined with the following morning eggs and were stored frozen (-20 ± 10 °C) prior to analysis. Hard-shelled internal eggs collected at sacrifice were combined with the Day-3 eggs. Following radioassay, samples were shipped to

Bayer Research Park (Stilwell, KS), where they were stored frozen (-20 ± 5 °C) prior to residue characterization.

Tissue samples were chopped, composited, homogenized in the presence of liquid nitrogen, and then stored frozen (≤-20 ± 10 °C). Following radioassay, the homogenized samples were shipped to Bayer Research Park, where they were stored frozen (-20 ± 5 °C) prior to residue characterization.

Subsamples of liver and muscle were extracted once with hexane and three times with acetonitrile:water (9:1, v:v). The acetonitrile/water extracts were combined, evaporated to dryness, and redissolved in acetonitrile:water (1:1, v:v). The extract was then applied to a solid phase extraction (SPE) cartridge which was eluted with acetonitrile:water (1:1, v:v) and then with acetonitrile (ACN). The ACN/water eluate was evaporated to dryness and redissolved in water:methanol (9:1, v:v) containing 0.1% acetic acid. The nonextractable residues were separately subjected to enzyme hydrolysis using protease (in 0.25 M TRIZMA® base and 5 mM calcium chloride solution; pH adjusted to 7.4 with 3 N HCl; at 37 °C for 16 hours). ACN was added to the hydrolysate, and the mixture was cooled. The ACN/hydrolysate was isolated by centrifugation, and the solids were washed with ACN. The combined ACN fractions were evaporated to dryness, dissolved in water, adjusted to pH 2 using 3 N HCl, and applied to an SPE cartridge. The cartridge was washed with water, and residues were eluted with methanol:water (7:3, v:v). The methanol/water eluate was evaporated to dryness and redissolved in water:methanol (9:1, v:v) containing 0.1% acetic acid.

Subsamples of muscle and liver were subjected to a separate trichloroacetic acid (TCA) extraction to determine protein-bound residues. Subsamples were extracted twice with dichloromethane:methanol (2:1, v:v), and the extracts were combined. The remaining nonextractable residues were extracted twice with 10% TCA at 0 °C, and the extracts were combined. The remaining solids were then hydrolyzed with 10% TCA at 90 °C for 15 min.

Subsamples of fat were extracted three times with ACN and then once with hexane. The combined ACN extracts were evaporated to dryness and redissolved in methanol:water (7:3, v:v) containing 0.1% acetic acid. The methanol/water extract was applied to an SPE cartridge which was eluted with methanol:water (7:3, v:v; containing 0.1% acetic acid) and methanol. The methanol/water eluate was evaporated to dryness and redissolved in water:methanol (9:1, v:v) containing 0.1% acetic acid. The nonextractable residues were hydrolyzed with 1.5% trifluoroacetic acid (TFA) for 16 hours under ambient conditions. The extract was evaporated to dryness and redissolved in water:methanol (9:1, v:v) containing 0.1% acetic acid.

Subsamples of eggs were extracted three times with ACN in the presence of Celite. The combined ACN extracts were concentrated and partitioned with ACN-saturated hexane. The hexane phase was partitioned with ACN (saturated with hexane), and all ACN phases were combined. The combined ACN extracts were evaporated to dryness and redissolved in methanol:water (7:3, v:v) containing 0.1% acetic acid. The methanol/water extract was applied to an SPE cartridge which was eluted with methanol:water (7:3, v:v) containing 0.1% acetic acid,

methanol, and hexane. The methanol/water eluate was evaporated to dryness and redissolved in water:methanol (9:1, v:v) containing 0.1% acetic acid. The nonextractable residues were hydrolyzed with 1.5% TFA as described for fat.

B.4.2. Analytical Methodology

Total radioactive residues (TRR) in egg and tissue samples were determined by combustion/LSC. Extracts and hydrolysates were radioassayed by LSC, and nonextractable residues were radioassayed by combustion/LSC. Detection limits were reported as 0.0005 ppm for eggs, and 0.0003-0.0005 ppm for tissues.

Extracts of hen matrices were analyzed by HPLC using a system equipped with a C-18 column, a UV detector, and a radiodetector; a gradient mobile phase of water and methanol, each containing 0.1% acetic acid, or water and ACN was used. Metabolites were identified by retention time comparisons with those of the following reference standards: [^{14}C]amicarbazone; [^{14}C]DA MKH 3586; [^{14}C]iPr-2-OH DA MKH 3586; [^{14}C]iPr-1,2-diOH DA MKH 3586; [^{14}C]tBu-OH DA MKH 3586; [^{14}C]tBu-OH MKH 3586; [^{14}C]tBu-Acid MKH 3586; [^{14}C]tBu-iPr-2-diOH DA MKH 3586; [^{14}C]iPr-Ene DA MKH 3586; N-Me DA MKH 3586; [^{14}C]triazolinone MKH 3586; and [^{14}C]triazolinone DA MKH 3586. The ^{14}C-labeled standards were obtained from the goat metabolism study (see DER for MRID 45121630).

Purified components from liver and muscle extracts were analyzed by LC/MS with electrospray ionization, using a C-18 column and a gradient mobile phase of water and methanol, each containing 0.1% formic acid, or 2 mM ammonium acetate and ACN containing 0.1% acetic acid. Isolated peaks from liver and muscle were analyzed by TLC using silica gel (60 F-254) plates and a solvent system of hexane:isopropanol (7:3 or 6:4, v:v), each containing 0.1% acetic acid. Radioactive areas were detected and quantified using a phosphorimager.

C. RESULTS AND DISCUSSION

Total radioactive residues (TRR) in hen eggs and tissues are reported in Table C.2.1. TRR were 0.009 ppm in Day-1 eggs, 0.023 ppm in Day-2 eggs, 0.034 ppm in Day-3 eggs, 0.061 ppm in muscle, 0.032 ppm in fat, and 0.476 ppm in liver from hens dosed orally with [^{14}C]amicarbazone at 15.3 ppm in the diet for 3 consecutive days. Radioactivity was highest in liver and lowest in eggs and fat. The majority of the administered dose was excreted; excreta accounted for ~78% of the administered dose.

The distribution of radioactivity in hen matrices is reported in Tables C.2.2.1 and C.2.2.2. A large portion of the TRR (~58-91% TRR) was extracted from hen commodities, except fat, using ACN/water (muscle and liver) or ACN (eggs); extraction of fat with ACN yielded 40% TRR. Hydrolysis using 1.5% TFA released additional radioactivity from fat (44% TRR) and eggs (5-22% TRR). Protease hydrolysis released additional radioactivity from liver and muscle (34-39% TRR). These methods adequately extracted residues from hen matrices. Nonextractable residues accounted for 3-14% TRR (0.0004-0.019 ppm) in hen matrices. The

petitioner normalized the extraction results, yielding material balances of 99-101%; however, reported accountability prior to normalization was 84-105%.

The characterization and identification of radioactive residues is summarized in Table C.2.3. Total residues amounting to 67-91% TRR were identified in liver and eggs, with lower amounts identified in muscle (55% TRR) and fat (39% TRR). Parent amicarbazone was a minor residue in the extracts of all matrices, at 1-7% TRR (0.0006-0.010 ppm); however, amicarbazone was identified in the protease hydrolysate of liver at 30% TRR (0.143 ppm). The major residue identified in eggs and tissues was iPr-2-OH DA MKH 3586, at 24% TRR (0.015 ppm) in muscle, 9% TRR (0.043 ppm) in liver, 10% TRR (0.0032 ppm) in fat, and 20-35% TRR (0.0032-0.0068 ppm) in eggs. Metabolite DA MKH 3586 was a significant residue in eggs (19-26% TRR, 0.0023-0.0065 ppm) but was found in lesser amounts in tissues (4-9% TRR, 0.0029-0.019 ppm). Metabolite iPr-1-OH DA MKH 3586 was a significant residue in liver (24% TRR, 0.114 ppm) and was also found in muscle (2% TRR, 0.001 ppm). Metabolite iPr-1-Ene DA MKH 3586 was a significant residue in fat and eggs (11-15% TRR, 0.0010-0.0048 ppm) but was found in lesser amounts in muscle and liver (≤8% TRR, ≤0.010 ppm). Metabolite iPr-Acid DA MKH 3586 was identified in liver only (11% TRR, 0.052 ppm). Other identified metabolites, each ≤7% TRR (≤0.005 ppm) were triazolinone MKH 3586, triazolinone DA MKH 3586, tBu-OH MKH 3586, and iPr-1,2-diOH MKH 3586 (muscle and liver only).

LC/MS analyses were used to confirm the identification of the following compounds, by comparing the LC/MS spectrum with that of the reference standard: amicarbazone in liver; DA MKH 3586 in liver; iPr-2-OH DA MKH 3586 in muscle and liver; tBu-OH MKH 3586 in muscle; iPr-2-diOH DA MKH 3586 in muscle and liver; and iPr-Ene DA MKH 3586 in liver. All metabolites in fat and eggs were identified by comparison of the HPLC chromatogram with a chromatogram of reference standards and with the chromatogram of the liver and/or muscle extract. The petitioner did not confirm the identification of any compound in fat or eggs, but all residues identified in fat and eggs were individually present at <0.01 ppm.

The metabolites iPr-Acid DA MKH 3586 and iPr-1-OH DA MKH 3586, for which no reference standards were available, were identified in liver using LC/MS analyses. In addition, metabolite iPr-1-OH DA MKH 3586 was isolated from liver and subjected to oxidation using potassium permanganate. An acetone:water (1:9, v:v) solution of the metabolite was mixed with 0.4 M potassium permangate for 8 hours, and the filtrate was evaporated to dryness and redissolved in water:methanol (9:1, v:v) containing 0.1% acetic acid. Oxidation converted the metabolite to iPr-Acid DA MKH 3586. Metabolite iPr-1-OH DA MKH 3586 was identified in muscle via retention time comparison with the metabolite in liver.

Under the HPLC conditions used, triazoline MKH 3586 and triazolinone DA MKH 3586 had identical retention times. TLC analysis of the isolated peaks in liver and muscle indicated that both components were present as a 1:3 mixture of triazolinone MKH 3586 and triazolinone DA MKH 3586 in liver and a 3:1 mixture in muscle. The relative quantities of the two metabolites in fat and eggs was not determined.

HPLC analysis of the protease hydrolysate of muscle nonextractable residues yielded one broad peak, termed M11. The petitioner attempted to partition M11 into organic solvents, which was not successful. M11 was subjected to hydrolysis in 6 N HCl (at 100 °C for 16 hours), which yielded a single polar residue (as determined by HPLC), termed M12. Attempts to partition M12 into organic solvents were unsuccessful. Based on the HPLC retention time of M12, the petitioner concluded that the residue was very polar and possibly a hydroxy or carboxylic acid analog of triazolinone DA MKH 3586.

HPLC analysis of the 1.5% TFA hydrolysate of fat nonextractable residues yielded two polar peaks, one of which had a similar retention time to M12. Based on the retention times, the petitioner concluded that the residues were possibly hydroxy or carboxylic acid analogs of triazolinone DA MKH 3586.

The petitioner indicated that the TCA extraction was employed to further characterize residues released by protease. According to the petitioner, TCA extraction procedures are commonly used to solubilize all non-protein components of a tissue and precipitate all protein-containing components. TCA extraction of liver yielded 75% TRR in the dichloromethane/methanol extract and 2% and 4% TRR in the TCA extracts at 0 °C and 90 °C, respectively, for a total of 81% TRR, and precipitated 19% TRR as solids. When this result was compared to the extraction profile of liver, in which 61% TRR was extractable using hexane and ACN/water, the petitioner concluded that not all of the nonextractable residues from the hexane and ACN/water extractions were bound to proteins; based on TCA extraction results, only approximately 20% of the nonextractable residues were bound to proteins.

TCA extraction of muscle yielded 48% TRR in the dichloromethane/methanol extract and <1% and 0% TRR in the TCA extracts at 0 °C and 90 °C, respectively, for a total of 48% TRR; 52% TRR precipitated as solids. When this result was compared to the extraction profile of muscle, in which 58% TRR was extractable using hexane and ACN/water, the petitioner concluded that all of the residues which were not extractable using hexane and ACN/water were bound to proteins.

C.1. Storage Stability

Egg and tissue samples were stored frozen at -20 ± 5 °C until analysis. The petitioner stated that all egg and tissue samples were extracted and initially profiled by HPLC within 6 weeks of collection, and that preliminary identification of tissue and egg residues was completed within 4 months of sample collection. The petitioner did not provide dates of initial and final sample extraction and analysis. This information should be submitted before it can be determined whether storage stability data are required to support the hen metabolism study.

TABLE C.1. Summary of Storage Conditions.

Matrix	Storage Temp. (°C)	Actual Storage Duration	Interval of Demonstrated Storage Stability
Eggs and tissues	-20 ± 5	Not reported; dates of sample extraction and analysis were not included in the submission.	No supporting storage stability data were provided.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRR) in Eggs, Tissues, and Excreta.

Matrix	Collection Timing	% Administered dose	ppm
Excreta	Day 1	27.07	–
	Day 2	29.54	–
	Day 3	21.48	–
Total excreta	Study duration	78.09	–
Eggs	Day 1	0.01	0.009
	Day 2	0.03	0.023
	Day 3	0.01	0.034
Muscle	At termination	0.39	0.061
Fat	At termination	0.04	0.032
Liver	At termination	0.37	0.476
Total recovery	–	78.94	–

TABLE C.2.2.1. Distribution of the Parent and the Metabolites in Hen Muscle and Liver Following Dosing with Radiolabeled Amicarbazone at 15.3 ppm.

Metabolite Fraction	Muscle		Liver	
	TRR = 0.061 ppm		TRR = 0.476 ppm	
	%TRR	ppm	%TRR	ppm
ACN/water extract	58	0.035	59	0.281
Amicarbazone	1	0.001	2	0.010
DA MKH 3586	6	0.004	4	0.019
iPr-2-OH DA MKH 3586	24	0.015	9	0.043
Triazolinone/Triazolinone DA	6	0.004	1	0.005
tBu-OH MKH 3586	6	0.004	–	–
iPr-1,2-diOH DA MKH 3586	2	0.001	1	0.005
iPr-Acid DA MKH 3586	–	–	11	0.052
iPr-1-OH DA MKH 3586	2	0.001	24	0.114
iPr-Ene DA MKH 3586	8	0.005	2	0.010
Unknowns	4	0.002	5	0.024
Protease hydrolysate	39	0.024	34	0.162
Amicarbazone	–	–	30	0.143
Unknowns	39	0.024	4	0.019
Hexane	<1	<0.001	2	0.010

TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Hen Fat and Eggs Following Dosing with Radiolabeled Amicarbazone at 15.3 ppm. [a]

Metabolite Fraction	Fat		Eggs - Day 1		Eggs - Day 2		Eggs - Day 3	
	TRR = 0.032 ppm		TRR = 0.009 ppm		TRR = 0.023 ppm		TRR = 0.034 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN	40	0.0128	91	0.0080	79	0.0181	67	0.0228
Amicarbazone	3	0.0010	7	0.0006	5	0.0012	4	0.0014
DA MKH 3586	9	0.0029	26	0 0023	21	0.0048	19	0.0065
iPr-2-OH DA MKH 3586	10	0.0032	35	0.0032	27	0.0062	20	0.0068
Triazolinone/Triazolinone DA	1	0.0003	6	0.0005	6	0.0014	7	0.0024
tBu-OH MKH 3586	1	0.0003	6	0.0005	6	0.0014	5	0.0017
iPr-Ene DA MKH 3586	15	0.0048	11	0.0010	14	0.0032	12	0.0041
1.5% TFA hydrolysate	44	0.0141	5 [b]	0 0004	15 [b]	0.0034	22 [b]	0.0075
Unknowns	44	0.0141						
Hexane	1	0.0003						

[a] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.

[b] Because of difficulties with cleanup and concentration, the hydrolysate could not be analyzed by HPLC.

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Hen Matrices Following Dosing with Radiolabeled Amicarbazone at 15.3 ppm.

Compound	Muscle		Liver		Fat		Eggs - Day 1		Eggs - Day 2		Eggs - Day 3	
	TRR=0.061 ppm		TRR=0.476 ppm		TRR=0.032 ppm		TRR=0.009 ppm		TRR=0.023 ppm		TRR=0.034 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Amicarbazone	1	0.001	32	0.153	3	0.0010	7	0.0006	5	0.0012	4	0.0014
DA MKH 3586	6	0.004	4	0.019	9	0.0029	26	0.0023	21	0.0048	19	0.0065
iPr-2-OH DA MKH 3586	24	0.015	9	0.043	10	0.0032	35	0.0032	27	0.0062	20	0.0068
Triazolinone/ Triazolinone DA	6	0.004	1	0.005	1	0.0003	6	0.0005	6	0.0014	7	0.0024
tBu-OH MKH 3586	6	0.004	–	–	1	0.0003	6	0.0005	6	0.0014	5	0.0017
iPr-1,2-diOH DA MKH 3586	2	0.001	1	0.005	–	–	–	–	–	–	–	–
iPr-Acid DA MKH 3586	–	–	11	0.052	–	–	–	–	–	–	–	–
iPr-1-OH DA MKH 3586	2	0.001	24	0.114	–	–	–	–	–	–	–	–
iPr-Ene DA MKH 3586	8	0.005	2	0.010	15	0.0048	11	0.0010	14	0.0032	12	0.0041
Unknowns	4	0.002	9	0.043	–	–	–	–	–	–	–	–
Protease hydrolysate	39	0.024	–	–	–	–	–	–	–	–	–	–
1.5% TFA hydrolysate	–	–	–	–	44	0.0141	5	0.0004	15	0.0034	22	0.0075
Hexane	<1	<0.001	2	0 010	1	0.0003	–	–	–	–	–	–
Total identified	55	0.035	84	0.401	39	0.0125	91	0.0081	79	0.0182	67	0.0229
Total characterized	43	0.026	11	0.053	45	0.0144	5	0.0004	15	0.0034	22	0.0075
Total extractable	97	0.059	95	0.453	85	0.0272	96	0 0084	94	0.0215	89	0.0303
Unextractable (PES) [1]	3	0.002	4	0.019	14	0.0045	4	0.0004	7	0.0016	11	0.0037
Accountability [2]	100		99		99		100		101		100	

[1] Residues remaining after exhaustive extractions.

[2] Accountability = (Total extractable + Total unextractable)/(TRR from combustion analysis; see TABLE C.2.1) * 100.

C.3. Proposed Metabolic Profile

FIGURE C.3.1. Proposed Metabolic Profile of Amicarbazone in Laying Hens.

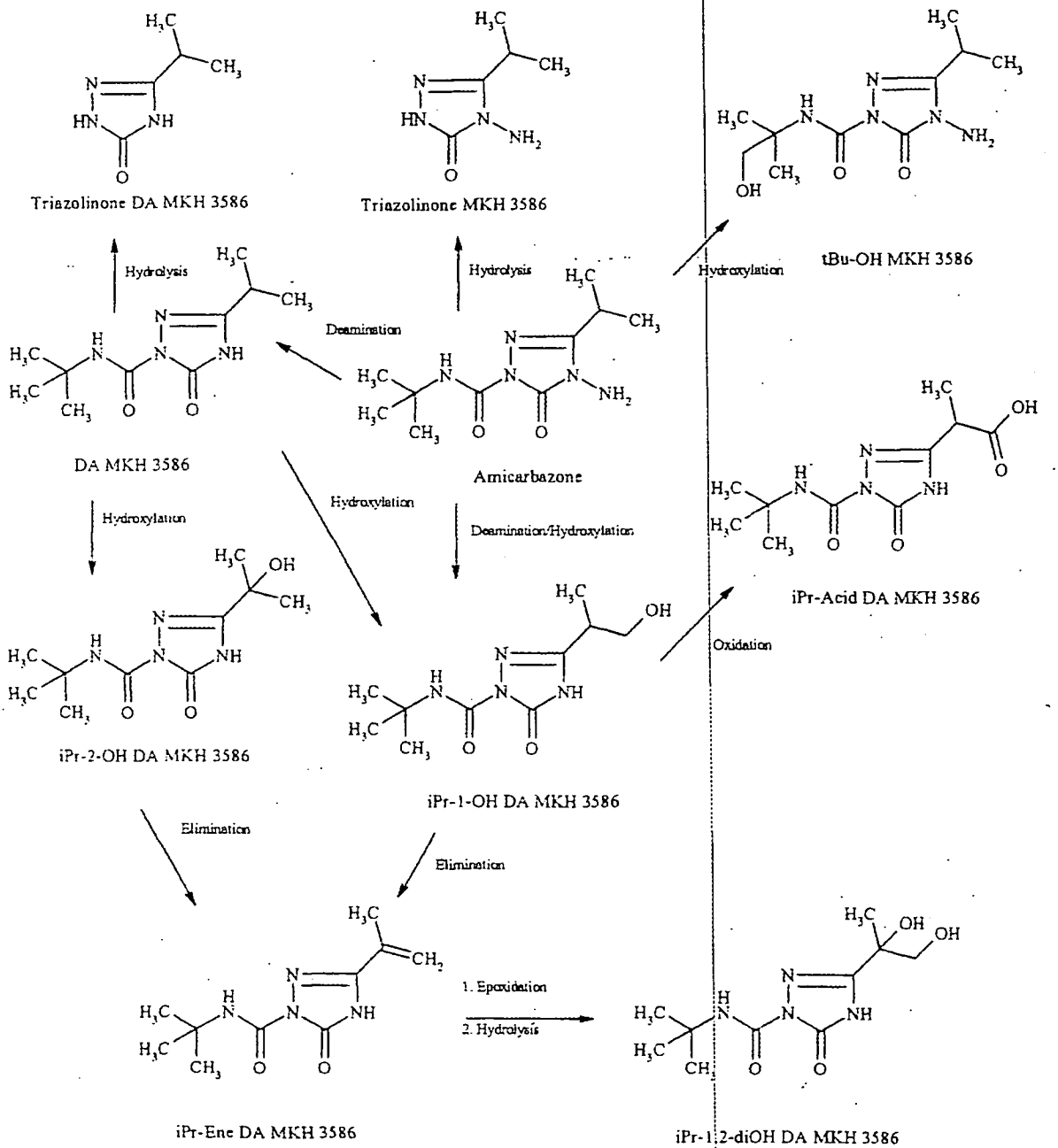


TABLE C.3. Identification of Compounds from Metabolism Study.

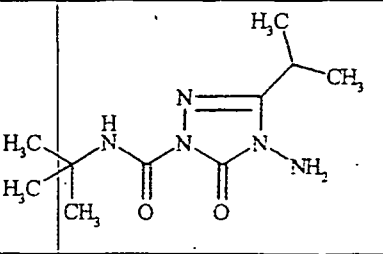
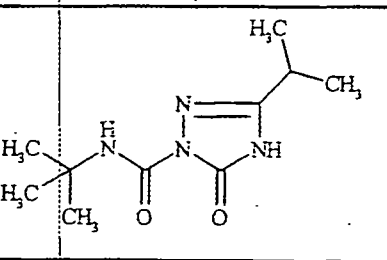
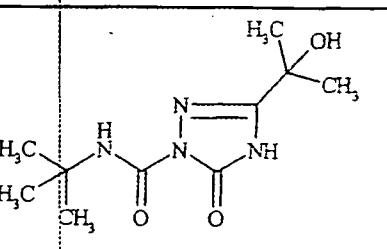
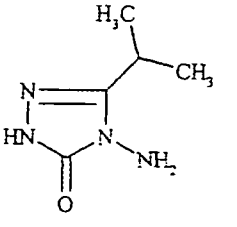
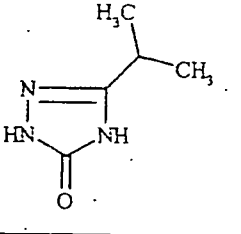
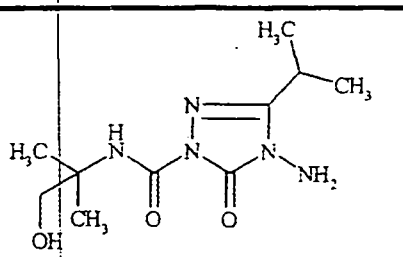
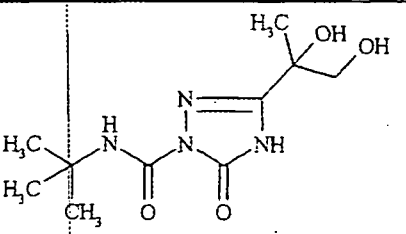
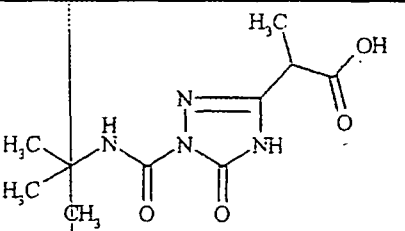
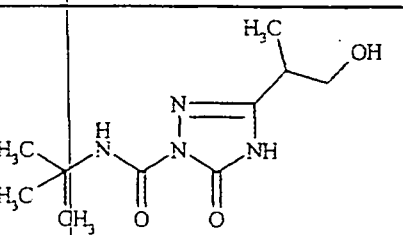
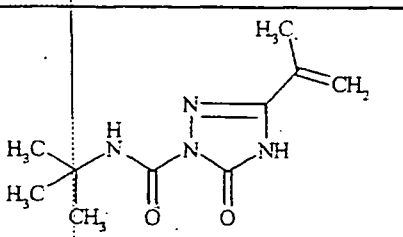
Common name/code Figure C.3.1. ID No.	Chemical name	Chemical structure
Amicarbazone; MKH 3586	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
DA MKH 3586	*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
iPr-2-OH DA MKH 3586	*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
Triazolinone MKH 3586	4-amino-2,4-dihydro-5-(1-methylethyl)-3*H*-1,2,4-triazol-3-one	
Triazolinone DA MKH 3586	1,2-dihydro-5-(1-methylethyl)-3*H*-1,2,4-triazol-3-one	

TABLE C.3. Identification of Compounds from Metabolism Study.

Common name/code Figure C.3.1. ID No.	Chemical name	Chemical structure
tBu-OH MKH 3586	4-amino-4,5-dihydro-*N*-(2-hydroxy-1,1-dimethylethyl)-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
iPr-1,2-diOH DA MKH 3586	3-(1,2-dihydroxy-1-methylethyl)-*N*-(1,1-dimethylethyl)-4,5-dihydro-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
iPr-Acid DA MKH 3586	2-(1-tert-butylcarbamoyl-5-oxo-4,5-dihydro-1H-[1,2,4]triazol-3-yl)propionic acid	
iPr-1-OH DA MKH 3586	3-(2-hydroxy-1-methylethyl)-5-oxo-4,5-dihydro-[1,2,4]triazole-1-carboxylic acid tert-butylamide	
iPr-Ene DA MKH 3586	N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethenyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide	

D. CONCLUSION

Total radioactive residues (TRR) were 0.009 ppm in Day-1 eggs, 0.023 ppm in Day-2 eggs, 0.034 ppm in Day-3 eggs, 0.061 ppm in muscle, 0.032 ppm in fat, and 0.476 ppm in liver from hens dosed orally with [^{14}C]amicarbazone at 15.3 ppm in the diet for 3 consecutive days. A large portion of the TRR (~58-91% TRR) was extracted from hen commodities, except fat; using ACN/water (muscle and liver) or ACN (eggs); extraction of fat with ACN yielded 40% TRR. Hydrolysis using 1.5% TFA released additional radioactivity from fat (44% TRR) and eggs (5-22% TRR). Protease hydrolysis released additional radioactivity from liver and muscle (34-39% TRR). The reported accountability was 84-105%.

Total residues amounting to 67-91% TRR were identified in liver and eggs, with lower amounts identified in muscle (55% TRR) and fat (39% TRR). Parent amicarbazone was a minor residue in the extracts of all matrices; however, amicarbazone was the major component in the protease hydrolysate of liver. The major residue identified in eggs and tissues was iPr-2-OH DA MKH 3586. Metabolite DA MKH 3586 was a significant residue in eggs but was found in lesser amounts in tissues. Metabolite iPr-1-OH DA MKH 3586 was a significant residue in liver and was also found in muscle in minor amounts; iPr-1-Ene DA MKH 3586 was a significant residue in fat and eggs but was found in lesser amounts in muscle and liver; iPr-Acid DA MKH 3586 was identified in liver only; triazolinone MKH 3586, triazolinone DA MKH 3586, and tBu-OH MKH 3586 were found in minor amounts in eggs and tissues; and iPr-1,2-diOH MKH 3586 was a minor residue detected only in muscle and liver. Nonextractable residues accounted for 3-14% TRR (0.0004-0.019 ppm) in hen matrices. Based on the submitted data, the analytical methods used were adequate to determine the identity of the major components in hen eggs and tissues treated with [^{14}C]amicarbazone.

Based on the results of the study, the petitioner proposed that amicarbazone is metabolized in hens via loss of the triazole amino group (deamination), yielding DA MKH 3586, followed by hydroxylation of the isopropyl methine carbon to form iPr-2-OH DA MKH 3586. Dehydration would then result in iPr-Ene DA MKH 3586, which may undergo epoxidation and hydrolysis to form iPr-1,2-diOH DA MKH 3586. Hydroxylation of DA MKH 3586 at an isopropyl methyl carbon would yield iPr-1-OH DA MKH 3586, which may then undergo oxidation to form iPr-Acid DA MKH 3586. Minor pathways involve hydroxylation of the tert-butyl group of amicarbazone to form tBu-OH MKH 3586, and hydrolytic cleavage of the carboxamide side chain of amicarbazone or DA MKH 3586 to form triazolinone MKH 3586 or triazolinone DA MKH 3586.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
DP Barcode: D288216
PC Code: 114004

Template Version September 2003

Primary Evaluator: Manying Xue, Chemist
RAB3/HED (7509C) Date: 03/23/05

Approved by: Leung Cheng, Senior Chemist
RAB3/HED (7509C) Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; updated draft submitted 09/21/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

45121630 De Haan, R. and Brown, F. (1996) The Distribution and Metabolism of (Triazolinone-3-(carbon 14) MKH 3586 in Lactating Goats: Lab Project Number: 107329: M6041001. Unpublished study prepared by Bayer Corp. 135 p.

45121632 De Haan, R. and Brown, F. (1996) The Isolation, Characterization, and Spectroscopic Identification of (Triazolinone-3-(carbon 14)) MKH 3586 Metabolites for use as Reference Standards: Lab Project Number: M6041001: 107328. Unpublished study prepared by Bayer Corp. 55 p.

EXECUTIVE SUMMARY:

Arvesta Corporation has submitted a study investigating the metabolism of [triazolinone-3-^{14}C]amicarbazone (specific activity 100,000 dpm/µg) in goats. Radiolabeled amicarbazone was administered orally to two lactating goats at an average of 101 ppm in the diet. The goats were dosed once per day for three consecutive days. Milk was collected twice daily throughout the study, and tissues (muscle, fat, liver, and kidney) were collected at sacrifice; milk and tissues from the two goats were pooled by matrix (and study day in the case of milk). The in-life phase of the study was conducted by Southwest Bio-Labs, Inc. (Las Cruces, NM), and the analytical phase of the study was conducted by Bayer (Stilwell, KS).

Total radioactive residues (TRR) were 0.202 ppm in Day-1 milk, 0.183 ppm in Day-2 milk, 1.066 ppm in muscle, 0.718 ppm in fat, 4.634 ppm in kidney, and 4.876 ppm in liver from two goats dosed orally with [^{14}C]amicarbazone. The majority of the TRR (~85-99% TRR) was extracted from goat tissues using methanol/acetic acid and acetone (liver, kidney, and muscle) or ACN (milk and fat). Methanol/water extraction at reflux of the nonextractable residues of liver and kidney released small amounts of radioactivity (2-7% TRR); subsequent acid hydrolysis of nonextractable residues released an additional 2-4% TRR. These methods adequately extracted residues from goat matrices. Nonextractable residues accounted for <1-3% TRR (0.004-<0.049 ppm) in goat matrices. The petitioner normalized the extraction results; however, reported

accountability prior to normalization was 80-103%. No storage stability data were submitted to support the study, and the actual storage intervals prior to final analysis were not reported.

Parent amicarbazone was a major residue in all matrices except kidney, accounting for 23-24% TRR (0.042-0.048 ppm) in milk, 29% TRR (0.309 ppm) in muscle, 45% TRR (0.323 ppm) in fat, and 11% TRR (0.537 ppm) in liver; amicarbazone was identified in kidney at 8% TRR (0.370 ppm). Metabolite DA MKH 3586 was a major residue in all matrices, at 18% TRR (0.033-0.036 ppm) in milk, 40% TRR (0.426 ppm) in muscle, 42% TRR (0.302 ppm) in fat, 28% TRR (1.298 ppm) in kidney, and 60% TRR (2.926 ppm) in liver. Metabolite iPr-2-OH DA MKH 3586 was a major residue in kidney, at 26% TRR (1.205 ppm), but was a minor residue in all other matrices (4-8% TRR, 0.015-0.244 ppm). Metabolite tBu-OH MKH 3586 was also identified as a major residue, at 28% TRR (0.051-0.057 ppm) in milk, 16% TRR (0.171 ppm) in muscle, and 10% TRR (0.463 ppm) in kidney, but was a minor residue at 4% TRR (0.029 ppm) in fat and 3% TRR (0.146 ppm) in liver. Metabolite iPr-Ene DA MKH 3586 was a major residue in liver, at 10% TRR (0.488 ppm), but was a minor residue in all other matrices (1-3% TRR, 0.002-0.093 ppm). Other identified metabolites were found at ≤9% TRR; these included triazolinone MKH 3586, triazolinone DA MKH 3586, tBu-Acid MKH 3586, tBu-iPr-2-diOH DA MKH 3586 (kidney and liver only), iPr-1,2-diOH DA MKH 3586, and tBu-OH DA MKH 3586.

The petitioner proposed that amicarbazone is metabolized in goats via loss of the triazole amino group (deamination), yielding DA MKH 3586, followed by hydroxylation of the isopropyl methine carbon to form iPr-2-OH DA MKH 3586. Dehydration would then result in iPr-Ene DA MKH 3586, which may undergo epoxidation and hydrolysis to form iPr-1,2-diOH DA MKH 3586. Minor pathways involve hydroxylation of the tert-butyl group of amicarbazone or DA MKH 3586 to form tBu-OH MKH 3586 or tBu-OH DA MKH 3586, and hydrolytic cleavage of the carboxamide side chain of amicarbazone or DA MKH 3586 to form triazolinone MKH 3586 or triazolinone DA MKH 3586.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Additional information pertaining to sample storage intervals should be submitted before the goat metabolism data may be classified as scientifically acceptable. The dates of sample extraction and analysis should be provided for all samples before HED can determine whether storage stability data are required to support this study.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D288216.

COMPLIANCE:

Signed and dated 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

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis.

TABLE A.1. Amicarbazone Nomenclature.

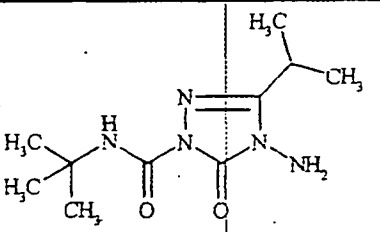
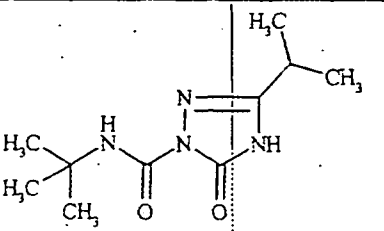
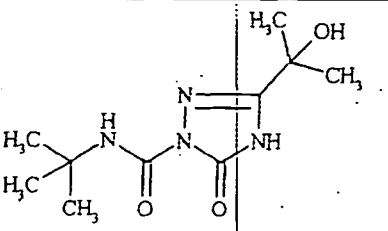
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-tert-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS registry number	129909-90-6
End-use formulation (EP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)
DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
*i*Pr-2-OH DA MKH 3586 (metabolite proposed for regulation)	 *N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-*H*-1,2,4-triazole-1-carboxamide

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.		
Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility (g/L)	4.6	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-6} Pa @ 25°C 1.30×10^{-6} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, $Log(K_{OW})$	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. EXPERIMENTAL DESIGN

B.1. Livestock

TABLE B.1.1. General Test Animal Information.					
Species	Breed	Age	Weight (kg; average for study Days 1-3)	Health Status	Description of housing/holding area
Goat	Alpine	4 years	52.6, 57.6	Appeared healthy throughout study; however, one goat lost 15 lb prior to dosing due to diarrhea which led to a lower feed intake.	Metabolism cages housed at Southwest Bio-Labs, Inc. (Las Cruces, NM), with average temperatures ranging 20-24 °C, relative humidity of 87%, and photoperiod of 14 hours light and 10 hours dark

TABLE B.1.2. Test Animal Dietary Regime.				
Composition of Diet	Food consumption (kg/day)	Water	Acclimation period	Predosing
Purina® Goat Chow, Purina® Alfalfa Pellets, and alfalfa hay *ad libitum*	2.383 - Goat 1 0.715 - Goat 2 *	Potable water, *ad libitum*	13 days	None

* This goat had low feed consumption on Day 1 and 3 of the study.

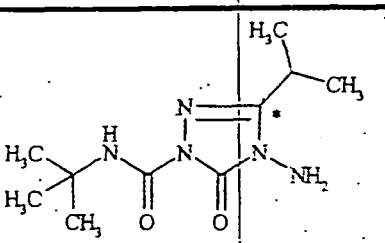
TABLE B.1.3. Test Animal Dosing Regime.			
Treatment Type	Feeding Level (ppm test material in food)	Vehicle	Timing/Duration

Oral	40.4 - Goat 1 162 - Goat 2	Gelatine capsules administered using a balling gun	Once per day following the morning milking for three consecutive days

The petitioner reported the feeding level to be 31.7 ppm in the diet. Instead of using the actual feed consumption of the goats in the study, the petitioner calculated the feeding level based on the assumption that a goat consumes 6% of its body weight in feed per day.

B.2. Test Materials

TABLE B.2.1 Test Material Characteristics.

Chemical structure	
Radiolabel position	Triazolinone-3-^{14}C
Lot No.	Vial C-693
Purity	100%
Specific activity	208,000 dpm/µg (prior to isotopic dilution); 100,000 dpm/µg (dosing solution)

The test substance in acetonitrile was isotopically diluted with unlabeled amicarbazone in ethanol. The mixture was evaporated to dryness and redissolved in ethanol.

B.3. Sampling Information

TABLE B.3.1. Sample Collection Information.

Milk collected	Urine, feces and cage wash collected	Interval from last dose to sacrifice	Tissues harvested and analyzed
Milk was collected twice daily. During the dosing period, 2281-3618 g were collected daily (composite of 2 goats); the amount of milk collected during normal production was not stated.	Urine and feces collected daily.	5 hours	Kidney, liver, fat (composite of omental, renal, and subcutaneous), and muscle (composite of loin, round, and flank) were collected from each goat and pooled by tissue

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Milk was collected twice daily: in the morning prior to dosing, and in the afternoon approximately 8 hours after dosing. The afternoon milk was refrigerated overnight and combined with the morning milk; milk samples from the two goats were then composited into one sample and stored frozen (≤-20 °C) prior to analysis. Because afternoon and morning milk

collections were combined and because the goats were sacrificed within 5 hours of the final dose, only two milk samples were generated. Following radioassay, samples were shipped to Bayer Research Park (Stilwell, KS), where they were stored frozen (-24 ± 4 °C) prior to residue characterization.

Tissue samples were chopped, composited, homogenized in the presence of liquid nitrogen, and then stored frozen (≤-20 °C). Following radioassay, the homogenized samples were shipped to Bayer Research Park, where they were stored frozen (-24 ± 4 °C) prior to residue characterization.

Subsamples of Day-1 and Day-2 milk were extracted three times with acetonitrile (ACN). The combined extracts were concentrated and partitioned with hexane (saturated with ACN), and the resulting hexane fraction was partitioned with ACN (saturated with hexane). All ACN fractions were combined, evaporated to dryness, and redissolved in methanol:water (1:9, v:v) containing 0.1% acetic acid for HPLC analysis.

Subsamples of liver, kidney, and muscle were extracted three times with methanol containing 0.1% acetic acid and then once with acetone. The combined extracts were concentrated, diluted with water, and then concentrated again to remove the organic solvent. The remaining aqueous phase was diluted with water and applied to a strong anion exchange column which was eluted with water and methanol. The methanol eluate was evaporated to dryness and redissolved in methanol:water (1:9, v:v) containing 0.1% acetic acid for HPLC analysis.

The kidney and liver solids remaining after extraction were heated at reflux in methanol:water:acetic acid (4:1:0.05, v:v:v) for 16 hours, and the extract was isolated by filtration. The remaining solids were extracted with acetone, and the acetone extract was combined with the methanol/water/acetic acid extract. The combined extracts were evaporated to dryness and redissolved in methanol:water (1:9, v:v) containing 0.1% acetic acid for HPLC analysis. The remaining solids were hydrolyzed in 0.5 N HCl at reflux for 4 hours and then filtered.

Subsamples of fat were extracted once with hexane and twice with ACN. The extracts were combined and allowed to separate. The hexane fraction was partitioned with ACN (saturated with hexane), and the ACN phase was combined with the ACN fraction. The ACN extract was evaporated to dryness and redissolved in methanol:water (1:9, v:v) containing 0.1% acetic acid for HPLC analysis.

B.4.2. Analytical Methodology

Total radioactive residues (TRR) in milk samples were determined by LSC, and TRR in tissue samples were determined by combustion/LSC. Extracts and hydrolysates were radioassayed by LSC, and nonextractable residues were radioassayed by combustion/LSC. Detection limits were reported as 0.0006 ppm for milk and 0.0003-0.0005 ppm for tissues.

Extracts of goat matrices were analyzed by HPLC using a system equipped with a C-18 column, a UV detector, and a radiodetector. A gradient mobile phase of water and methanol, each containing 0.1% acetic acid, or a gradient mobile phase of water and ACN was used. Metabolites were identified by retention time comparisons with those of the following reference standards: [^{14}C]amicarbazone, [^{14}C]DA MKH 3586, and N-Me DA MKH 3586.

Purified components from liver were analyzed by LC/MS with electrospray ionization using a C-18 column and a gradient mobile phase of water and methanol, each containing 0.1% formic acid. A single isolated peak from liver was analyzed by TLC using silica gel plates and a solvent system of hexane:isopropanol (7:3 or 6:4, v:v), each containing 0.1% acetic acid. Radioactive areas were detected and quantified using a phosphorimager.

Because many of the peaks initially found in goat matrices did not match those of the available reference standards, the petitioner isolated six compounds from goat urine. Each compound was characterized by LC/MS and ^{1}H and ^{13}C NMR, and the compounds were used as reference standards for the identification of metabolites in goat tissues and milk. The following compounds were isolated and identified: [^{14}C]iPr-2-OH DA MKH 3586, [^{14}C]tBu-OH DA MKH 3586, [^{14}C]iPr-1,2-diOH DA MKH 3586, [^{14}C]tBu-OH MKH 3586, [^{14}C]tBu-Acid MKH 3586, and [^{14}C]tBu-iPr-2-diOH DA MKH 3586. Isolated [^{14}C]tBu-Acid MKH 3586 was also methylated (using diazomethane), LC/MS and NMR spectra of the methylated metabolite confirmed the identification as tBu-Acid MKH 3586. In addition, identification of isolated [^{14}C]tBu-OH MKH 3586 in goat urine was confirmed by deamination (using HCl and sodium nitrite) and LC/MS and NMR analyses of the deaminated product.

C. RESULTS AND DISCUSSION

Total radioactive residues (TRR) in goat milk and tissues are reported in Table C.2.1. TRR were 0.202 ppm in Day-1 milk, 0.183 ppm in Day-2 milk, 1.066 ppm in muscle, 0.718 ppm in fat, 4.634 ppm in kidney, and 4.876 ppm in liver from two goats dosed orally with [^{14}C]amicarbazone at an average of 101 ppm in the diet for 3 consecutive days. Radioactivity was highest in kidney and liver and lowest in milk. The majority of the administered dose was excreted; urine and feces accounted for ~69% of the administered dose.

The distribution of radioactivity in goat commodities is reported in Tables C.2.2.1 and C.2.2.2. The majority of the TRR (~85-99% TRR) was extracted from goat tissues using methanol/acetic acid and acetone (liver, kidney, and muscle) or ACN (milk and fat). Methanol/water extraction at reflux of the nonextractable residues of liver and kidney released small amounts of radioactivity (2-7% TRR); subsequent acid hydrolysis of nonextractable residues released an additional 2-4% TRR. These methods adequately extracted residues from goat matrices. Nonextractable residues accounted for <1-3% TRR (0.004-<0.049 ppm) in goat matrices. The petitioner normalized the extraction results, yielding material balances of 100%; however, reported accountability prior to normalization was 80-103%.

The characterization and identification of radioactive residues is summarized in Table C.2.3. Total residues amounting to 91-99% TRR were identified in goat matrices. Parent amicarbazone was a major residue in all matrices except kidney, accounting for 23-24% TRR (0.042-0.048 ppm) in milk; 29% TRR (0.309 ppm) in muscle, 45% TRR (0.323 ppm) in fat, and 11% TRR (0.537 ppm) in liver; amicarbazone was identified in kidney at 8% TRR (0.370 ppm). Metabolite DA MKH 3586 was a major residue in all matrices, at 18% TRR (0.033-0.036 ppm) in milk, 40% TRR (0.426 ppm) in muscle, 42% TRR (0.302 ppm) in fat, 28% TRR (1.298 ppm) in kidney, and 60% TRR (2.926 ppm) in liver. Metabolite iPr-2-OH DA MKH 3586 was a major residue in kidney, at 26% TRR (1.205 ppm), but was a minor residue in all other matrices (4-8% TRR, 0.015-0.244 ppm). Metabolite tBu-OH MKH 3586 was also identified as a major residue, at 28% TRR (0.051-0.057 ppm) in milk, 16% TRR (0.171 ppm) in muscle, and 10% TRR (0.463 ppm) in kidney, but as a minor residue at 4% TRR (0.029 ppm) in fat and 3% TRR (0.146 ppm) in liver. Metabolite iPr-Ene DA MKH 3586 was a major residue in liver, at 10% TRR (0.488 ppm), but was a minor residue in all other matrices (1-3% TRR, 0.002-0.093 ppm). Other identified metabolites were found at ≤9% TRR; these included triazolinone MKH 3586; triazolinone DA MKH 3586; tBu-Acid MKH 3586; tBu-iPr-2-diOH DA MKH 3586 (kidney and liver only); iPr-1,2-diOH DA MKH 3586; and tBu-OH DA MKH 3586.

LC/MS analyses were used to confirm the identification of the following compounds in liver: amicarbazone; DA MKH 3586; iPr-2-OH DA MKH 3586; tBu-iPr-2-diOH DA MKH 3586; tBu-OH MKH 3586; iPr-1,2-diOH DA MKH 3586; and tBu-OH DA MKH 3586. The LC/MS spectra of the isolated metabolites from liver were compared to the LC/MS spectra of the reference standards (in the case of amicarbazone and DA MKH 3586) or of the isolated compound from goat urine. tBu-Acid MKH 3586 was only tentatively identified in milk and tissue extracts by comparison of HPLC retention times with the isolated urine metabolite.

Metabolite iPr-Ene DA MKH 3586, for which no reference standards were available, was identified in liver using LC/MS analyses. In addition, the metabolite was isolated from liver and subjected to hydrogenation (a methanol solution of the metabolite was mixed with a palladium catalyst and purged with hydrogen) and oxidation (a tert-butanol/water solution of the metabolite was mixed with osmium tetraoxide for 2 hours). LC/MS analyses indicated that the hydrogenation product was DA MKH 3586 and the oxidation product was iPr-1,2-diOH DA MKH 3586; these results were consistent with the presence of a double bond on the isopropyl group. This metabolite was identified in kidney, muscle, fat, and milk by comparison of retention times with that of the metabolite in liver.

Under the HPLC conditions used, triazolinone MKH 3586 and triazolinone DA MKH 3586 had identical retention times. TLC analysis of the isolated peak in liver indicated that both components were present as a 1:3 mixture of triazolinone MKH 3586 to triazolinone DA MKH 3586. The relative quantities of the two metabolites in muscle, fat, kidney, and milk was not determined. The petitioner stated that reference standards of [^{14}C]triazolinone MKH 3586 and [^{14}C]triazolinone DA MKH 3586 were obtained from goat urine. In MRID 45121630 (page 35) it was stated that the LC/MS spectra of these compounds could be found in MRID 45121632; however, these compounds are not discussed in MRID 45121632. Because these compounds

were not found in goat matrices at significant levels, we will not require the petitioner to identify the source of these two compounds or provide the LC/MS spectra at this time.

All metabolites in muscle, fat, kidney, and milk were identified by comparison of the HPLC chromatogram with a chromatogram of reference standards and with the chromatogram of the liver extract. The petitioner did not confirm the identification of any compound in muscle, fat, kidney, or milk. Currently Agency guidance (OPPTS 860.1300) requires that: "identification of metabolites should be established using two different analytical techniques except when unambiguous identification is made using a spectroscopic method such as gas or liquid chromatography/mass spectrometry (GC/MSX or LC/MS), or the metabolite is determined to be of minimal importance due to its low absolute level (<0.05 ppm) or percentage of the TRR (<10 percent of TRR)." Based on these requirements, the petitioner should have confirmed the identification of the following: amicarbazone in muscle, fat, and milk; DA MKH 3586 in muscle, fat, kidney, and milk; t-Bu-OH MKH 3586 in muscle, kidney, and milk; and iPr-2-OH DA MKH 3586 in kidney. For the purposes of this petition only, the Agency will not require the petitioner to go back and conduct additional confirmation because the metabolite profiles (HPLC chromatograms) were similar for all tissues. However, the petitioner is reminded that the confirmation requirements specified in 860.1300 should be met for all future metabolism studies.

C.1. Storage Stability

Milk and tissue samples were stored frozen at ≤-20 °C until analysis. The petitioner stated that all milk and tissue samples were extracted and initially profiled by HPLC within 6 weeks of collection, and that preliminary identification of tissue and milk residues was completed within 4 months of sample collection. The petitioner did not provide dates of initial and final sample extraction and analysis. This information should be submitted before it can be determined whether storage stability data are required to support the goat metabolism study.

TABLE C.1. Summary of Storage Conditions.

Matrix	Storage Temp. (°C)	Actual Storage Duration	Interval of Demonstrated Storage Stability
Milk and tissues	≤-20	Not reported; dates of sample extraction and analysis were not included in the submission.	No supporting storage stability data were provided.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRR) in Milk, Tissues, and Excreta.

Matrix	Collection Timing	% Administered Dose	ppm
Urine	Day 1	23.56	–
	Day 2	40.98	–
	Day 3	0.30	–

TABLE C.2.1. Total Radioactive Residues (TRR) in Milk, Tissues, and Excreta.

Matrix	Collection Timing	% Administered Dose	ppm
Feces	Day 1	1.07	–
	Day 2	2.66	–
	Day 3	0.48	–
Total excreta	Study duration	69.05	–
Muscle	At termination	0.26	1.066
Fat	At termination	0.13	0.718
Kidney	At termination	0.24	4.634
Liver	At termination	1.64	4.876
Milk	Day 1	0.11	0.202
	Day 2	0.10	0.183
Bile	At termination	0.02	–
Total recovery	–	71.55	–

TABLE C.2.2.1. Distribution of the Parent and the Metabolites in Goat Muscle, Kidney, and Liver Following Dosing with Radiolabeled Amicarbazone at an Average of 101 ppm.[a]

Metabolite Fraction	Muscle		Kidney		Liver	
	TRR = 1.066 ppm		TRR = 4.634 ppm		TRR = 4.876 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
Methanol eluate	98	1.045	85	3.939	94	4.583
Amicarbazone	29	0.309	7	0.324	10	0.488
DA MKH 3586	40	0.426	26	1.205	59	2.877
iPr-2-OH DA MKH 3586	5	0.053	24	1.112	5	0.244
Triazolinone/Triazolinone DA	2	0.021	1	0.046	1	0.049
tBu-Acid MKH 3586[b]	<1	<0.011	2	0.093	<1	<0.049
tBu-iPr-2-diOH DA MKH 3586	--	--	2	0.093	<1	<0.049
tBu-OH MKH 3586	16	0.171	9	0.417	3	0.146
iPr-1,2-diOH DA MKH 3586	1	0.011	8	0.371	2	0.098
tBu-OH DA MKH 3586	1	0.011	3	0.139	<1	<0.049
iPr-Ene DA MKH 3586	3	0.032	2	0.093	10	0.488
Unknowns	1	0.011	3	0.138	2	0.098
Methanol/water reflux			7	0.324	2	0.098
Amicarbazone			1	0.046	1	0.049
DA MKH 3586			2	0.093	1	0.049
iPr-2-OH DA MKH 3586			2	0.093	<1	<0.049
Triazolinone/Triazolinone DA			<1	<0.046	<1	<0.049
tBu-Acid MKH 3586			<1	<0.046	<1	<0.049
tBu-iPr-2-diOH DA MKH 3586			<1	<0.046	<1	<0.049
tBu-OH MKH 3586			1	0.046	<1	<0.049
iPr-1,2-diOH DA MKH 3586			1	0.046	<1	<0.049
tBu-OH DA MKH 3586			<1	<0.046	<1	<0.049
iPr-Ene DA MKH 3586			<1	<0.046	<1	<0.049
Unknowns			<1	<0.046	<1	<0.049
Aqueous eluate	<1	<0.011	3	0.139	2	0.098
0.5 N HCl hydrolysate			4	0.185	2	0.098

[a] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[b] Tentatively identified by HPLC.

TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Goat Fat and Milk Following Dosing with Radiolabeled Amicarbazone at an Average of 101 ppm.

Metabolite Fraction	Fat		Milk - Day 1		Milk - Day 2	
	TRR = 0.718 ppm		TRR = 0.202 ppm		TRR = 0.183 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN	99	0.711	98	0.198	97	0.173
Amicarbazone	45	0.323	24	0.048	23	0.042

TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Goat Fat and Milk Following Dosing with Radiolabeled Amicarbazone at an Average of 101 ppm.

Metabolite Fraction	Fat		Milk - Day 1		Milk - Day 2	
	TRR = 0.718 ppm		TRR = 0.202 ppm		TRR = 0.183 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
DA MKH 3586	42	0.302	18	0.036	18	0.033
iPr-2-OH DA MKH 3586	4	0.029	8	0.016	8	0.015
Triazolinone/Triazolinone DA	1	0.007	3	0.006	5	0.009
tBu-Acid MKH 3586 *	<1	<0.007	2	0.004	2	0.004
tBu-OH MKH 3586	4	0.029	28	0.057	28	0.051
iPr-1,2-diOH DA MKH 3586	1	0.007	7	0.014	8	0.015
tBu-OH DA MKH 3586	<1	<0.007	3	0.006	2	0.004
iPr-Ene DA MKH 3586	2	0.014	1	0.002	1	0.002
Unknowns	<1	<0.007	4	0.008	3	0.005
Hexane	<1	<0.007	<1	<0.002	<1	<0.002

* Tentatively identified by HPLC.

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Livestock Matrices Following Dosing with Radiolabeled Amicarbazone at an Average of 101 ppm.

Compound	Muscle		Fat		Kidney		Liver		Milk - Day 1		Milk - Day 2	
	TRR=1.066 ppm		TRR=0.718 ppm		TRR=4.634 ppm		TRR=4.876 ppm		TRR=0.202 ppm		TRR=0.183 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Amicarbazone	29	0.309	45	0.323	8	0.370	11	0.537	24	0.048	23	0.042
DA MKH 3586	40	0.426	42	0.302	28	1.298	60	2.926	18	0.036	18	0.033
iPr-2-OH DA MKH 3586	5	0.053	4	0.029	26	1.205	5	0.244	8	0.016	8	0.015
Triazolinone/ Triazolinone DA	2	0.021	1	0.007	1	0.046	1	0.049	3	0.006	5	0.009
tBu-Acid MKH 3586	<1	<0.011	<1	<0.007	2	0.093	<1	<0.049	2	0.004	2	0.004
tBu-iPr-2-diOH DA MKH 3586	–	–	–	–	2	0.093	<1	<0.049	–	–	–	–
tBu-OH MKH 3586	16	0.171	4	0.029	10	0.463	3	0.146	28	0.057	28	0.051
iPr-1,2-diOH DA MKH 3586	1	0.011	1	0.007	9	0.417	2	0.098	7	0.014	8	0.015
tBu-OH DA MKH 3586	1	0.011	<1	<0.007	3	0.139	<1	<0.049	3	0.006	2	0.004
iPr-Ene DA MKH 3586	3	0.032	2	0.014	2	0.093	10	0.488	1	0.002	1	0.002
Unknowns	<1	<0.011	<1	<0.007	3	0.138	2	0.098	4	0.008	3	0.005
Aqueous eluate	<1	<0.011	–	–	3	0.139	2	0.098	–	–	–	–
0.5 N HCl hydrolysate	–	–	–	–	4	0.185	2	0.098	–	–	–	–
Total identified	97	1.034	99	0.711	91	4.217	92	4.488	94	0.189	95	0.175
Total characterized	1	0.011	<1	<0.007	10	0.462	6	0.294	4	0.008	3	0.005
Total extractable	98	1.045	99	0.711	99	4.587	100	4.877	98	0.198	97	0.173
Unextractable (PES) [1]	1	0.011	<1	<0.007	1	0.046	<1	<0.049	2	0.004	3	0.005
Accountability [2]	99		99		100		100		100		100	

[1] Residues remaining after exhaustive extractions.
[2] Accountability = (Total extractable + Total unextractable)/(TRR from combustion analysis; see TABLE C.2.1) * 100.

C.3. Proposed Metabolic Profile

FIGURE C.3 Proposed Metabolic Profile of Amicarbazone in Lactating Goats.

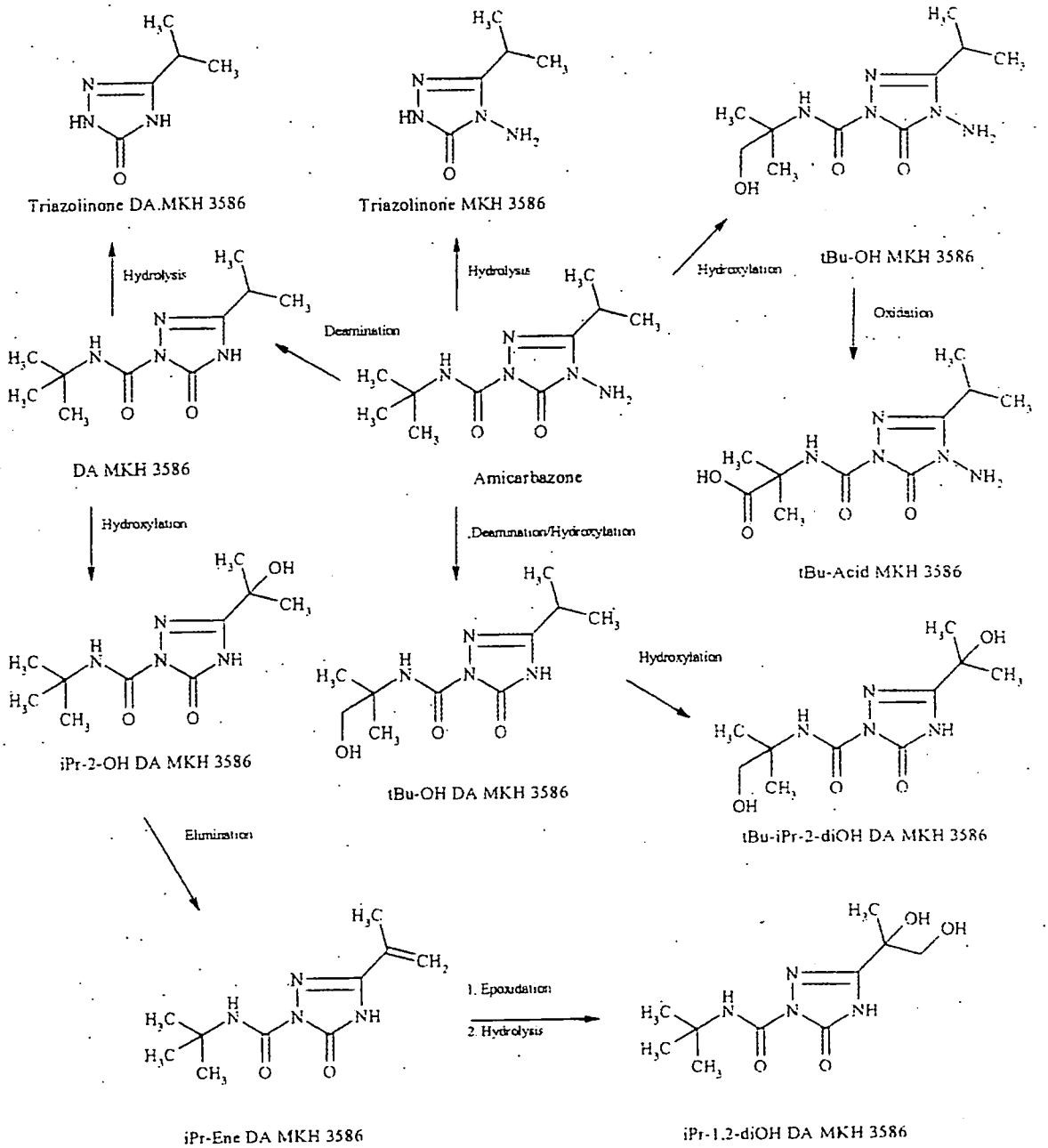


TABLE C.3.1. Identification of Compounds from Metabolism Study.

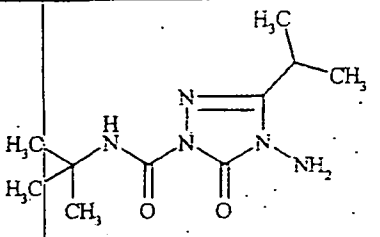
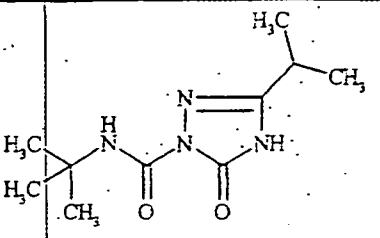
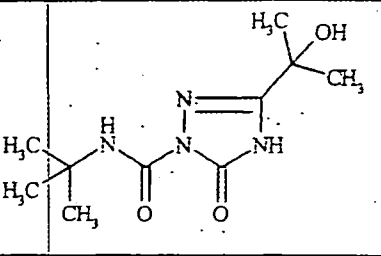
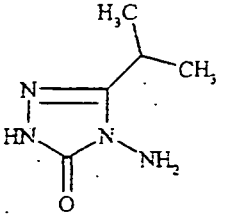
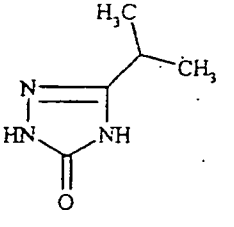
Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
Amicarbazone; MKH 3586	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
DA MKH 3586	*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
iPr-2-OH DA MKH 3586	*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
Triazolinone MKH 3586	4-amino-2,4-dihydro-5-(1-methylethyl)-3*H*-1,2,4-triazol-3-one	
Triazolinone DA MKH 3586	1,2-dihydro-5-(1-methylethyl)-3*H*-1,2,4-triazol-3-one	

TABLE C.3.1. Identification of Compounds from Metabolism Study.

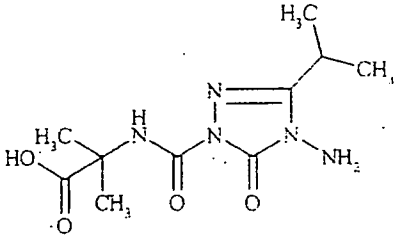
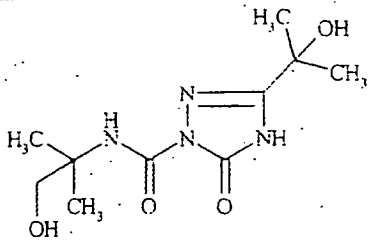
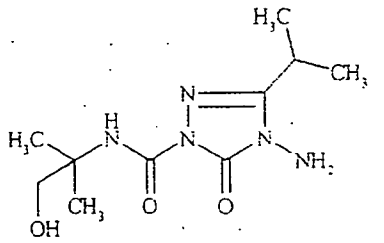
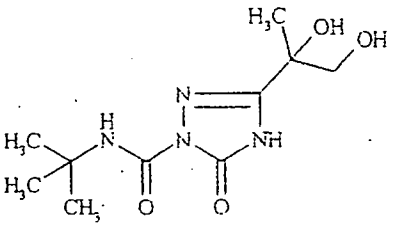
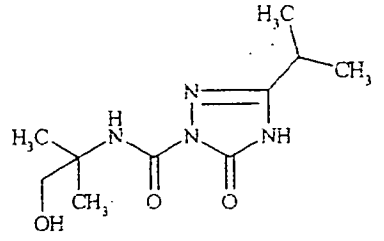
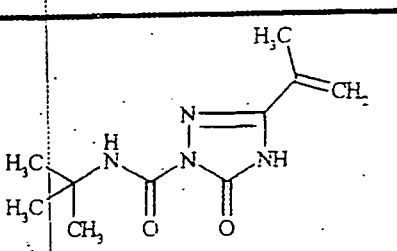
Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
tBu-Acid MKH 3586	*N*-[[4-amino-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-yl]carbonyl]-2-methylalanine	
tBu-iPr-2-diOH DA MKH 3586	4,5-dihydro-*N*-(2-hydroxy-1,1-dimethylethyl)-3-(1-hydroxy-1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
tBu-OH MKH 3586	4-amino-4,5-dihydro-*N*-(2-hydroxy-1,1-dimethylethyl)-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
iPr-1,2-diOH DA MKH 3586	3-(1,2-dihydroxy-1-methylethyl)-*N*-(1,1-dimethylethyl)-4,5-dihydro-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
tBu-OH DA MKH 3586	4,5-dihydro-*N*-(2-hydroxy-1,1-dimethylethyl)-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	

TABLE C.3.1. Identification of Compounds from Metabolism Study.		
Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
iPr-Ene DA MKH 3586	N-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethenyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide	

D. CONCLUSION

Total radioactive residues (TRR) were 0.202 ppm in Day-1 milk, 0.183 ppm in Day-2 milk, 1.066 ppm in muscle, 0.718 ppm in fat, 4.634 ppm in kidney, and 4.876 ppm in liver from two goats dosed orally with [^{14}C]amicarbazone at an average of 101 ppm in the diet for 3 consecutive days. The majority of the TRR (~85-99% TRR) was extracted from goat tissues using methanol/acetic acid and acetone (liver, kidney, and muscle) or ACN (milk and fat). Methanol/water extraction at reflux of the nonextractable residues of liver and kidney released small amounts of radioactivity (2-7% TRR); subsequent acid hydrolysis of nonextractable residues released an additional 2-4% TRR. The reported accountability was 80-103%.

Total residues amounting to 91-99% TRR were identified in goat matrices. Parent amicarbazone was identified in all matrices (11% - 45% TRR), and was a major residue in all matrices except kidney. Metabolite DA MKH 3586 was a major residue in all matrices (18% - 40% TRR). Metabolite iPr-Ene DA MKH 3586 was a major residue in liver (10% TRR), but a minor residue in all other matrices. Metabolite tBu-OH MKH 3586 was identified in all matrices and was a major residue in milk, muscle, and kidney (10%-28%); iPr-2-OH DA MKH 3586 was a major residue in kidney (26% TRR), but was a minor residue in all other matrices. Other identified metabolites were found at ≤9% TRR; these included triazolinone MKH 3586, triazolinone DA MKH 3586, tBu-Acid MKH 3586, tBu-iPr-2-diOH DA MKH 3586 (kidney and liver only), iPr-1,2-diOH DA MKH 3586, and tBu-OH DA MKH 3586. Based on the submitted data, the analytical methods used were adequate to determine the identity of the major components in goat milk and tissues treated with radiolabeled amicarbazone.

Based on the results of the study, the petitioner proposed that amicarbazone is metabolized in goats via loss of the triazole amino group (deamination), yielding DA MKH 3586, followed by hydroxylation of the isopropyl methine carbon to form iPr-2-OH DA MKH 3586. Dehydration would then result in iPr-Ene DA MKH 3586, which may undergo epoxidation and hydrolysis to form iPr-1,2-diOH DA MKH 3586. Minor pathways involve hydroxylation of the tert-butyl group of amicarbazone or DA MKH 3586 to form tBu-OH MKH 3586 or tBu-OH DA MKH 3586, and hydrolytic cleavage of the carboxamide side chain of amicarbazone or DA MKH 3586 to form triazolinone MKH 3586 or triazolinone DA MKH 3586.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: PP#0F6131
DP Barcode: D288216
PC Code: 114004

Template Version September 2003

15

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 6.3/OPPTS 860.1300/OECD II 6.2.2, 6.2.3 & IIIA 8.2, 8.4.1, 8.4.2
Nature of the Residues in Plants - Corn

Primary Evaluator: Manying Xue, Chemist
RAB3/HED (7509C) Date: 03/23/05

Approved by: Leung Cheng, Senior Chemist
RAB3/HED (7509C) Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; updated draft submitted 09/21/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

45121633 Matthew, A. and Nguyen, T. (1997) Metabolism of (Triazolinone-3-(carbon 14))MKH 3586 in Corn: Lab Project Number: 107531: M6041601. Unpublished study.prepared by Bayer Corp. 129 p.

EXECUTIVE SUMMARY:

Arvesta Corporation has submitted a study investigating the metabolism of [triazolinone-3-^{14}C]amicarbazone (specific activity 208,500 dpm/µg) in corn. The radiolabeled test substance was applied to the soil at the equivalent of 0.72 lb ai/A four hours after planting corn seed. The plants were grown in containers in a greenhouse. The in-life and analytical phases of the study were conducted by Bayer Corporation (Stilwell, KS).

The total radioactive residues (TRR) were 0.988, 2.369, and 0.054 ppm in corn forage, fodder, and grain, respectively, harvested 104 days (forage) or 126 days (fodder and grain) following treatment. The majority of the TRR (~82-92% TRR) was extracted from corn matrices using acetonitrile/water/acetic acid or acetonitrile/water/acetic acid and methanol. Additional residues were released by Soxhlet extraction with acetonitrile/water/acetic acid (3-7% TRR), and hydrolysis with 2 N HCl at reflux (5% TRR; forage) or with 1 N or 2 N KOH at reflux (3-8% TRR; fodder and grain). Total extractable residues were 91-100% TRR. Because extraction values were normalized by the petitioner, reported accountabilities were 100%; however, information presented in the raw data indicated that actual accountabilities were 99-104%. Residues were characterized/identified primarily by HPLC analysis with confirmatory analysis by TLC and LC/MS. Adequate storage stability data were submitted demonstrating the stability of the metabolite profiles in corn forage, fodder, and grain for ~8, 7, and 11 months, respectively, of frozen storage. However, the dates of sample extraction and analysis were not included in the submission.

Total identified residues were 74-79% TRR in corn commodities. Parent, amicarbazone, was the major residue identified in forage (45%TRR) and fodder (20% TRR); amicarbazone was

identified in smaller amounts in grain (13% TRR). The major residue identified in grain was iPr-2-OH DA MKH 3586 (38% TRR); this compound was also found in forage (15% TRR) and fodder (16% TRR). Other identified residues were DA MKH 3586 at 14% TRR in grain, 6% TRR in forage and 10% TRR in fodder; 4-*N*-glu-DA MKH 3586 at 4% in grain, 5% TRR in forage and 7% TRR in fodder; tBu-iPr-2-diOH DA MKH 3586 at 4% TRR in forage only; and iPr-2-O-glu-DA MKH 3586 at 4% TRR in forage, 5% TRR in fodder, and 7% TRR in grain. Four additional glucose/glucoside conjugates were identified in small amounts (≤5% TRR) in fodder.

Based on the results of the study, the petitioner proposed that the major metabolic pathway in corn involves the deamination of the triazole amino group followed by hydroxylation at the tertiary carbon of the isopropyl group to form iPr-2-OH DA MKH 3586. Additional pathways involve hydroxylation of the isopropyl methyl to form iPr-1-OH DA MKH 3586, followed by glucosidation; hydroxylation of the t-butyl and isopropyl groups to form tBu-iPr-2-diOH DA MKH 3586. In addition, DA MKH 3586 formed an *N*-glucoside; and glucosidation of hydroxylated DA MKH 3586 formed several minor *O*-glucosides. The proposed metabolic pathway is presented in Figure C.3.1.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Additional information pertaining to sample storage intervals should be submitted before the corn metabolism data may be classified as scientifically acceptable. The dates of initial and final sample extraction and analysis should be submitted before it can be determined whether the storage stability data included in the study are adequate to support the corn metabolism study.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D288216.

COMPLIANCE:

Signed and dated 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

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis.

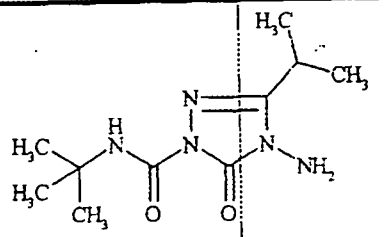
TABLE A.1. Amicarbazone Nomenclature.	
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-*tert*-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS #	129909-90-6
End-use formulation (EUP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.

Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-6} Pa @ 25°C 1.30×10^{-6} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, $Log(K_{OW})$	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

TABLE B.1.1. Test Site Information.

Testing Environment	Soil characteristics			
	Type	%OM	pH	CEC
Five-gallon buckets in a greenhouse	Silt loam	1.8	6.0	13.6 (no units reported)

The petitioner included temperature and humidity data for the greenhouse during the crop growth period; average temperature and humidity were 24 ± 1.3 °C and 18 ± 8.6%, respectively.

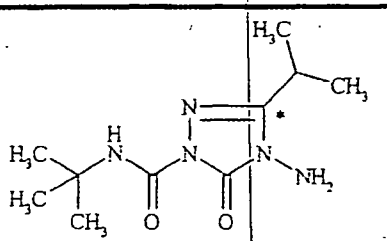
TABLE B.1.2. Crop Information.

Crop; crop group	Variety	Growth stage at application	Growth stage at harvest [a]	Harvested RAC	Harvesting procedure
Corn; Cereal Grain group and Forage, Fodder, and Straw of Cereal Grain group	N5901, hybrid seed corn	Preemergence; application was made four hours after planting seeds.	Immature; 104 DAP	Forage	Entire plants were cut 3-4 inches above soil surface
			Mature; 126 DAP	Grain and fodder	Plants were cut 3-4 inches above soil surface

[a] DAP = Days after planting.

B.2. Test Materials

TABLE B.2 Test Material Characteristics.

Chemical structure	
Radiolabel position	Triazolinone-3-^{14}C
Lot No.	Vials C-657B and C-693
Purity	>99% (HPLC)
Specific activity	208,500 dpm/µg

B.3. Study Use Pattern

TABLE B.3 Use Pattern Information.	
Chemical name	[triazolinone-3-^{14}C]amicarbazone
Application method	The test substance was formulated as a 70% water-dispersible granule (dry-flowable) formulation by mixing with formulation blank. The mixture was then concentrated and diluted with water for application. The soil in the buckets was sprayed with the test substance 4 hours after planting corn seed. The buckets to be used for controls were sprayed with formulation blank. A total of 14 buckets (2 controls and 12 treated) were used.
Application rate	0.72 lb ai/A
Number of applications	One
Timing of applications	Preemergence; four hours after planting seed
PHI	Forage: 104 days after treatment (plants from 3 buckets) Grain/Fodder: 126 days after treatment (plants from remaining 9 buckets)

The petitioner stated that plants were thinned to two plants per bucket 21 days after planting, and then thinned to one plant per bucket one week later.

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Forage samples were cut into pieces and then homogenized in the presence of liquid nitrogen. Mature plants were separated into stalks and ears. The husks were removed from the ears, and the grain was separated from the cobs; cobs and husks were added to the stalks to generate the fodder sample. Fodder and grain samples were homogenized in the presence of liquid nitrogen.

Forage: A subsample of forage was extracted with acetontrile:water:acetic acid (80:19.9:0.1, v:v:v) three times, and the extract was isolated by centrifugation and filtration, then evaporated to dryness and redissolved in acetonitrile (ACN) for HPLC analysis. The nonextractable residues were subjected to Soxhlet extraction using acetontrile:water:acetic acid (80:19.9:0.1, v:v:v) for 16 hours. The Soxhlet extract was concentrated and reserved for HPLC analysis. The remaining solids were separately subjected to: (i) cellulase hydrolysis (in pH 5 sodium acetate buffer at 37° C for 7 hours); (ii) acid hydrolysis (2 N HCl at room temperature for 5 hours); and (iii) acid hydrolysis at reflux (2 N HCl for 5 hours). The hydrolysates were isolated by filtration.

Fodder: A subsample of fodder was extracted with acetontrile:water:acetic acid (80:19.9:0.1, v:v:v) three times, and the extract was isolated by centrifugation and filtration. The remaining solids were extracted three times with methanol, and the methanol extract was combined with the ACN/water/acetic acid extract. The combined extracts were evaporated to dryness and redissolved in ACN for HPLC analysis. The nonextractable residues were subjected to Soxhlet extraction using acetontrile:water:acetic acid (80:19.9:0.1, v:v:v) for 16 hours. The Soxhlet extract was concentrated and reserved for HPLC analysis. The remaining solids were separately subjected to: (i) cellulase hydrolysis (in pH 5 sodium acetate buffer at 37 °C for 6 hours); (ii)

acid hydrolysis (2 N HCl at room temperature for 5 hours); and (iii) base hydrolysis (1 N KOH at reflux for 3 hours). The hydrolysates were isolated by filtration.

Grain: A subsample of grain was extracted with acetontrile:water:acetic acid (80:19.9:0.1, v:v:v) three times and methanol (methanol extraction was not described), and the extract was isolated by centrifugation and filtration. The extract was evaporated to near dryness and redissolved in ACN for HPLC analysis. The nonextractable residues were subjected to Soxhlet extraction using acetontrile:water:acetic acid (80:19.9:0.1, v:v:v) for 16 hours. The Soxhlet extract was concentrated for radioassay. According to the extraction flowchart presented in the submission, the remaining solids were subjected to hydrolysis using 2 N KOH; however, this procedure was not described.

B. 4.2. Analytical Methodology

Total radioactive residues (TRR) in forage, fodder, and grain samples were determined by combustion/LSC. Extracts and hydrolysates were radioassayed by LSC, and nonextractable residues were radioassayed by combustion/LSC. Detection limits were reported as 0.0018 ppm for liquid samples and 0.002 ppm for solid samples.

Extracts of corn matrices were analyzed by HPLC using a system equipped with a variable wavelength UV detector, a radioactivity detector, and a C-8 column, and using a gradient mobile phase of water and ACN, each containing 0.1% acetic acid. Extracts were first evaporated to dryness and redissolved in the mobile phase. Metabolites were identified by cochromatography with the following reference standards: [^{14}C]amicarbazone; [^{14}C]DA MKH 3586; [^{14}C]iPr-2-OH DA MKH 3586; [^{14}C]tBu-iPr-2-diOH DA MKH 3586; iPr-1-OH DA MKH 3586; 4-*N*-glucosyl-DA MKH 3586; and DCA DA MKH 3586.

Metabolites were isolated by cleaning up the extract on a C-18 column, applying the extract on the HPLC (as described above), and collecting individual peaks. The isolated metabolites were then purified by HPLC using a C-18 column and a gradient mobile phase of water and ACN or methanol, each containing 0.1% acetic acid.

Extracts and purified metabolites were also analyzed by TLC using silica-gel plates and a solvent system of hexane:isopropanol:acetic acid (70:29:1 or 90:9:1, v:v:v). Radioactivity was detected and quantified using a phosphorimager.

Metabolite identifications/confirmations were made using LC/MS, operating in the electrospray ionization mode. Analyses were conducted using a C-18 column and a mobile phase of water and methanol, each containing 0.1% acetic acid.

C. RESULTS AND DISCUSSION

Total radioactive residues (TRR) in corn matrices are reported in Table C.2.1. The total radioactive residues were 0.988, 2.369, and 0.054 ppm in corn forage, fodder, and grain,

respectively, harvested 104-126 days following a single preemergence application of [^{14}C]amicarbazone at 0.72 lb ai/A.

The majority of the TRR (~82-92% TRR) was extracted from corn matrices using ACN/water/acetic acid or ACN/water/acetic acid and methanol. Additional residues were released by Soxhlet extraction with ACN/water/acetic acid (3-7% TRR), and hydrolysis with 2 N HCl at reflux (5% TRR; forage) or with 1 N or 2 N KOH at reflux (3-8% TRR; fodder and grain). Total extractable residues were 91-100% TRR. Because extraction values were normalized by the petitioner, reported accountabilities were 100%; however, information presented in the raw data indicated that actual accountabilities were 99-104%. Residues were characterized/identified primarily by HPLC analysis with confirmatory analysis by TLC and LC/MS.

The distribution of the radioactivity in corn matrices is presented in Table C.2.2; characterization and identification of residues are summarized in Table C.2.3. For corn forage and fodder, in which nonextractable residues were subjected to three separate hydrolysis procedures, only the results from the most successful procedure are reported.

Total identified residues were 74-79% TRR in corn commodities. Parent, amicarbazone, was the major residue identified in forage (45%TRR) and fodder (20% TRR); amicarbazone was identified in smaller amounts in grain (13% TRR). The major residue identified in grain was iPr-2-OH DA MKH 3586 (38% TRR); this compound was also found in forage (15% TRR) and fodder (16% TRR). Other identified residues were DA MKH 3586 at 14% TRR in grain, 6% TRR in forage and 10% TRR in fodder; 4-*N*-glu-DA MKH 3586 at 4% in grain, 5% TRR in forage and 7% TRR in fodder; tBu-iPr-2-diOH DA MKH 3586 at 4% TRR in forage only; and iPr-2-O-glu-DA MKH 3586 at 4% TRR in forage, 5% TRR in fodder, and 7% TRR in grain. Four additional glucose/glucoside conjugates were identified in small amounts (≤5% TRR) in fodder.

TLC analyses were used to confirm the identification of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 in the Soxhlet extract of fodder and the identification of amicarbazone and DA MKH 3586 in grain.

LC/MS analyses were used to confirm the identification of amicarbazone, DA MKH 3586, iPr-2-OH DA MKH 3586, 4-*N*-glucosyl-DA MKH 3586, and tBu-iPr-2-diOH DA MKH 3586 in forage; amicarbazone, DA MKH 3586, iPr-2-OH DA MKH 3586, and 4-*N*-glucosyl-DA MKH 3586 in fodder; and iPr-2-OH DA MKH 3586 in grain.

The petitioner also subjected glucoside metabolites to hydrolysis (using 2 N HCl or cellulase, as described above). Acid hydrolyses were used to confirm the identification of 4-*N*-glucosyl-DA MKH 3586 in forage and fodder. In addition, cellulase hydrolyses and LC/MS analyses of the hydrolysis products were used to identify iPr-2-O-glu-DA MKH 3586 in forage and fodder, and O-glucosides of iPr-1-OH DA MKH 3586, tBu-iPr-2-diOH DA MKH 3586 and hydroxy DA MKH 3586 in fodder. An O-glucoside of iPr-2-OH DA MKH 3586 was also identified in fodder

with cellulase and LC/MS analysis; the petitioner postulates that this glucoside could be an anomeric form (one of two sterioisomers that differs in the configuration at C-1 of the sugar) of iPr-2-O-glu-DA MKH 3586.

The base hydrolysate of Soxhlet-extracted residues of fodder was subjected to HPLC analysis, which revealed one major peak. Based on retention time comparison, the peak was tentatively identified as DCA DA MKH 3586; no quantitative data were provided.

C.1. Storage Stability

Homogenized samples were stored at -24 ± 4 °C. The petitioner stated that samples were initially profiled by HPLC within 20 days of harvest and that the major components in corn matrices were identified within 3 months of sample collection. To demonstrate the stability of amicarbazone residues during frozen storage, samples of forage, fodder, and grain were reextracted and analyzed (HPLC) after 246, 224, and 345 days, respectively, of storage. Comparison of the extraction profile and HPLC chromatograms from the initial and final analyses indicates that the metabolite profiles were generally stable in corn forage, fodder, and grain for ~8, 7, and 11 months, respectively.

The petitioner did not provide dates of initial and final sample extraction and analysis. This information should be submitted before it can be determined whether the submitted storage stability data are adequate to support the corn metabolism study.

TABLE C.1 Summary of Storage Conditions.

Matrix	Storage Temp. (°C)	Actual Study Duration	Interval of Demonstrated Storage Stability
Corn forage	-24 ± 4	Not reported; dates of sample extraction and analysis were not included in the submission	246 days
Corn fodder			224 days
Corn grain			345 days

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRR) in Corn Matrices.

Matrix	PHI (days)	ppm, [^{14}C]amicarbazone equivalents
Forage	104	0.988
Fodder	126	2.369
Grain	126	0.054

TABLE C.2.2. Distribution of the Parent and the Metabolites in Corn Matrices Following Soil Application of Radiolabeled Amicarbazone at 0.72 lb ai/A.[a]

Metabolite Fraction	Forage		Fodder		Grain	
	TRR = 0.988 ppm		TRR = 2.369 ppm		TRR = 0.054 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN:water:acetic acid	92	0.909	82	1.943	84	0.045
Amicarbazone	44	0.436	18	0.426	13	0.007
DA MKH 3586	6	0.065	9	0.213	14[b]	0.008
iPr-2-OH DA MKH 3586	14	0.136	15	0.355	38	0.020
iPr-2-O-glu-DA MKH 3586	4	0.040	5	0.118	7[b]	0.004
O-glucoside of iPr-2-OH DA MKH 3586	–	–	2	0.047	–	–
iPr-1-O-glu-DA MKH 3586	–	–	3	0 071	–	–
4-N-glu-DA MKH 3586	5	0.054	7	0 166	4[b]	0.002
tBu-iPr-2-diOH DA MKH 3586	4	0.040	–	–	–	–
tBu-iPr-2-diOH-glu-DA MKH 3586	–	–	3	0.071	–	–
O-glucoside of hydroxy DA MKH 3586 [FOD-7]	–	–	5[b]	0.118	–	–
O-glucoside of hydroxy DA MKH 3586 [FOD-6]	–	–	3[b]	0.071	–	–
Unknowns	14[c]	0.140	12[d]	0.284	8[e]	0.004
Soxhlet extract	3	0.030	7	0.166	4	0 002
Amicarbazone	1[b]	0.010	2	0.047		
DA MKH 3586	–	–	1	0.024		
iPr-2-OH DA MKH 3586	1[b]	0.010	1	0.024		
Unknowns	<1[f]	<0.010	<2[g]	<0 047		
2 N HCl (reflux) hydrolysate	5	0.049				
1 N or 2 N KOH hydrolysate			8[h]	0.189	3	0.002

[a] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[b] Tentative identification.
[c] A total of seven unknowns, each ≤3% TRR (≤0.030 ppm).
[d] A total of nine unknowns, each ≤2% TRR (≤0.047 ppm).
[e] Two unknowns, each 4% TRR (0.002 ppm).
[f] A total of ten unknowns, each <1% TRR (<0.010 ppm).
[g] A total of thirteen unknowns, each <1% TRR (<0.024 ppm).
[h] HPLC analysis indicated one major peak, which had a retention time similar to that of DCA DA MKH 3586.

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Corn Matrices Following Soil Application of Radiolabeled Amicarbazone at 0.72 lb ai/A.

Compound	Forage		Fodder		Grain	
	TRR = 0.988 ppm		TRR = 2.369 ppm		TRR = 0.054 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
Amicarbazone	45	0.446	20	0.473	13	0.007
DA MKH 3586	6	0.065	10	0.237	14	0.008
iPr-2-OH DA MKH 3586	15	0.146	16	0.379	38	0.020
iPr-2-O-glu-DA MKH 3586	4	0.040	5	0.118	7	0.004
O-glucoside of iPr-2-OH DA MKH 3586	–	–	2	0.047	–	–
iPr-1-O-glu-DA MKH 3586	–	–	3	0.071	–	–
4-N-glu-DA MKH 3586	5	0.054	7	0.166	4	0.002
tBu-iPr-2-diOH DA MKH 3586	4	0.040	–	–	–	–
tBu-iPr-2-diOH-glu-DA MKH 3586	–	–	3	0.071	–	–
O-glucoside of hydroxy DA MKH 3586 [FOD-7]	–	–	5	0.118	–	–
O-glucoside of hydroxy DA MKH 3586 [FOD-6]	–	–	3	0.071	–	–
Unknowns	14	0.140	12	0.284	8	0.004
Soxhlet extract	–	–	–	–	4	0.002
HCl hydrolysate	5	0.049	–	–	–	–
KOH hydrolysate	–	–	8	0.189	3	0.002
Total identified	79	0.791	74	1.751	76	0.041
Total characterized	19	0.189	22	0.52	15	0.008
Total extractable	100	0.988	97	2.298	91	0.049
Unextractable (PES) [1]	<1	0.001	2	0.047	9	0.005
Accountability [2]	100		99		100	

[1] Residues remaining after exhaustive extractions.
[2] Accountability = (Total extractable + Total unextractable)/(TRR from combustion analysis, see TABLE C.2.1) * 100.

C.3. Proposed Metabolic Profile

FIGURE C.3.1 Proposed Metabolic Profile of Amicarbazone in Corn.

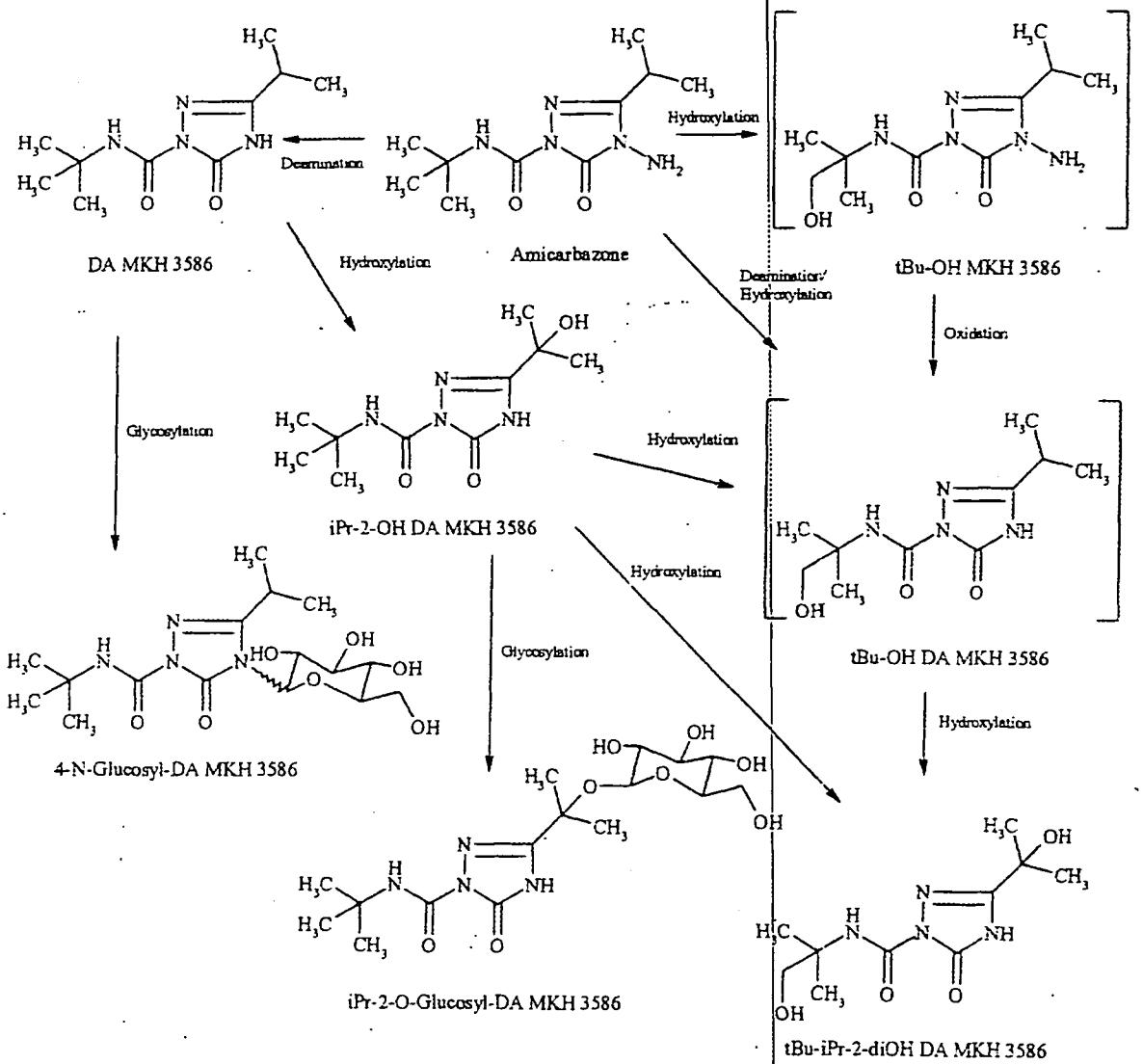


TABLE C.3 Identification of Compounds from Metabolism Study.

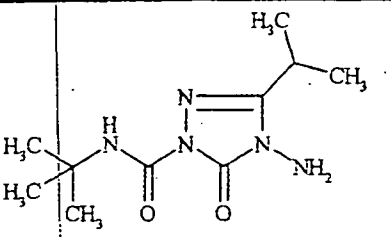
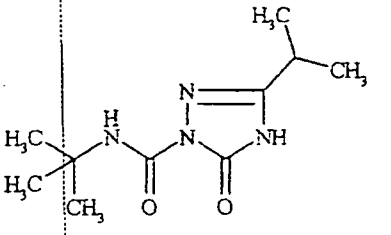
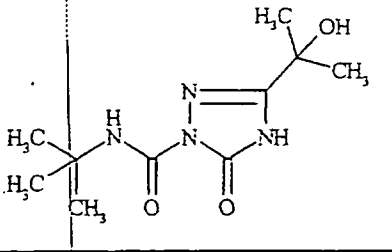
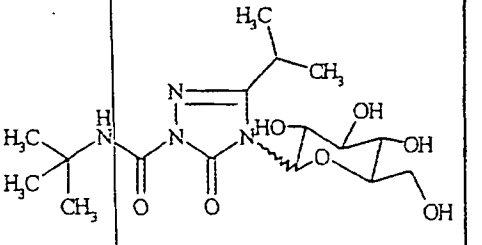
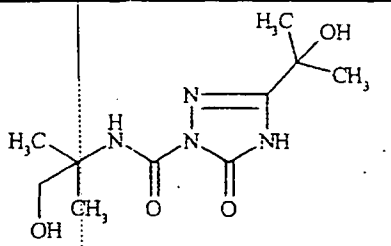
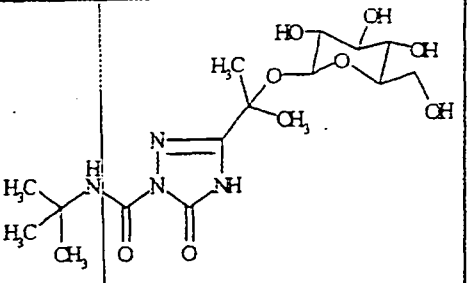
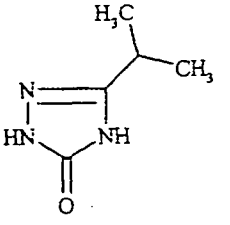
Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
Amicarbazone; MKH 3586	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
DA MKH 3586	*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
iPr-2-OH DA MKH 3586	*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
4-*N*-Glucosyl-DA MKH 3586	*N*-(1,1-dimethylethyl)-4,5-dihydro-4-N-glucosyl-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
tBu-iPr-2-diOH DA MKH 3586	4,5-dihydro-*N*-(2-hydroxy-1,1-dimethylethyl)-3-(1-hydroxy-1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	

TABLE C.3 Identification of Compounds from Metabolism Study.

Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
iPr-2-O-Glucosyl-DA MKH 3586	3-[1-Methyl-1-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-ethyl]-5-oxo-4,5-dihydro-[1,2,4]triazole-1-carboxylic acid tert-butylamide [a]	
DCA DA MKH 3586; triazolinone DA MKH 3586 [b]	1,2-dihydro-5-(1-methylethyl)-3*H*-1,2,4-triazol-3-one	

[a] Chemical name generated using naming software of ISIS/Draw.
[b] Tentatively identified in the base hydrolysate of corn fodder.

D. CONCLUSION

The total radioactive residues were 0.988, 2.369, and 0.054 ppm in corn forage, fodder, and grain, respectively, harvested 104-126 days following a single preemergence application of [^{14}C]amicarbazone at 0.72 lb ai/A. The majority of the TRR (~82-92% TRR) was extracted from corn matrices using ACN/water/acetic acid or ACN/water/acetic acid and methanol. Additional residues were released by Soxhlet extraction with ACN/water/acetic acid (3-7% TRR), and hydrolysis with 2 N HCl at reflux (5% TRR; forage) or with 1 N or 2 N KOH at reflux (3-8% TRR; fodder and grain). Total extractable residues were 91-100% TRR.

Total identified residues were 74-79% TRR in corn commodities. Parent, amicarbazone, was the major residue identified in forage (45%TRR) and fodder (20% TRR); amicarbazone was identified in smaller amounts in grain (13% TRR). The major residue identified in grain was iPr-2-OH DA MKH 3586 (38% TRR); this compound was also found in forage (15% TRR) and fodder (16% TRR). Other identified residues were DA MKH 3586 at 14% TRR in grain, 6% TRR in forage and 10% TRR in fodder; 4-*N*-glu-DA MKH 3586 at 4% in grain, 5% TRR in forage and 7% TRR in fodder; tBu-iPr-2-diOH DA MKH 3586 at 4% TRR in forage only; and iPr-2-O-glu-DA MKH 3586 at 4% TRR in forage, 5% TRR in fodder, and 7% TRR in grain. Four additional glucose/glucoside conjugates were identified in small amounts (≤5% TRR) in fodder.

Based on the results of the study, the petitioner proposed that the major metabolic pathway in corn involves the deamination of the triazole amino group followed by hydroxylation at the tertiary carbon of the isopropyl group to form iPr-2-OH DA MKH 3586. Additional pathways involve hydroxylation of the isopropyl methyl to form iPr-1-OH DA MKH 3586, followed by glucosidation; hydroxylation of the t-butyl and isopropyl groups to form tBu-iPr-2-diOH DA MKH 3586. In addition, DA MKH 3586 formed an *N*-glucoside; and glucosidation of hydroxylated DA MKH 3586 formed several minor *O*-glucosides.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: PP#0F6131
DP Barcode: D288216
PC Code: 114004

Template Version September 2003

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.2.1, 7.2.2, and 7.2.3/OPPTS 860.1340/OECD IIA 4.2.5, 4.2.6 and 4.3
Residue Analytical Method - Plant Commodities

Primary Evaluator	Manying Xue, Chemist RAB3/HED (7509C)	Date: 03/23/05
Approved by	Leung Cheng, Senior Chemist RAB3/HED (7509C)	Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 09/21/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

45121702 Matthew, A. (2000) Extraction Efficiency of the Analytical Method for the Determination of MKH 3586 Residues in Plant Matrices: Lab Project Number: M6221601: 108737. Unpublished study prepared by Bayer Corp. 46 p.

45121708 Mathew, A., Gould, T. & Bucklin, D. et al. (2000) Analytical Residue Method for the Determination of MKH 3586 Residues in Plant Matrices: Lab Project Number: M6111601: 108340. Unpublished study prepared by Bayer Corp. 185 p.

45121709 Perez, R. (1999) Independent Laboratory Validation of Analytical Method 108340 for Analytical Residue Method for the Determination of MKH 3586 Residues in Plant Matrices: Lab Project Number: M6111602: 109594: 982-1101-99-002. Unpublished study prepared by ADPEN Labs., Inc. 74 p.

EXECUTIVE SUMMARY:

Arvesta Corporation has proposed an LC/MS/MS method for the enforcement of tolerances for residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 in plant commodities. The method, entitled "Analytical Residue Method for the Determination of MKH 3586 Residues in Plant Matrices" (Bayer Report Number 108340), was used for the determination of residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in the field corn crop field trial and processing studies associated with DP Barcode D288216..

The method includes instructions for analysis of samples of corn forage, corn fodder, corn grain, and corn grain processed products. Briefly, samples are extracted using accelerated solvent extraction (ASE) with water or 0.05% phosphoric acid as the extraction solvent. The extracts are cleaned up by solid phase extraction (SPE) and dissolved in 2 mM ammonium acetate for LC/MS/MS analysis. The validated limit of quantitation (LOQ) is 0.01 ppm for each analyte in all matrices. The reported limits of detection (LODs) were 0.001 ppm for each analyte in corn

forage, grain and processed fractions, and 0.006 ppm for each analyte in corn fodder. The results for all analytes are reported as parent equivalents. The method includes instructions for confirmatory analysis, using LC/MS/MS with the same chromatographic system as the main method but different MS conditions.

Method validation data for the LC/MS/MS method demonstrated adequate method recoveries of residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 at the LOQ and 5x the LOQ from field corn forage, fodder, and grain, at 60x the LOQ from field corn forage, at 50x the LOQ from field corn stover, and at the LOQ from field corn processed commodities starch, grits, meal, flour, and refined oil. Overall recovery ranges (and CVs) from these matrices were 70-114% (12%) for amicarbazone, 71-119% (12%) for DA MKH 3586, and 68-119% (10%) for iPr-2-OH DA MKH 3586. The fortification levels and samples used in method validation are adequate to bracket expected residue levels.

Adequate independent laboratory validation data have been submitted for this method using corn grain samples. The submitted radiovalidation data are adequate to demonstrate that the method adequately extracts incurred residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 from corn grain.

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 proposed enforcement method has been forwarded to ACB for petition method validation.

We note that the petitioner included names, structures, and molecular weights for the deuterated compounds to be used as internal standards. The chemical names and molecular weights for the amicarbazone-d_9 and DA MKH 3586-d_9 compounds do not match the chemical structures and the chemical structures do not correspond to the non-deuterated compounds. The petitioner should correct the chemical structures in the method for amicarbazone-d_9 and DA MKH 3586-d_9.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D288216.

COMPLIANCE:

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

A. BACKGROUND INFORMATION

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis.

TABLE A.1. Amicarbazone Nomenclature.

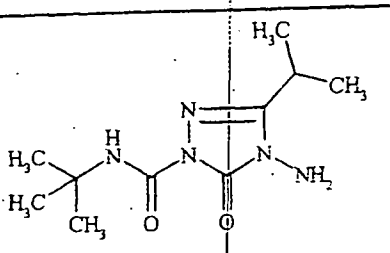
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-tert-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS registry number	129909-90-6
End-use formulation (EP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.

Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility (g/L)	4.6	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-6} Pa @ 25°C 1.30×10^{-6} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, Log(K_{ow})	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

An LC/MS/MS method, entitled "Analytical Residue Method for the Determination of MKH 3586 Residues in Plant Matrices" (Bayer Report Number 108340), was used for the determination of residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA

MKH 3586 in the field corn crop field trial and processing studies associated with DP Barcode D288216.

B.1.1. Principle of the Method:

The method includes instructions for analysis of samples of corn forage, corn fodder, corn grain, and corn grain processed products. Briefly, samples are extracted using ASE (accelerated solvent extraction) with water or 0.05% phosphoric acid as the extraction solvent. Following addition of deuterated internal standards, the extracts are cleaned up by SPE (solid phase extraction) and dissolved in 2 mM ammonium acetate for LC/MS/MS analysis.

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

Method ID	Bayer Report Number 108340
Analytes	Amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586
Extraction solvent/technique	If not already homogenized, samples are homogenized in the presence of dry ice. Corn oil samples are not subjected to extraction procedures. Corn forage and fodder: The homogenized sample is mixed with aluminum oxide (alumina) and extracted using ASE, using water as the extraction solvent and conducting extraction at 150 °C and 1500 psi. A solution of mixed internal standards [amicarbazone-d_9, DA MKH 3586-d_4, and iPr-2-OH DA MKH 3586-d_5] is added to the extract and the mixture is centrifuged. Corn grain and processed commodities other than oil: The homogenized sample is mixed with Celite and extracted using ASE, using 0.05% phosphoric acid as the extraction solvent and conducting extraction at 150 °C and 1500 psi. A solution of mixed internal standards [amicarbazone-d_9, DA MKH 3586-d_4, and iPr-2-OH DA MKH 3586-d_5] is added to the extract and the mixture is centrifuged.
Cleanup strategies	Corn forage, fodder, grain, and processed commodities other than oil: Extracts are cleaned up by C-18 SPE, using methanol to elute residues. The eluate is concentrated and mixed with 2 mM ammonium acetate for LC/MS/MS analysis. Corn oil: The sample is mixed with 0.05% phosphoric acid and internal standards [amicarbazone-d_9, DA MKH 3586-d_4, and iPr-2-OH DA MKH 3586-d_5] and applied to a C-18 SPE cartridge; residues are eluted with methanol. The eluate is concentrated and mixed with 2 mM ammonium acetate for LC/MS/MS analysis.
Instrument/Detector	HPLC utilizing a reverse-phase C-18 column and a gradient mobile phase of 2mM ammonium acetate and methanol, with tandem mass spectrometry (MS/MS) detection using electrospray ionization operating in the positive ion mode. The daughter ion transitions monitored are: amicarbazone: m/z 242 to m/z 143 amicarbazone-d_9: m/z 251 to m/z 143 DA MKH 3586: m/z 227 to m/z 128 DA MKH 3586-d_4: m/z 236 to m/z 128 iPr-2-OH DA MKH 3586: m/z 243 to m/z 144 iPr-2-OH DA MKH 3586-d_5: m/z 249 to m/z 150 For each analyte, the daughter ion is used for quantitation.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Amicarbazone Residues in Plant Matrices.	
Standardization method	External and internal standards are used. Internal standard solutions are added to samples and standards such that internal standard concentration is the same in each. Standards are injected before and after each sample (maximum of 10 sample injections between standards), and concentrations of analytes in samples are calculated by comparing the relative response of analyte and corresponding internal standard with the relative response in the standard solution. The concentrations of standards of DA MKH 3586 and iPr-2-OH DA MKH 3586 are calculated to be equivalent to amicarbazone on a molar basis. Therefore, all residue results for those compounds are reported in parent equivalents. Linearity of the detector response is to be determined by injecting a blank and 6 standards; the relative response of analyte and internal standard is calculated for each analyte and standard and plotted against concentration. The method specifies that the correlation coefficient should be ≥0.99.
Stability of std solutions	Standard solutions are to be stored refrigerated (4 ± 3 °C); standards are reportedly stable under these conditions for at least 3 months. The petitioner noted that secondary standards and linearity standards contain acetonitrile at approximately 10% as a bacteriocide.
Retention times	Approximately 6 minutes for amicarbazone, 7 minutes for DA MKH 3586, and 5 minutes for iPr-2-OH DA MKH 3586.

The method includes instructions for confirmatory analysis, using LC/MS/MS with the same chromatographic system as the main method but different MS conditions. For amicarbazone, positive ion MS/MS of the ammonium adduct is conducted, and for DA MKH 3586 and iPr-2OH DA MKH 3586, negative ion MS/MS is conducted. The daughter ion transitions to be monitored are:

amicarbazone:	m/z 259 to m/z 143
amicarbazone-d_9:	m/z 268 to m/z 143.
DA MKH 3586:	m/z 225 to m/z 126
DA MKH 3586-d_9:	m/z 234 to m/z 126
iPr-2-OH DA MKH 3586:	m/z 241 to m/z 142
iPr-2-OH DA MKH 3586-d_6:	m/z 247 to m/z 148

We note that the petitioner included names, structures, and molecular weights for the deuterated compounds to be used as internal standards. The chemical names and molecular weights for the amicarbazone-d_9 and DA MKH 3586-d_9 compounds do not match the chemical structures and the chemical structures do not correspond to the non-deuterated compounds (the structures represent deuterated versions of compounds created by adding one methyl group to the 1-methylethyl substituent on the triazole ring for amicarbazone and DA MKH 3586). The petitioner should correct the chemical structures in the method for amicarbazone-d_9 and DA MKH 3586-d_9.

B.2. Enforcement Method

The proposed enforcement method for plant commodities is the same as the data-gathering method.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

TABLE C.1.1. Recovery Results from Method Validation of Corn Matrices using the Data-Gathering Analytical Method.[1]

Matrix	Analyte	Spiking Level (ppm)	Recoveries Obtained (%)	Mean Recovery ± SD [CV] (%)
Method Validation [2]				
Field corn forage	Amicarbazone	0.010	73, 84, 88	82 ± 5 [6]
		0.050	80, 84, 85	
	DA MKH 3586	0.010	80, 91, 92	90 ± 5 [6]
		0.050	91, 94, 94	
	iPr-2-OH DA MKH 3586	0.010	91, 91, 119	96 ± 12 [12]
		0.050	85, 94, 95	
Field corn stover	Amicarbazone	0.010	71, 78, 82	80 ± 5 [7]
		0.050	82, 83, 86	
	DA MKH 3586	0.010	90, 91, 93	93 ± 2 [2]
		0.050	92, 94, 95	
	iPr-2-OH DA MKH 3586	0.010	78, 89, 90	88 ± 6 [7]
		0.050	85, 90, 96	
Field corn grain	Amicarbazone	0.010	70, 87, 92	84 ± 10 [12]
		0.050	74, 90, 93	
	DA MKH 3586	0.010	74, 86, 96	87 ± 9 [10]
		0.050	79, 93, 94	
	iPr-2-OH DA MKH 3586	0.010	80, 90, 95	89 ± 8 [9]
		0.050	78, 95, 97	
Concurrent Method Recovery [3]				
Field corn forage	Amicarbazone	0.010	74, 82, 82, 85, 86, 87, 88, 90, 91, 94, 98	84 ± 8 [10]
		0.600	71, 73, 75	
	DA MKH 3586	0.010	85, 88, 90, 90, 92, 95, 99, 100, 102, 107, 111	93 ± 10 [10]
		0.600	80, 80, 84	
	iPr-2-OH DA MKH 3586	0.010	79, 83, 83, 85, 87, 89, 89, 96, 94, 98, 100	88 ± 7 [8]
		0.600	79, 82, 83	

TABLE C.1.1. Recovery Results from Method Validation of Corn Matrices using the Data-Gathering Analytical Method.[1]

Matrix	Analyte	Spiking Level (ppm)	Recoveries Obtained (%)	Mean Recovery ± SD [CV] (%)
Field corn stover	Amicarbazone	0.010	70, 73, 75, 78, 79, 82, 88, 96, 106	80 ± 12 [14]
		0.500	70, 70, 71	
	DA MKH 3586	0.010	77, 87, 90, 91, 92, 93, 94, 98, 115	90 ± 10 [11]
		0.500	79, 82, 84	
	iPr-2-OH DA MKH 3586	0.010	68, 76, 80, 84, 85, 85, 85, 95, 102	84 ± 9 [10]
		0.500	78, 81, 86	
Field corn grain	Amicarbazone	0.010	70, 71, 72, 76, 76, 76, 85, 85, 89, 90, 91, 91, 95	82 ± 9 [10]
		0.050	70, 83, 85	
	DA MKH 3586	0.010	71, 76, 76, 77, 79, 79, 82, 84, 86, 88, 93, 98, 100	84 ± 9 [10]
		0.050	72, 87, 89	
	iPr-2-OH DA MKH 3586	0.010	69, 70, 78, 80, 87, 87, 88, 93, 97, 105, 105, 110, 105	90 ± 13 [14]
		0.050	77, 91, 91	
Corn, starch	Amicarbazone	0.01	89, 100, 102, 106	99 ± 7 [7]
	DA MKH 3586	0.01	78, 82, 92, 95	87 ± 8 [9]
	iPr-2-OH DA MKH 3586	0.01	85, 90, 96, 98	92 ± 6 [7]
Corn, grits	Amicarbazone	0.01	75, 83, 83	80 ± 5 [6]
	DA MKH 3586	0.01	77, 79, 81	79 ± 2 [3]
	iPr-2-OH DA MKH 3586	0.01	78, 86, 94	86 ± 8 [9]
Corn, meal	Amicarbazone	0.01	70, 81, 82, 84	79 ± 6 [8]
	DA MKH 3586	0.01	73, 76, 87, 94	83 ± 10 [12]
	iPr-2-OH DA MKH 3586	0.01	81, 83, 90, 96	88 ± 7 [8]
Corn, flour	Amicarbazone	0.01	79, 80, 108	89 ± 16 [18]
	DA MKH 3586	0.01	74, 75, 97	82 ± 13 [16]
	iPr-2-OH DA MKH 3586	0.01	85, 85, 87	86 ± 1 [1]
Refined corn oil	Amicarbazone	0.01	76, 81, 92, 103, 108, 114	96 ± 15 [16]
	DA MKH 3586	0.01	91, 100, 104, 107, 118, 119	107 ± 11 [10]
	iPr-2-OH DA MKH 3586	0.01	89, 93, 93, 97, 100, 107	97 ± 6 [6]

[1] Standards were prepared in acetonitrile:water (approximately 1:10, v:v).
[2] The method validation and concurrent method recovery data for field corn forage, stover, and grain are identical to those submitted with the field corn field trial study (refer to the DER for MRID 45121710).

The fortification levels and samples used in method validation are adequate to bracket expected residue levels.

The method contains procedures for confirming the identification of residues of amicarbazone and its metabolites in/on plant commodities. In addition, the petitioner addressed the issue of interference from other pesticides. Of the 111 compounds identified by the petitioner as registered for use on corn, only six compounds (ametryn, bentazon, cyanazine, ethoprop, mevinphos, and prometryn) were found to have a molecular weight within 2 amu of the parent ions for amicarbazone and its metabolites. Based on a examination of potential fragmentation, the petitioner concluded that none of the six compounds would form a daughter ion that would interfere with the determination of residues of amicarbazone, DA MKH 3586, or iPr-2-OH DA MKH 3586.

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Amicarbazone Residues in Corn Matrices.	
Analytes	Amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586
Equipment ID	TSQ 7000 LC/MS; Phenomenex Prodigy C18 5 µm column
Limit of quantitation (LOQ)	0.01 ppm; represents the lowest fortification level at which acceptable recoveries were obtained.
Limit of detection (LOD)	0.001 ppm for corn forage and grain; 0.006 ppm for corn fodder LOD was determined by first calculating the mean and standard deviation of measured residues in unfortified samples, adding 3 std. dev. to the average residue, and then rounding up to one significant figure.
Accuracy/Precision	Percent recoveries and coefficients of variance (CVs) indicate acceptable accuracy/precision at the LOQ and 5xLOQ for residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 from field corn forage, fodder, and grain, at 60xLOQ from field corn forage, at 50xLOQ from field corn stover, and at the LOQ from field corn processed commodities starch, grits, meal, flour, and refined oil. Overall recovery ranges (and CVs) from these matrices were 70-114% (12%) for amicarbazone, 71-119% (12%) for DA MKH 3586, and 68-119% (10%) for iPr-2-OH DA MKH 3586.
Reliability of the Method/ [ILV]	An independent laboratory method validation [ILV] of the proposed enforcement method was conducted to verify the reliability of the method for the determination of residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 in corn grain. The values obtained are indicative that the method is reliable. See Section C.3 below.
Linearity	The method/detector response was linear (coefficient of determination, r^2= 0.9944-0.9999) within the range of: 0.0-0.10, 0.0-0.5, and 0.0-1.0 ppm for each analyte in solvent blank; 0.0-0.5 ppm for each analyte in corn forage extract; 0.0-1.0 ppm for each analyte in corn fodder extract; and 0.0-0.10 ppm for each analyte in the extracts of corn grain, flour, grits, meal, oil, and starch.
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.

The extraction solvents used in the proposed enforcement method are not similar to those used in the field corn metabolism study (refer to the DER for MRIDs 45121633). In the metabolism study, the majority of the TRR (~82-92% TRR) was extracted from corn matrices using

acetonitrile/water/acetic acid (approximately 80:20:0.1) or acetonitrile/water/acetic acid and methanol. This differs from the ASE extraction procedures of the enforcement method, in which water or 0.05% phosphoric acid is used as the extraction solvent.

Arvesta has submitted radiovalidation data for the proposed enforcement method (MRID 45121702) using samples of corn fodder and grain from the field corn metabolism study. Samples of corn fodder and grain, which had been stored frozen at -20 ± 4 °C since collection, were reextracted and analyzed according to the procedures of the metabolism study, as follows. Corn fodder subsamples were extracted with acetonitrile:water:acetic acid (80:19.9:0.1, v:v:v) three times, and the extracts were isolated by centrifugation and filtration. The remaining solids were extracted three times with methanol, and the methanol extract was combined with the ACN/water/acetic acid extract. The combined extracts were concentrated and redissolved in HPLC mobile phase for analysis. Corn grain subsamples were extracted with acetonitrile:water:acetic acid (80:19.9:0.1, v:v:v) three times, and the extracts were isolated by centrifugation and filtration. The combined extracts were concentrated and redissolved in HPLC mobile phase for analysis.

Subsamples of corn fodder and grain were also extracted according to the procedures of the proposed enforcement method, as described above in Table B.1.1.

Extracts were analyzed by HPLC using a system equipped with a variable wavelength UV detector, a radioactivity detector, and a C-18 column, and using a gradient mobile phase of water and ACN, each containing 0.1% acetic acid. Metabolites were identified by comparison of retention times with those of reference standards of [^{14}C]amicarbazone, [^{14}C]DA MKH 3586, and [^{14}C]iPr-2-OH DA MKH 3586 in extracts of untreated corn grain and fodder. The results of the study are reported below in Table C.1.3.

TABLE C.1.3. Extraction Efficiency of the Enforcement Analytical Method Using Radiolabeled Samples from the Field Corn Metabolism Study.

Matrix	Original Metabolism Study Results (ppm)	Radiovalidation Study Results			
		Extraction using procedures of metabolism study [1] (ppm)	Extraction using procedures of enforcement method [2] (ppm)	Extraction efficiency (%), using current metabolism study results [3]	Extraction efficiency (%), using results from original metabolism study [4]
Corn fodder					
TRR	2.369	2.34	–	–	–
Extracted residues	1.943	1.673	1.909	114.1	98.3
Amicarbazone	0.473	0.461	0.576	124.9	121.8
DA MKH 3586	0.237	0.244	0.149	61.1	62.9
iPr-2-OH DA MKH 3586	0.379	0.425	0.175	41.2	46.2
Total toxic residue	1.089	1.130	0.900	79.6	82.6

TABLE C.1.3. Extraction Efficiency of the Enforcement Analytical Method Using Radiolabeled Samples from the Field Corn Metabolism Study.

Matrix	Original Metabolism Study Results (ppm)	Radiovalidation Study Results			
		Extraction using procedures of metabolism study [1] (ppm)	Extraction using procedures of enforcement method [2] (ppm)	Extraction efficiency (%), using current metabolism study results [3]	Extraction efficiency (%), using results from original metabolism study [4]
Corn grain					
TRR	0.054	0.047	–	–	–
Extracted residues	0.045	0.034	0.028	82.4	62.2
Amicarbazone	0.007	0.004	0.005	125.0	71.4
DA MKH 3586	0.008	0.008	0.005	62.5	62.5
iPr-2-OH DA MKH 3586	0.020	0.021	0.012	57.1	60.0
Total toxic residue	0.035	0.033	0.022	66.7	62.9

[1] Average of two replicate samples
[2] Average of three replicate samples
[3] Extraction efficiency = (ppm, extraction using procedures of enforcement method) ÷ (ppm, extraction using procedures of metabolism study) x 100.
[4] Extraction efficiency = (ppm, extraction using procedures of enforcement method) ÷ (ppm, original metabolism study results) x 100.

We note that for both matrices, the extraction efficiency was >100% for amicarbazone and <65% for the metabolites. The petitioner did not address this issue in their submission. Because the data indicate that the extraction procedures of the proposed enforcement method adequately extract aged residues from field corn fodder and grain (based on a comparison of the residues extracted using procedures of the proposed enforcement method with the residues extracted using the procedures of the metabolism study), HED concludes that the method has been adequately radiovalidated.

C.2. Enforcement Method

The proposed enforcement method for plant commodities is the same as the data-gathering method.

C.3. Independent Laboratory Validation

An independent laboratory validation (ILV) of the proposed enforcement method was conducted by ADPEN Laboratories (Jacksonville, FL) using samples of corn grain (MRID 45121709).

Samples of homogenized untreated corn grain (obtained by ADPEN from the petitioner) were fortified with amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 each at 0.01 ppm (LOQ) and 0.02 ppm. Fortified and unfortified (control) samples were analyzed using the proposed enforcement method as described in Table B.1.1 (draft version, dated 11/18/1999).

Corn grain samples were chosen for the ILV study because grain was considered to be a difficult matrix to analyze.

The first ILV trial was successful. Recoveries of amicarbazone, DA MKH 3586, and iPr-2OH DA MKH 3586 from corn grain samples are reported in Table C.3.1. Detectable residues of amicarbazone were observed in/on two samples of unfortified corn grain at 0.0016 and 0.0030 ppm; residues of DA MKH 3586 and iPr-2-OH DA MKH 3586 were nondetectable (<0.001 ppm) in/on these samples.

The laboratory reported that a set of 6-8 samples required 7 person-hours for extraction; LC/MS/MS analyses could be conducted unattended overnight and an additional 1.5 hours are needed for data analysis. No critical steps were identified by the ILV laboratory. The laboratory recommended that the method be revised to include additional information regarding calibration and optimization of the LC/MS/MS instrument, and that an introduction, recovery data, and additional representative chromatograms should be added. We note that the ILV laboratory used a draft version of the method and that the final version has been modified as suggested.

TABLE C.3.1. **Recovery Results Obtained by an Independent Laboratory Validation of the Enforcement Method for the Determination of Amicarbazone Residues in Corn Matrices.**

Matrix	Analyte	Spiking Level (ppm)	Recoveries Obtained [1] (%)	Mean Recovery ± SD [CV] (%)
Corn grain	Amicarbazone	0.01	75.0, 97.0	81.6 ± 10.8 [13.2]
		0.02	73.3, 81.5	
	DA MKH 3586	0.01	67.0, 73.5	74.0 ± 5.5 [7.5]
		0.02	74.8, 80.5	
	iPr-2-OH DA MKH 3586	0.01	87.0, 116	95.2 ± 14.2 [14.9]
		0.02	85.3, 92.5	

[1] Recoveries were corrected for apparent residues in control samples.

D. CONCLUSION

Adequate method validation data have been submitted for the proposed LC/MS/MS enforcement method for the determination of residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in plant (field corn) commodities.

Adequate independent laboratory validation data have been submitted for this method using corn grain samples. The submitted radiovalidation data are adequate to demonstrate that the method adequately extracts incurred residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 from corn grain.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: PP#0F6131
DP Barcode: D288216
PC Code: 114004

Template Version September 2003

17

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.2.4/OPPTS 860.1360/OECD IIIA 5.3.1
Multiresidue Analytical Methods

Primary Evaluator	Manying Xue, Chemist RAB3/HED (7509C)	Date: 03/23/05
Approved by	Leung Cheng, Senior Chemist RAB3/HED (7509C)	Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 09/21/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

45121703 Perez, R.; Thiam, M. (1999) Evaluation of MKH 3586 through the FDA Multiresidue Method: Lab Project Number: M6161601: 109352. Unpublished study prepared by ADPEN Labs., Inc. 123 p.

EXECUTIVE SUMMARY:

Arvesta Corporation has submitted multiresidue method data for amicarbazone. Amicarbazone and its DA MKH 3586 and iPr-2-OH DA MKH 3586 metabolites were analyzed according to the FDA Multi-Residue Method Test guidelines in PAM, Vol. I (dated 1/94). Amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 were tested through Protocol C; and as a result of Protocol C, all compounds were tested through Protocols D and F. Based on the results of the Protocol D testing, testing under Protocol E was not required for any of the test substances. Because the test substances are not N-methyl carbamates, naturally fluorescent, acids, phenols, or substituted ureas, no testing under Protocols A, B, or G is required.

Amicarbazone was not recovered using Protocol D, and interference caused high recoveries of DA MKH 3586 and iPr-2-OH MKH 3586 under Protocol D. DA MKH 3586 and iPr-2-OH MKH 3586 could not be recovered using the Florisil column cleanup procedures of Protocol D. No recovery of the test substances was obtained using the Florisil column cleanup procedures of Protocol F.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the multiresidue method residue data are classified as scientifically acceptable. These data will be forwarded to the U.S. FDA for further evaluation.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D288216.

COMPLIANCE:

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

A. BACKGROUND INFORMATION

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis.

TABLE A.1. Amicarbazone Nomenclature.

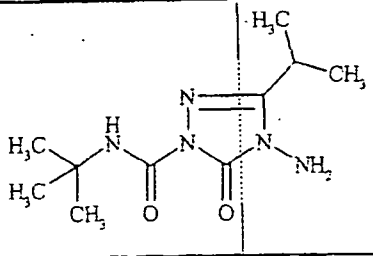
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-tert-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS registry number	129909-90-6
End-use formulation (EP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.

Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-4} Pa @ 25°C 1.30×10^{-4} Pa @ 20°C	MRID 45121501

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.		
Parameter	Value	Reference*
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, Log(K_{ow})	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. MATERIALS AND METHODS

Amicarbazone, des amino MKH 3586 (DA MKH 3586), and isopropyl 2-hydroxy (3') MKH 3594 DA (iPr-2-OH DA MKH 3586) were tested through Protocol C, and as a result of Protocol C testing, all compounds were tested through Protocols D and F. Based on the results of the Protocol D testing, testing under Protocol E was not required for any of the test substances. Because the test substances are not N-methyl carbamates, naturally fluorescent, acids, phenols, or substituted ureas, no testing under Protocols A, B, or G is required.

C. RESULTS AND DISCUSSION

TABLE C.1. Results of Multiresidue Methods Testing with Amicarbazone and its Metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586.		
PAM I Protocol	Results	Comments
A	Not tested because the test substances are not N-methyl carbamates or naturally fluorescent.	
B	Not tested because the test substances are not acids or phenols.	
C	Amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 demonstrated acceptable chromatography using a DB-17 column with a nitrogen/phosphorus detector (NPD).	Further work was conducted using Protocols D, E, and F
D	Amicarbazone and its metabolites were tested using corn kernels under Section 302 with module E4 cleanup; samples were fortified at 0.02 ppm and 0.075 ppm. There was no recovery of amicarbazone using this procedure; interference was observed in the control sample which would interfere with amicarbazone determination. For DA MKH 3586 and iPr-2-OH DA MKH 3586, interferences were observed in both the reagent blanks and the control samples which yielded high recoveries in the fortified samples (up to 315%). DA MKH 3586 and iPr-2-OH DA MKH 3586 were tested for their recovery using Florisil column cleanup; no recovery of these analytes was observed using either of the eluant systems (C1 or C5).	No further testing of analytes under Protocol D was conducted.

TABLE C.1. Results of Multiresidue Methods Testing with Amicarbazone and its Metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586.

PAM I Protocol	Results	Comments
E	Because amicarbazone was not recovered using Protocol D and DA MKH 3586 and iPr-2-OH MKH 3586 were not recovered during Florisil column cleanup, no testing under Protocol E was conducted.	
F	The test substances were first evaluated for recovery via Florisil cleanup procedures 304 C1 and C2. No recovery of amicarbazone, DA MKH 3586, or iPr-2-OH MKH 3586 was observed using either of the elution systems.	Because the test substances could not be recovered during Florisil cleanup, no further testing was conducted.
G	Not tested because the test substances are not substituted ureas.	

D. CONCLUSION

Amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 were adequately evaluated for their recovery through FDA multiresidue methods. Because the test substances are not N-methyl carbamates, naturally fluorescent, acids, phenols, or substituted ureas, testing under Protocols A, B, or G was not conducted. Protocal C is applicable to determine the GLC characteristics. Amicarbazone and its metabolite DA MKH 3586 chromatographed using Protocal C. Amicarbazone was not recovered using Protocol D, and interference caused high recoveries of DA MKH 3586 and iPr-2-OH MKH 3586 under Protocol D. DA MKH 3586 and iPr-2-OH MKH 3586 could not be recovered during Florisil column cleanup. Based on the results of Protocol D testing, testing under Protocol E was not conducted. No recovery of the test substances was obtained using the Florisil column cleanup procedures of Protocol F.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: PP#0F6131
DP Barcode: D288216
PC Code: 114004

Template Version September 2003

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.4.3/OPPTS 860.1850/OECD IIA 6.6.3, 6.8.7 and IIIA 8.6
Confined Accumulation in Rotational Crops - Kale, Turnip, and Spring Wheat

Primary Evaluator: Manying Xue, Chemist
RAB3/HED (7509C) Date: 03/23/05

Approved by: Leung Cheng, Senior Chemist
RAB3/HED (7509C) Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; updated draft submitted 09/21/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

45121704 Krolski, M. and Bosnak, L. (1999) The Accumulation of (Triazolinone-3-(carbon 14))MKH 3586 Residues in Confined Rotational Crops: Lab Project Number: M6051601: 109220. Unpublished study prepared by Bayer Corp. 202 p.

EXECUTIVE SUMMARY:

Arvesta Corporation has submitted a confined rotational crop study with [triazolinone-3-^{14}C]amicarbazone (specific activity 209,000 dpm/µg). The radiolabeled test substance was applied directly to silt loam soil in metal tubs at 0.42 lb ai/A, and rotational kale, turnip, and wheat were planted 47, 138, and 364 days after treatment (DAT). The in-life and analytical phases of the study were conducted by Bayer Corporation (Stilwell, KS).

Total radioactive residues (TRR) accumulated at 0.01-7.42 ppm in all rotated crops planted 47, 138, and 364 days following a single soil application of [^{14}C]amicarbazone. Crops which had been planted 30 days following treatment failed due to amicarbazone phytotoxicity. In addition, only small amounts of sample could be collected at the 47-day plantback interval (PBI) due to phytotoxicity. TRR were generally highest in samples from the 47-day PBI and lower in those from the 138- and 364-day PBIs. For the 47-day PBI, TRR were highest in turnip tops (7.42 ppm), and wheat hay and straw (2.42 and 4.18 ppm); residues in other commodities ranged 0.10-1.59 ppm. In crop matrices from the 138-day PBI, TRR were highest in wheat hay and straw (2.78 and 3.49 ppm); residues in other commodities ranged 0.01-0.72 ppm. In crop matrices from the 364-day PBI, TRR were again highest in wheat hay and straw (1.53 and 1.72 ppm), and residues in other commodities ranged 0.02-0.74 ppm.

Methanol (MeOH) extraction released 60->100% TRR from rotational crop matrices, with the exception of wheat grain. For wheat grain, MeOH extraction released 28-29% TRR, and extraction with MeOH at reflux released 53-57% TRR. For 138- and 364-DAT samples, additional extraction and hydrolysis procedures were conducted for selected crop matrices; however, these procedures released ≤9% TRR, except for MeOH extraction at reflux, which released 15% TRR (<0.01 ppm) from 138-DAT turnip roots. Nonextractable residues in 138-

and 364-DAT rotational crop commodities ranged <1-9% TRR (<0.01-0.03 ppm). The extraction procedures extracted sufficient residues from rotational crop matrices from the 138- and 364-day PBIs. The procedures did not extract sufficient residues from rotational crop matrices from the 47-day PBI; nonextractable residues in rotational crop matrices from the 47-day PBI ranged 7-22% TRR (0.01-1.64 ppm). The petitioner stated that the small sample sizes for the 47-DAT samples prevented further extraction of the samples. Adequate supporting storage stability data were submitted to support the storage conditions and intervals of samples from this study.

No characterization/identification of residues could be conducted for 47-DAT turnip root and wheat grain because of sample size and low radioactivity levels. Total identified residues ranged 22-104% TRR in remaining rotated crop commodities. Residues were characterized/identified primarily by HPLC analysis with confirmatory LC/MS analysis. In 47-DAT matrices, amicarbazone was the major identified residue, at 22-40% TRR (0.55-2.2 ppm). Metabolite iPr-2-OH DA MKH 3586 was also a significant residue in all rotational commodities, at 11-19% TRR (0.25-0.80 ppm). Metabolite DA MKH 3586 was identified in kale, turnip top, wheat forage, and wheat straw, at 5-18% TRR (0.16-1.07 ppm). Glucose conjugates DA OH Glu I MKH 3586 and DA OH Glu II MKH 3586 were major residues in wheat hay and straw (each at 11-15% TRR; 0.36-0.59 ppm), but were found in smaller quantities in kale, turnip top, and wheat forage (≤5% TRR, ≤0.14 ppm). Other identified residues, each present at ≤3% TRR, included triazolinone MKH 3586 (turnip top and wheat forage and straw), tBu-OH DA MKH 3586 (kale), N-Me DA MKH 3586 (turnip top and wheat hay), and a conjugate of triazolinone MKH 3586 (wheat forage).

In 138-DAT matrices, amicarbazone was a major residue in all commodities except turnip root and wheat grain, at 10-37% TRR (0.16-0.64 ppm); amicarbazone was found in turnip root at 2% TRR (<0.01 ppm) and was not found in wheat grain. Metabolite iPr-2-OH DA MKH 3586 was found to be a significant residue in all rotational commodities at 12-42% TRR (<0.01-0.78 ppm). Metabolite DA MKH 3586 was identified in all commodities except wheat grain, at <1-18% TRR (<0.01-0.13 ppm). Glucose conjugates DA OH Glu I MKH 3586 and DA OH Glu II MKH 3586 were major residues in wheat hay, grain, and straw (each at 11-28% TRR; 0.02-0.96 ppm), but were found in smaller quantities in kale, turnip top, and wheat forage (≤7% TRR, ≤0.05 ppm). Other identified residues, each present at ≤5% TRR, included triazolinone MKH 3586 (all commodities except turnip root), tBu-OH DA MKH 3586 (kale), N-Me DA MKH 3586 (kale, turnip top, and wheat hay), and a conjugate of triazolinone MKH 3586 (wheat forage).

In 364-DAT matrices, amicarbazone was a major residue in kale, turnip top, and wheat forage, at 12-18% TRR (0.02-0.09 ppm); amicarbazone was found in wheat hay and straw at ≤7% TRR (≤0.10 ppm) and was not found in turnip root or wheat grain. Metabolite iPr-2-OH DA MKH 3586 was found to be a significant residue in all rotational commodities, at 12-41% TRR (<0.01-0.47 ppm). Metabolite DA MKH 3586 was identified in all commodities except wheat grain, at <1-35% TRR (<0.01-0.22 ppm). Glucose conjugates DA OH Glu I MKH 3586 and DA OH Glu II MKH 3586 were major residues in wheat hay, grain, and straw (each at 13-39% TRR; <0.01-0.67 ppm), but were found in smaller quantities in kale, turnip top, and wheat forage (≤7% TRR,

≤0.05 ppm). Other identified residues, each present at ≤10% TRR, included triazolinone MKH 3586 (turnip top and wheat forage, grain, and straw), tBu-OH DA MKH 3586 (kale), N-Me DA MKH 3586 (kale, turnip top, and wheat hay), and a conjugate of triazolinone MKH 3586 (wheat forage).

The petitioner did not propose a metabolic profile for rotational crops. The petitioner noted that the metabolites identified in rotational crop were similar to those found in the primary crop metabolism study (corn; see DER for MRID 45121633) and stated that the major metabolites were those in which the tertiary carbon of the isopropyl moiety had undergone deamination and oxidation. We note that two metabolites identified in rotational crops, triazolinone MKH 3586 and N-Me DA MKH 3586, were not found in the corn metabolism study.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the confined rotational crop residue data are classified as scientifically acceptable.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D288216.

COMPLIANCE:

Signed and dated 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

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis.

TABLE A.1. Amicarbazone Nomenclature.

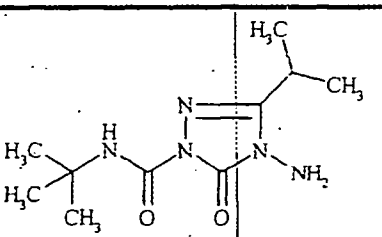
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-*tert*-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide

TABLE A.1. Amicarbazone Nomenclature.	
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS #	129909-90-6
End-use formulation (EUP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.		
Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-6} Pa @ 25°C 1.30×10^{-6} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient. Log(K_{ow})	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

TABLE B.1.1. Test Site Information.							
Testing Environment and location.	Soil characteristics						
	Type	% Sand	% Silt	% Clay	%OM	pH	CEC (meq/ 100 g)
Metal tubs maintained in a greenhouse at Bayer (Stilwell, KS)	Silt loam	10	64	26	Not specified	6.0	13.6

The petitioner included temperature data for the greenhouse during the crop growth period. No unusual temperature variations were noted.

TABLE B.1.2. Crop Information.					
Crop; crop group	Variety	Plantback intervals (days)	Growth stage at harvest *	Harvested RAC	Harvesting procedure
Kale; vegetable, Brassica, leafy	Dwarf curly	47, 138, 364	Maturity; 47-71 DAP	Leaves	Manually cut at the soil surface

TABLE B.1.2. Crop Information.

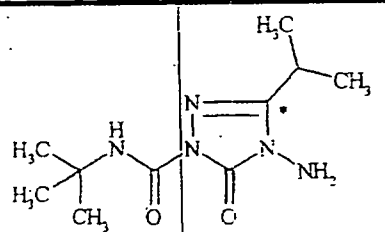
Crop; crop group	Variety	Plantback intervals (days)	Growth stage at harvest [a]	Harvested RAC	Harvesting procedure
Turnip; vegetable, root and tuber, and leaves of root and tuber vegetable	Purple top	47, 138, 364	Maturity; 62-71 DAP	Roots and tops	Pulled from the soil and separated into roots and tops; roots gently rinsed with water
Spring wheat; cereal grains and forage, fodder, and straw of cereal grains	Butte	47, 138, 364	6- to 8-inches tall; 20-28 DAP	Forage	Manually cut at soil surface
			Early flower to soft dough; 38-70 DAP	Hay	Manually cut at soil surface; dried for 3 days in a laboratory fume hood
			Maturity; 83-96 DAP	Grain and straw	Heads cut from stalks manually and grain removed from heads; stalks cut at soil surface

[a] DAP = Days after planting.

Rotational crops were initially planted 30 days after treatment; however, kale and turnips failed at this interval due to phytotoxicity. The soil was turned and crops were replanted at a 47-day plantback interval for the first rotation.

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics.

Chemical structure	
Radiolabel position	Triazolinone-3-^{14}C
Lot No.	Vial C-693
Purity	100%
Specific activity	209,000 dpm/µg (22.7 mCi/mmol)

B.3. Study Use Pattern

TABLE B.3.1. Use Pattern Information.

Chemical name	[triazolinone-3-^{14}C]amicarbazone
Application method	The test substance was mixed with WP formulation blank and water, and was applied to the soil using a spray bottle.
Application rate	0.42 lb ai/A
Number of applications	One

TABLE B.3.1. Use Pattern Information.	
Timing of applications	47 days prior to planting first rotation
PHI	N/A; application to bare soil

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Immediately following harvest, samples of kale, turnip tops and root, and wheat forage, hay, and straw were cut into small pieces with a knife or scissors; hay was dried in a laboratory fume hood for three days prior to collection. All prepared samples were homogenized in the presence of liquid nitrogen or dry ice for all matrices except wheat grain and straw.

Kale: Samples were macerated in methanol (MeOH) using a tissumizer; the extraction was repeated two more times using MeOH. The combined extracts were evaporated to dryness and redissolved in MeOH:0.1% acetic acid (1:9 or 1:5, v:v) for HPLC analysis. For the kale samples harvested 138 and 364 days after treatment (DAT), the remaining solids were heated at reflux in MeOH for 2 hours.

Turnip root: Samples were macerated in MeOH using a tissumizer; the extraction was repeated two more times using MeOH. For 138- and 364-DAT samples, the combined extracts were concentrated, mixed with water, basified using 1 N NaOH and partitioned three times with chloroform. The combined chloroform fractions were evaporated to dryness and redissolved in MeOH:0.1% acetic acid (1:9, v:v) for HPLC analysis (138-DAT sample only). For the 364-DAT sample, the aqueous fraction (after chloroform partition) was acidified using 6 N sulfuric acid then partitioned three times with chloroform. The combined chloroform fractions were evaporated to dryness and redissolved in MeOH:0.1% acetic acid (1:9, v:v) for HPLC analysis. For the 138-DAT sample, the solids remaining after MeOH maceration were heated at reflux in MeOH for 2.5 hours.

Turnip top: Samples were macerated in MeOH using a tissumizer; the extraction was repeated two more times using MeOH. The combined extracts were evaporated to dryness and redissolved in MeOH:0.1% acetic acid (1:9, v:v; 47- and 364-DAT sample) or 0.1% acetic acid (138-DAT sample) for HPLC analysis. For the 138-DAT sample, the remaining solids were heated at reflux in MeOH for 2.5 hours. The remaining solids were then sequentially hydrolyzed with 1 N HCl and 2 N NaOH, at ambient temperature for 3 hours. The hydrolysates were neutralized to pH 7, and the acid hydrolysate was partitioned three times with chloroform. The solids remaining after base hydrolysis were sonicated in MeOH:water (3:1, v:v) for 2 hours.

Wheat forage: Samples were extracted three times with MeOH. The combined extracts were evaporated to dryness and redissolved in MeOH:0.1% acetic acid (50:5, v:v) for HPLC analysis. For 138- and 364-DAT samples, the remaining solids were heated at reflux in MeOH for 2.5 hours. The extract (364-DAT sample only) was evaporated to dryness, redissolved in MeOH, evaporated to dryness again, and redissolved in MeOH:0.1% acetic acid (1:9, v:v) for HPLC

analysis. The remaining solids were sequentially hydrolyzed with 1 N HCl and 2 N NaOH, each at ambient temperature for 3 hours. The hydrolysates were neutralized to pH 7, and the base hydrolysate was partitioned three times with chloroform. The solids remaining after base hydrolysis were sonicated in MeOH:water (3:1, v:v) for 2 hours.

Wheat hay: Samples were macerated in MeOH (47-DAT sample) or MeOH:water (3:1, v:v; 138- and 364-DAT samples) using a tissumizer; the extraction was repeated two more times using MeOH. The combined extracts were evaporated to dryness and redissolved in MeOH:0.1% acetic acid (1:9, v:v) for HPLC analysis. For 138- and 364-DAT hay samples, the remaining solids were heated at reflux in MeOH for 2.5 hours. The extract was evaporated to dryness and redissolved in MeOH:0.1% acetic acid (1:5, v:v) for HPLC analysis (138-DAT sample only). The remaining solids were then sequentially hydrolyzed with 1 N HCl and 2 N NaOH, at ambient temperature for 3 hours. The hydrolysates were neutralized to pH 7, and the base hydrolysate was partitioned three times with chloroform. The solids remaining after base hydrolysis were sonicated in MeOH:water (3:1, v:v) for 2 hours. The solids remaining after MeOH/water sonication were sequentially hydrolyzed with 6 N HCl and 6 N NaOH, each at reflux for 4 hours; the hydrolysates were neutralized to pH 7.

Wheat grain: Only 138- and 364-DAT samples were subjected to extraction procedures because of insufficient sample from the 47-DAT plants. Samples were macerated in MeOH using a tissumizer; the extraction was repeated two more times using MeOH. The combined extracts were evaporated to dryness and redissolved in 0.1% acetic acid (138-DAT sample) or MeOH:0.1% acetic acid (1:9, v:v; 364-DAT sample) for HPLC analysis. The remaining solids were heated at reflux in MeOH for 2.5 hours. The extract was evaporated to dryness and redissolved in 0.1% acetic acid (138-DAT sample) or MeOH:0.1% acetic acid (1:9, v:v; 364-DAT sample) for HPLC analysis. The remaining solids were then sequentially hydrolyzed with 1 N HCl and 2 N NaOH, at ambient temperature for 3 hours. The hydrolysates were neutralized to pH 7. For the 138-DAT sample, the acid hydrolysate was partitioned three times with chloroform, and the base hydrolysate was partitioned three times with ethyl acetate. The solids remaining after base hydrolysis were sonicated in MeOH (138-DAT sample) or MeOH:water (3:1, v:v; 364-DAT sample) for 2 hours.

Wheat straw: Samples were macerated in MeOH:water (4:1, v:v; 47-DAT sample) or MeOH:water (3:1, v:v; 138- and 364-DAT samples) using a tissumizer; the extraction was repeated two more times using MeOH. For 138- and 364-DAT samples, the combined extracts were evaporated to dryness and redissolved in MeOH:0.1% acetic acid (1:9, v:v) for HPLC analysis. For 138- and 364-DAT samples, the remaining solids were heated at reflux in MeOH for 2.5 hours. The extract was evaporated to dryness and redissolved in 0.1% acetic acid for HPLC analysis. The remaining solids were then sequentially hydrolyzed with 1 N HCl and 2 N NaOH, at ambient temperature for 3 hours. The hydrolysates were neutralized to pH 7, and separately partitioned three times with chloroform (138-DAT sample only). The combined chloroform fractions from the acid hydolysate were evaporated to dryness and redissolved in MeOH:0.1% acetic acid (1:9, v:v) for HPLC analysis. The solids remaining after base hydrolysis were sonicated in MeOH:water (3:1, v:v) for 2 hours. The solids remaining after MeOH/water

sonication were sequentially hydrolyzed with 6 N HCl and 6 N NaOH, each at reflux for 4 hours. The hydrolysates were neutralized to pH 7, and the base hydrolysate was partitioned three times with chloroform (138-DAT sample only).

B.4.2. Analytical Methodology

Samples of rotational crop commodities were subjected to combustion/LSC for determination of total radioactive residues (TRR). Reported TRR values for each RAC were calculated as the mean of 3-20 determinations per RAC. Extracts and hydrolysates were radioassayed directly by LSC, and nonextractable residues were radioassayed by combustion/LSC. Detection limits were reported as 0.0011 ppm for liquid samples and 0.0015 ppm for solid samples.

Extracts of rotational crop matrices were analyzed by HPLC using a system equipped with a C-18 column, UV detector, and radioactivity monitor. A gradient mobile phase of 0.1% acetic acid and 0.1% acetic acid in MeOH was used. Alternate systems, using a C8 column and a gradient mobile phase of 0.1% acetic acid and 0.1% acetic acid in methanol or acetonitrile, were used for metabolite identification, confirmation, and/or purification. Metabolites were identified by cochromatography and/or retention time comparison with the following reference standards: [^{14}C]amicarbazone; [^{14}C]DA MKH 3586; [^{14}C]iPr-2-OH DA MKH 3586; [^{14}C]iPr-1,2-diOH DA MKH 3586; [^{14}C]tBu-OH DA MKH 3586; [^{14}C]tBu-iPr-2-diOH DA MKH 3586; [^{14}C]triazolinone MKH 3586; and N-Me DA MKH 3586.

Selected isolated metabolites were purified by TLC for LC/MS analysis. TLC was conducted on silica gel 60 F_{254} plates using a solvent system of hexane:0.1% acetic acid in isopropanol (6:4, v:v). Radioactivity was detected using a radioscanner.

Selected purified components were analyzed by LC/MS with electrospray ionization, using a C8 column and a gradient mobile phase of ammonium acetate and acetonitrile. Radioactivity was monitored using a radioactivity detector.

C. RESULTS AND DISCUSSION

Total radioactive residues (TRR) in rotational crops are reported in Table C.2.1. TRR accumulated at ≥ 0.01 ppm in all rotated crops planted 47, 138, and 364 days following a single soil application of [^{14}C]amicarbazone at 0.42 lb ai/A. Crops which had been planted 30 days following treatment failed due to amicarbazone phytotoxicity. In addition, only small amounts of sample could be collected at the 47-day plantback interval (PBI) due to phytotoxicity. TRR were generally highest in samples from the 47-day PBI and lower in those from the 138- and 364-day PBIs. For the 47-day PBI, TRR were highest in turnip tops (7.42 ppm), and wheat hay and straw (2.42 and 4.18 ppm); residues in other commodities ranged 0.10-1.59 ppm. In crop matrices from the 138-day PBI, TRR were highest in wheat hay and straw (2.78 and 3.49 ppm); residues in other commodities ranged 0.01-0.72 ppm. In crop matrices from the 364-day PBI, TRR were again highest in wheat hay and straw (1.53 and 1.72 ppm), and residues in other commodities ranged 0.02-0.74 ppm.

The distribution of radioactivity in rotated crops is presented in Tables C.2.2.1 through C.2.2.3, Methanol extraction released 60->100% TRR from rotational crop matrices, with the exception of wheat grain. For wheat grain, MeOH extraction released 28-29% TRR, and extraction with MeOH at reflux released 53-57% TRR. For 138- and 364-DAT samples, additional extraction and hydrolysis procedures were conducted for selected crop matrices; however, these procedures released ≤9% TRR, except for MeOH extraction at reflux, which released 15% TRR (<0.01 ppm) from 138-DAT turnip roots. Nonextractable residues in 138- and 364-DAT rotational crop commodities ranged <1-9% TRR (<0.01-0.03 ppm). The extraction procedures extracted sufficient residues from rotational crop matrices from the 138- and 364-day PBIs. The procedures did not extract sufficient residues from rotational crop matrices from the 47-day PBI; nonextractable residues in rotational crop matrices from the 47-day PBI ranged 7-22% TRR (0.01-1.64 ppm). The petitioner stated that the small sample sizes for the 47-DAT samples prevented further extraction of the samples.

Characterization and identification of residues are summarized in Tables C.2.3.1 through C.2.3.3. No characterization/identification of residues could be conducted for 47-DAT turnip root and wheat grain because of sample size and low radioactivity levels. Total identified residues ranged 22->100% TRR in remaining rotated crop commodities. In 47-DAT matrices, amicarbazone was the major identified residue, at 22-40% TRR (0.55-2.2 ppm). Metabolite iPr-2-OH DA MKH 3586 was also a significant residue, in all rotational commodities, at 11-19% TRR (0.25-0.80 ppm). Metabolite DA MKH 3586 was identified in kale, turnip top, wheat forage, and wheat straw, at 5-18% TRR (0.16-1.07 ppm). Glucose conjugates DA OH Glu I MKH 3586 and DA OH Glu II MKH 3586 were major residues in wheat hay and straw (each at 11-15% TRR; 0.36-0.59 ppm), but were found in smaller quantities in kale, turnip top, and wheat forage (≤5% TRR, ≤0.14 ppm). Other identified residues, each present at ≤3% TRR, included triazolinone MKH 3586 (turnip top and wheat forage and straw), tBu-OH DA MKH 3586 (kale), N-Me DA MKH 3586 (turnip top and wheat hay), and a conjugate of triazolinone MKH 3586 (wheat forage).

In 138-DAT matrices, amicarbazone was a major residue in all commodities except turnip root and wheat grain, at 10-37% TRR (0.16-0.64 ppm); amicarbazone was found in turnip root at 2% TRR (<0.01 ppm) and was not found in wheat grain. Metabolite iPr-2-OH DA MKH 3586 was found to be a significant residue, in all rotational commodities, at 12-42% TRR (<0.01-0.78 ppm). Metabolite DA MKH 3586 was identified in all commodities except wheat grain, at <1-18% TRR (<0.01-0.13 ppm). Glucose conjugates DA OH Glu I MKH 3586 and DA OH Glu II MKH 3586 were major residues in wheat hay, grain, and straw (each at 11-28% TRR; 0.02-0.96 ppm), but were found in smaller quantities in kale, turnip top, and wheat forage (≤7% TRR, ≤0.05 ppm). Other identified residues, each present at ≤5% TRR, included triazolinone MKH 3586 (all commodities except turnip root), tBu-OH DA MKH 3586 (kale), N-Me DA MKH 3586 (kale, turnip top, and wheat hay), and a conjugate of triazolinone MKH 3586 (wheat forage).

In 364-DAT matrices, amicarbazone was a major residue in kale, turnip top, and wheat forage, at 12-18% TRR (0.02-0.09 ppm); amicarbazone was found in wheat hay and straw at ≤7% TRR (≤0.10 ppm) and was not found in turnip root or wheat grain. Metabolite iPr-2-OH DA MKH 3586 was found to be a significant residue in all rotational commodities, at 12-41% TRR (<0.01-

0.47 ppm). Metabolite DA MKH 3586 was identified in all commodities except wheat grain, at <1-35% TRR (<0.01-0.22 ppm). Glucose conjugates DA OH Glu I MKH 3586 and DA OH Glu II MKH 3586 were major residues in wheat hay, grain, and straw (each at 13-39% TRR; <0.01-0.67 ppm), but were found in smaller quantities in kale, turnip top, and wheat forage (≤7% TRR, ≤0.05 ppm). Other identified residues, each present at ≤10% TRR, included triazolinone MKH 3586 (turnip top and wheat forage, grain, and straw), tBu-OH DA MKH 3586 (kale), N-Me DA MKH 3586 (kale, turnip top, and wheat hay), and a conjugate of triazolinone MKH 3586 (wheat forage).

LC/MS analyses were used to confirm the identification of the following compounds: amicarbazone in 47-DAT turnip top and 364-DAT wheat forage; DA MKH 3586 in 47-DAT turnip top and 364-DAT wheat forage; and iPr-2-OH DA MKH 3586 in 47-DAT kale and 364-DAT wheat forage. In addition, LC/MS analyses were used to identify N-Me DA MKH 3586 in 364-DAT kale and wheat hay, and to characterize a metabolite in 364-DAT wheat forage as a conjugate of triazolinone MKH 3586.

The identification of DA OH Glu I MKH 3586 and DA OH Glu II MKH 3586 in wheat straw and hay was made using LC/MS analyses. The petitioner noted that the LC/MS analyses could not determine the site of glucose conjugation in either of the glucose conjugate compounds; however, isolation of two compounds indicated that both O- and N-glucosides had been formed. Hydrolysis with β-glucosidase was attempted; however, it was unsuccessful. The petitioner assigned DA OH Glu I MKH 3586 as the O-glucoside and DA OH Glu II MKH 3586 as the N-glucoside based on the results of the corn metabolism study (see DER for MRID 45121633), in which the O-glucoside was found to be more polar.

The identification of triazolinone MKH 3586 in 364-DAT wheat forage was confirmed by cochromatography with a reference standard in two HPLC systems.

C.1. Storage Stability

Samples of rotational crop commodities were stored frozen at <-10 °C prior to analysis. The petitioner stated that initial processing and extraction of samples occurred within 21 days of sample collection for all samples, and that samples were then stored up to 3 months prior to quantitative analysis. It was also stated that LC/MS analyses of some metabolites was conducted up to 30 months following sample collection for certain metabolites; however, the petitioner noted that these analyses were not quantitative.

The petitioner reextracted samples of 364-DAT rotational crop matrices (except turnip root) following 318 to 393 days of frozen storage (at the conclusion of analytical work). The extraction profiles for the stored samples were found to be similar to those for the samples from the initial extractions. The metabolite profiles were found to be stable in the stored samples, with the exception of amicarbazone in kale and wheat forage. It was found the amicarbazone converted to DA MKH 3586 and iPr-2-OH DA MKH 3586 in kale and wheat forage during frozen storage. Similar decomposition may have occurred in the other rotational crop matrices,

but to a lesser extent than in kale and wheat forage. We note that, based on the values reported with the storage stability data, the petitioner used the values from the initial analyses for the quantitative results for metabolites in the 364-DAT samples.

Although the petitioner did not provide information regarding actual storage intervals for samples from this study, HED concludes that the storage stability data provided by the petitioner are adequate to support the storage conditions and intervals of samples from this study. Although the storage stability data indicate some degradation of amicarbazone over storage, quantitative data for metabolite levels were obtained from analyses conducted within 3-4 months of sample collection.

TABLE C.1. Summary of Storage Conditions.

Matrix	Plantback interval (days)	Storage Temp.	Actual Storage Duration	Interval of Demonstrated Storage Stability (days)
Kale	47, 138, 364	<-10 °C	Not reported; dates of sample extraction and analysis were not included in the submission.	368
Turnip root				–
Turnip top				346
Wheat forage				393
Wheat hay				366
Wheat grain				318
Wheat straw				320

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRR) in Rotational Crop Matrices.

Matrix	Plantback interval (days)	ppm, [^{14}C]amicarbazone equivalents
Kale	47	1.59
	138	0.46
	364	0.17
Turnip top	47	7.42
	138	0.49
	364	0.15
Turnip root	47	0.10
	138	0.01
	364	0.02
Wheat forage	47	1.38
	138	0.72
	364	0.74
Wheat hay	47	2.42
	138	2.78
	364	1.53

TABLE C.2.1. Total Radioactive Residues (TRR) in Rotational Crop Matrices.

Matrix	Plantback interval (days)	ppm, [^{14}C]amicarbazone equivalents
Wheat grain	47	0.18
	138	0.24
	364	0.09
Wheat straw	47	4.18
	138	3.49
	364	1.72

TABLE C.2.2.1. Distribution of the Parent and the Metabolites in Rotational Crop Matrices Planted 47 Days Following Soil Application of Radiolabeled Amicarbazone at 0.42 lb ai/A. *

Metabolite Fraction	Kale		Turnip root		Turnip top		Wheat forage		Wheat hay		Wheat straw	
	TRR = 1.59 ppm		TRR = 0.10 ppm		TRR = 7.42 ppm		TRR = 1.38 ppm		TRR = 2.42 ppm		TRR = 4.18 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
MeOH	84	1.34	100	0.10	66	4.93	89	1.23	83	2.00	78	3.25
Amicarbazone	40	0.64			30	2.20	40	0.55	26	0.64	22	0.94
DA MKH 3586	10	0.16			14	1.07	18	0.25	ND	ND	5	0.22
iPr-2-OH DA MKH 3586	16	0.25			11	0.80	19	0.26	16	0.38	17	0.70
Triazolinone MKH 3586	–	–			1	0.07	<1	<0.01	–	–	1	0.04
DA OH Glu I MKH 3586	2	0.03			1	0.05	5	0.07	15	0.36	11	0.47
tBu-OH DA MKH 3586	1	0.02			–	–	–	–	–	–	–	–
DA OH Glu II MKH 3586	4	0.07			2	0.14	4	0.05	15	0.36	14	0.59
Triazolinone MKH 3586 conjugate	–	–			–	–	1	0.02	–	–	–	–
N-Me DA MKH 3586	ND	ND			1	0.09	–	–	3	0.07	–	–
Unknowns	9	0.14			7	0.49	4	0.05	6	0.15	6	0.24

* Shading indicates that the characterization step was not conducted for that matrix. The petitioner normalized %TRR values but not ppm values; the actual %TRR values were calculated by the study reviewer (from the ppm values or from raw data reported in the submission) and are reported in the table above.

TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Rotational Crop Matrices Planted 138 Days Following Soil Application of Radiolabeled Amicarbazone at 0.42 lb ai/A.

Metabolite Fraction	Kale		Turnip root		Turnip top		Wheat forage		Wheat hay		Wheat grain		Wheat straw	
	TRR = 0.46 ppm		TRR = 0.01 ppm		TRR = 0.49 ppm		TRR = 0.72 ppm		TRR = 2.78 ppm		TRR = 0.24 ppm		TRR = 3.49 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
MeOH	96	0.44	NR	NR	80	0.39	86	0.62	84	2.33	28	0.06	89	3.09
Amicarbazone	37	0.17			37	0.18	22	0.16	23	0.64	–	–	10	0.35
DA MKH 3586	15	0.07			4	0.02	18	0.13	2	0.05	–	–	<1	0.01
iPr-2-OH DA MKH 3586	24	0.11			24	0.12	25	0.18	25	0.70	15	0.04	21	0.73
Triazolinone MKH 3586	2	<0.01			4	0.02	<1	<0.01	<1	0.02	2	<0.01	5	0.17
DA OH Glu I MKH 3586	<1	<0.01			2	0.01	7	0.05	12	0.33	6	0.01	17	0.59
tBu-OH DA MKH 3586	2	0.01			–	–	–	–	–	–	–	–	–	–
DA OH Glu II MKH 3586	2	<0.01			2	0.01	7	0.05	14	0.39	3	<0.01	26	0.91
Triazolinone MKH 3586 conjugate	–	–			–	–	3	0.02	–	–	–	–	–	–
N-Me DA MKH 3586	2	<0.01			1	<0.01	–	–	1	0.03	–	–	–	–
Unknowns	10	0.04			4	0.02	3	0.02	5	0.15	<1	<0.01	6	0.21
Basic chloroform partition			22	<0.01										
Amicarbazone			2	<0.01										
DA MKH 3586			9	<0.01										
iPr-2-OH DA MKH 3586			12	<0.01										
Aqueous partition			38	<0.01										
MeOH reflux	1	<0.01	15	<0.01	1	<0.01	<1	<0.01	1	0.03	57	0.13	6	0.21
Amicarbazone											–	–	<1	0.01
DA MKH 3586											–	–	<1	0.03
iPr-2-OH DA MKH 3586											27	0.06	2	0.05
Triazolinone MKH 3586											3	<0.01	<1	0.02
DA OH Glu I MKH 3586											13	0.03	2	0.04
DA OH Glu II MKH 3586											8	0.02	2	0.05

TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Rotational Crop Matrices Planted 138 Days Following Soil Application of Radiolabeled Amicarbazone at 0.42 lb ai/A.*

Metabolite Fraction	Kale		Turnip root		Turnip top		Wheat forage		Wheat hay		Wheat grain		Wheat straw	
	TRR = 0.46 ppm		TRR = 0.01 ppm		TRR = 0.49 ppm		TRR = 0.72 ppm		TRR = 2.78 ppm		TRR = 0.24 ppm		TRR = 3.49 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Unknowns											3	<0.01	<1	0.02
1 N HCl hydrolysate					NR	NR	<1	<0.01	<1	0.02	NR	NR	NR	NR
Organic					<1	<0.01					<1	<0.01	2	0.07
Aqueous					<1	<0.01					2	<0.01	1	0.04
2 N NaOH hydrolysate					<1	<0.01	NR	NR	NR	NR	NR	NR	NR	NR
Organic							2	0.01	<1	0.01	2	<0.01	2	0.06
Aqueous							<1	<0.01	<1	0.02	3	<0.01	1	0.03
MeOH/water sonication					2	<0.01	<1	<0.01	<1	0.01	2	<0.01	<1	0.01
6 N HCl hydrolysate									<1	0.02			<1	0.02
6 N NaOH hydrolysate									<1	0.02			NR	NR
Organic													<1	<0.01
Aqueous													<1	0.02

* Shading indicates that the characterization step was not conducted for that matrix. The petitioner normalized %TRR values but not ppm values; the actual %TRR values were calculated by the study reviewer (from the ppm values or from raw data reported in the submission) and are reported in the table above.

TABLE C.2.2.3. Distribution of the Parent and the Metabolites in Rotational Crop Matrices Planted 364 Days Following Soil Application of Radiolabeled Amicarbazone at 0.42 lb ai/A. [a]

Metabolite Fraction	Kale		Turnip root		Turnip top		Wheat forage		Wheat hay		Wheat grain		Wheat straw	
	TRR = 0.17 ppm		TRR = 0.02 ppm		TRR = 0.15 ppm		TRR = 0.74 ppm		TRR = 1.53 ppm		TRR = 0.09 ppm		TRR = 1.72 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
MeOH	112	0.19	NR	NR	93	0.14	91	0.67	82	1.25	29	0.03	85	1.46
Amicarbazone	18	0.03			13	0.02	12	0.09	7	0.10	–	–	3	0.05
DA MKH 3586	35	0.06			6	<0.01	30	0.22	4	0.06	–	–	ND	ND
iPr-2-OH DA MKH 3586	29	0.05			40	0.06	22	0.16	31	0.47	14	0.01	13	0.22
Triazolinone MKH 3586	ND	ND			4	<0.01	2	0.01	ND	ND	3	<0.01	5	0.08
DA OH Glu I MKH 3586	ND	ND			1	<0.01	7	0.05	14	0.22	6	<0.01	21	0.36
tBu-OH DA MKH 3586	10	0.02			–	–	–	–	–	–	–	–	–	–
DA OH Glu II MKH 3586	2	<0.01			7	<0.01	5	0.04	22	0.33	4	<0.01	37	0.64
Triazolinone MKH 3586 conjugate	–	–			–	–	8	0.06	–	–	–	–	–	–
N-Me DA MKH 3586	10	0.02			7	0.01	–	–	3	0.04	–	–	–	–
Unknowns	6	<0.01			15	0.02	5	0.04	2	0.03	2	<0.01	7	0.12
Basic chloroform partition			4	<0.01										
Acidic chloroform partition			23	<0.01										
DA MKH 3586			10	<0.01										
iPr-2-OH DA MKH 3586			12	<0.01										
Unknown			2	<0.01										
Aqueous partition			54	<0.01										
MeOH reflux	1	<0.01					2	0.02	3	0.04	53	0.05	6	0.11
Amicarbazone											–	–	<1	<0.01
DA MKH 3586											–	–	<1	<0.01
iPr-2-OH DA MKH 3586											27	0.03	1	0.02
Triazolinone MKH 3586											4	<0.01	<1	<0.01

TABLE C.2.2.3. Distribution of the Parent and the Metabolites in Rotational Crop Matrices Planted 364 Days Following Soil Application of Radiolabeled Amicarbazone at 0.42 lb ai/A. *

Metabolite Fraction	Kale		Turnip root		Turnip top		Wheat forage		Wheat hay		Wheat grain		Wheat straw	
	TRR = 0.17 ppm		TRR = 0.02 ppm		TRR = 0.15 ppm		TRR = 0.74 ppm		TRR = 1.53 ppm		TRR = 0.09 ppm		TRR = 1.72 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
DA OH Glu I MKH 3586											10	0.01	2	0.02
DA OH Glu II MKH 3586											9	<0.01	2	0.03
Unknowns											2	<0.01	<1	<0.01
1 N HCl hydrolysate							<1	<0.01	<1	0.01	9	<0.01	1	0.02
2 N NaOH hydrolysate							NR	NR	NR	NR	4	<0.01	2	0.03
Organic							<1	<0.01	1	0.01				
Aqueous							<1	<0.01	<1	0.01				
MeOH/water sonication							<1	<0.01	<1	<0.01	3	<0.01	1	0.02
6 N HCl hydrolysate									<1	<0.01			<1	<0.01
6 N NaOH hydrolysate									<1	0.01			<1	0.01

* Shading indicates that the characterization step was not conducted for that matrix. The petitioner normalized %TRR values but not ppm values; the actual %TRR values were calculated by the study reviewer (from the ppm values or from raw data reported in the submission) and are reported in the table above.

TABLE C.2.3.2. Summary of Characterization and Identification of Radioactive Residues in Rotational Crop Matrices Planted 138 Days Following Soil Application of Radiolabeled Amicarbazone at 0.42 lb ai/A.

Compound	Kale		Turnip root		Turnip top		Wheat forage		Wheat hay		Wheat grain		Wheat straw	
	TRR = 0.46 ppm		TRR = 0.01 ppm		TRR = 0.49 ppm		TRR = 0.72 ppm		TRR = 2.78 ppm		TRR = 0.24 ppm		TRR = 3.49 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Amicarbazone	37	0.17	2	<0.01	37	0.18	22	0.16	23	0.64	–	–	10	0.36
DA MKH 3586	15	0.07	9	<0.01	4	0.02	18	0.13	2	0.05	–	–	<1	0.04
iPr-2-OH DA MKH 3586	24	0.11	12	<0.01	24	0.12	25	0.18	25	0.70	42	0.09	23	0.78
Triazolinone MKH 3586	2	<0.01	–	–	4	0.02	<1	<0.01	<1	0.02	5	<0.01	5	0.19
DA OH Glu I MKH 3586	<1	<0.01	–	–	2	0.01	7	0.05	12	0.33	19	0.04	19	0.63
tBu-OH DA MKH 3586	2	0.01	–	–	–	–	–	–	–	–	–	–	–	–
DA OH Glu II MKH 3586	2	<0.01	–	–	2	0.01	7	0.05	14	0.39	11	0.02	28	0.96
Triazolinone MKH 3586 conjugate	–	–	–	–	–	–	3	0.02	–	–	–	–	–	–
N-Me DA MKH 3586	2	<0.01	–	–	1	<0.01	–	–	1	0.03	–	–	–	–
Unknowns	10	0.04	–	–	4	0.02	3	0.02	5	0.15	3	<0.01	6	0.23
Aqueous partition (MeOH extract)	–	–	38	<0.01	–	–	–	–	–	–	–	–	–	–
MeOH reflux	1	<0.01	15	<0.01	1	<0.01	<1	<0.01	1	0.03	–	–	–	–
1N HCl hydrolysate	–	–	–	–	<1	<0.01	<1	<0.01	<1	0.02	2	<0.01	3	0.11
2 N NaOH hydrolysate	–	–	–	–	<1	<0.01	2	0.01	<1	0.03	5	<0.01	1	0.09
MeOH/water sonication	–	–	–	–	2	<0.01	<1	<0.01	<1	0.01	2	<0.01	<1	0.01
6 N HCl hydrolysate	–	–	–	–	–	–	–	–	<1	0.02	–	–	<1	0.02
6 N NaOH hydrolysate	–	–	–	–	–	–	–	–	<1	0.02	–	–	<1	0.02
Total identified	84	0.36	23	<0.01	74	0.36	82	0.59	77	2.15	77	0.18	85	2.97
Total characterized	10	0.04	53	<0.01	7	0.03	5	0.03	10	0.28	12	0.03	12	0.48
Total extractable	97	0.44	75	<0.01	83	0.39	88	0.63	88	2.46	94	0.23	102	3.55
Unextractable (PES) [1]	2	0.01	7	<0.01	<1	<0.01	2	0.01	<1	0.02	7	0.02	1	0.03
Accountability [2]	99		82		84		90		89		101		103	

[1] Residues remaining after exhaustive extractions.
[2] Accountability = (Total extractable + Total unextractable)/(TRR from combustion analysis; see TABLE C.2.1) * 100.

TABLE C.2.3.3. Summary of Characterization and Identification of Radioactive Residues in Rotational Crop Matrices Planted 364 Days Following Soil Application of Radiolabeled Amicarbazone at 0.42 lb ai/A.

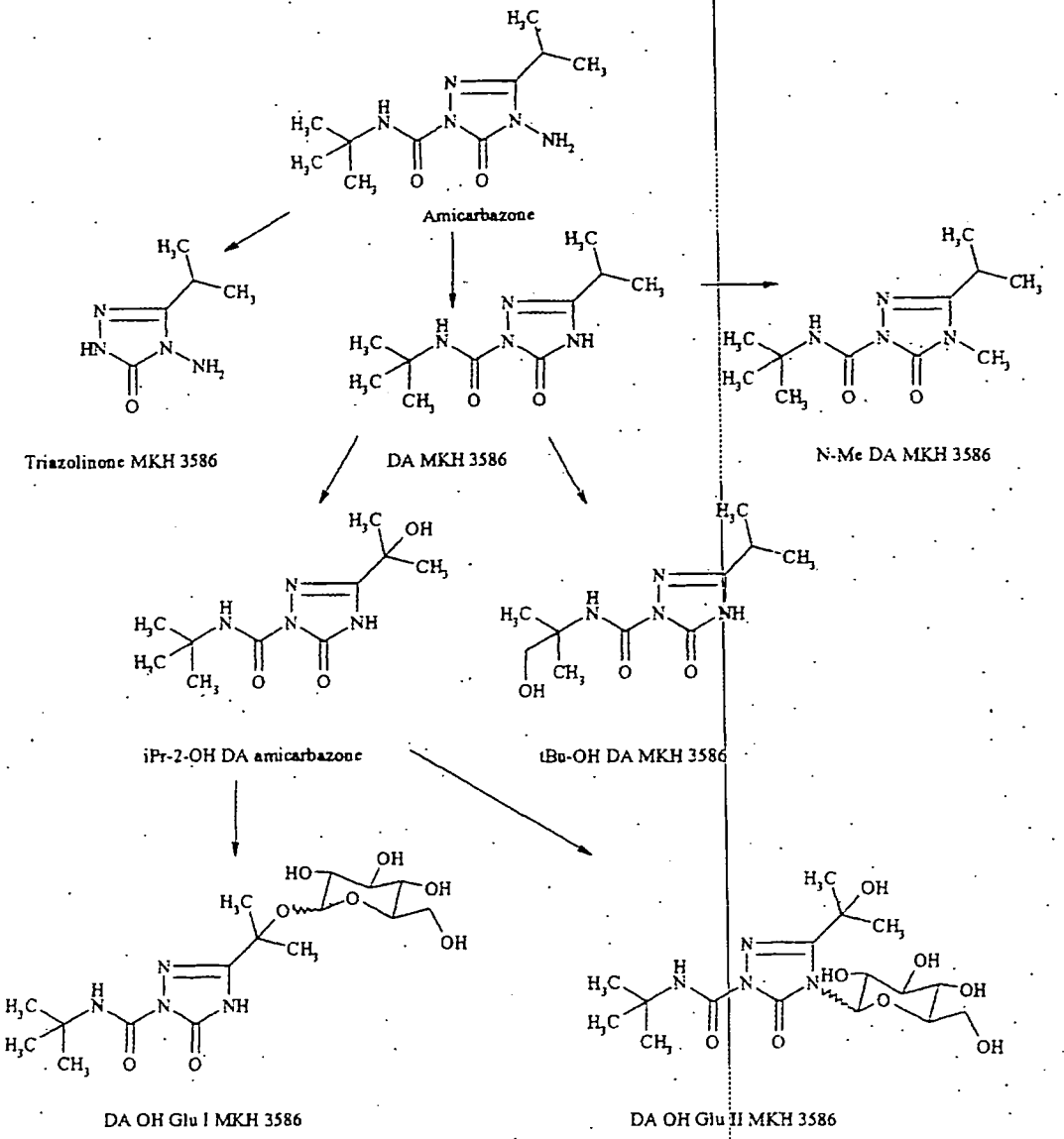
Compound	Kale		Turnip root		Turnip top		Wheat forage		Wheat hay		Wheat grain		Wheat straw	
	TRR = 0.17 ppm		TRR = 0.02 ppm		TRR = 0.15 ppm		TRR = 0.74 ppm		TRR = 1.53 ppm		TRR = 0.09 ppm		TRR = 1.72 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Amicarbazone	18	0.03	–	–	13	0.02	12	0.09	7	0.10	–	–	3	0.05
DA MKH 3586	35	0.06	10	<0.01	6	<0.01	30	0.22	4	0.06	–	–	<1	<0.01
iPr-2-OH DA MKH 3586	29	0.05	12	<0.01	40	0.06	22	0.16	31	0.47	41	0.03	14	0.24
Triazolinone MKH 3586	–	–	–	–	4	<0.01	2	0.01	–	–	7	<0.01	5	0.08
DA OH Glu I MKH 3586	–	–	–	–	1	<0.01	7	0.05	14	0.22	16	<0.01	23	0.38
tBu-OH DA MKH 3586	10	0.02	–	–	–	–	–	–	–	–	–	–	–	–
DA OH-Glu II MKH 3586	2	<0.01	–	–	7	<0.01	5	0.04	22	0.33	13	<0.01	39	0.67
Triazolinone MKH 3586 conjugate	–	–	–	–	–	–	8	0.06	–	–	–	–	–	–
N-Me DA MKH 3586	10	0.02	–	–	7	0.01	–	–	3	0.04	–	–	–	–
Unknowns	6	<0.01	2	<0.01	15	0.02	5	0.04	2	0.03	3	<0.01	7	0.12
Basic chloroform partition	–	–	4	<0.01	–	–	–	–	–	–	–	–	–	–
Acidic aqueous partition	–	–	54	<0.01	–	–	–	–	–	–	–	–	–	–
MeOH reflux	<1	<0.01	–	–	–	–	2	0.02	3	0.04	–	–	–	–
1N HCl hydrolysate	–	–	–	–	–	–	<1	<0.01	<1	0.01	9	<0.01	1	0.02
2 N NaOH hydrolysate	–	–	–	–	–	–	<1	<0.01	1	0.01	4	<0.01	2	0.03
MeOH/water sonication	–	–	–	–	–	–	<1	<0.01	<1	0.01	3	<0.01	1	0.02
6 N HCl hydrolysate	–	–	–	–	–	–	–	–	<1	<0.01	–	–	<1	<0.01
6 N NaOH hydrolysate	–	–	–	–	–	–	–	–	<1	0.01	–	–	<1	0.01
Total identified	104	0.18	22	<0.01	78	0.12	86	0.63	80	1.22	78	0.07	84	1.42
Total characterized	6	<0.01	60	<0.01	15	0.02	7	0.06	7	0.11	20	0.02	11	0.20
Total extractable	112	0.19	81	<0.01	93	0.14	93	0.69	87	1.33	98	0.09	95	1.65
Unextractable (PES) [1]	1	<0.01	9	<0.01	3	<0.01	2	0.01	<1	<0.01	5	<0.01	<1	0.01
Accountability [2]	113		90		96		95		88		103		96	

[1] Residues remaining after exhaustive extractions.

[2] Accountability = (Total extractable + Total unextractable)/(TRR from combustion analysis; see TABLE C.2.1) * 100

C.3. Proposed Metabolic Profile

FIGURE C.3.1. Proposed Metabolic Profile of Amicarbazone in Rotational Kale, Turnip, and Wheat.



We note that the petitioner did not propose a metabolic pathway. The above figure was generated by the study reviewer based on the compounds identified in rotational crops.

TABLE C.3.1. Identification of Compounds from the Confined Rotational Crop Study

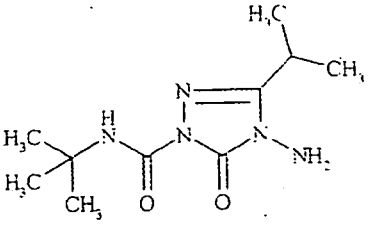
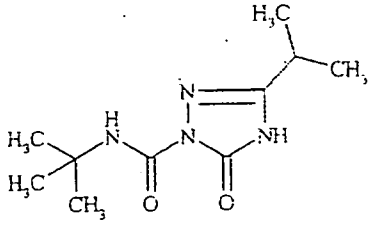
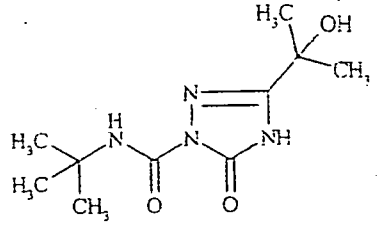
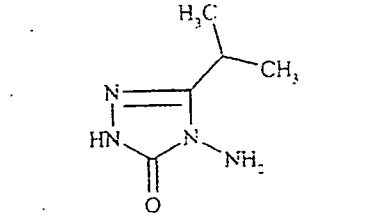
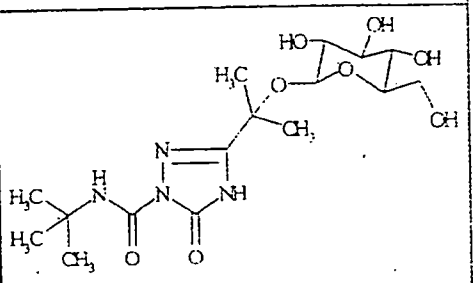
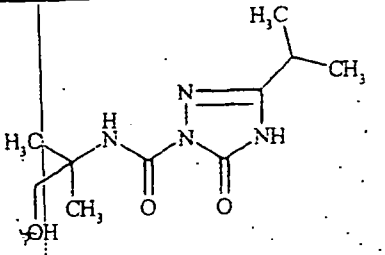
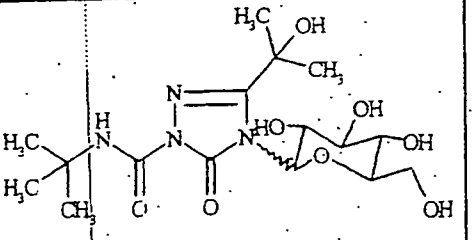
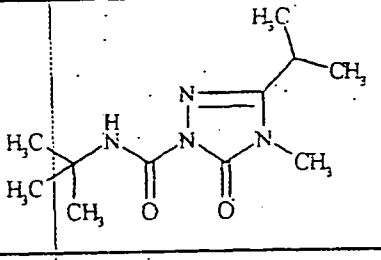
Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
Amicarbazone	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
DA MKH 3586	*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
iPr-2-OH DA MKH 3586	*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-hydroxy-1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
Triazolinone MKH 3586	4-amino-2,4-dihydro-5-(1-methylethyl)-3*H*-1,2,4-triazol-3-one	
DA OH Glu I MKH 3586; iPr-2-O-Glucosyl-DA MKH 3586	3-[1-Methyl-1-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-ethyl]-5-oxo-4,5-dihydro-[1,2,4]triazole-1-carboxylic acid-tert-butylamide	

TABLE C.3.1. Identification of Compounds from the Confined Rotational Crop Study

Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
tBu-OH DA MKH 3586	4,5-dihydro-*N*-(2-hydroxy-1,1-dimethylethyl)-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide	
DA OH Glu II MKH 3586	3-(1-hydroxy-1-methyl-ethyl)-5-oxo-4-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yl)-4,5-dihydro-[1,2,4]triazole-1-carboxylic acid tert-butylamide *	
N-Me DA MKH 3586	N-(1,1-dimethylethyl)-4,5-dihydro-4-methyl-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazol-1-carboxamide *	

* Chemical name generated using naming software of ISIS/Draw.

D. CONCLUSION

TRR accumulated at 0.01-7.42 ppm in all rotated crops planted 47, 138, and 364 days following a single application of [^{14}C]amicarbazone to silt loam soil at 0.42 lb ai/A. Crops which had been planted 30 days following treatment failed due to phytotoxicity. In addition, only small amounts of sample could be collected at the 47-day plantback interval (PBI), due to amicarbazone phytotoxicity. TRR were generally highest in samples from the 47-day PBI and lower in those from the 138- and 364-day PBI. For the 47-day PBI, TRR were highest in turnip tops (7.42 ppm) and wheat hay and straw (2.42 and 4.18 ppm); residues in other commodities ranged 0.10-1.59 ppm. In crop matrices from the 138-day PBI, TRR were highest in wheat hay and straw (2.78 and 3.49 ppm); residues in other commodities ranged 0.01-0.72 ppm. In crop matrices from the 364-day PBI, TRR were again highest in wheat hay and straw (1.53 and 1.72 ppm); residues in other commodities ranged 0.02-0.74 ppm.

Total identified residues ranged 22-104% TRR in rotated crop commodities. Amicarbazone, DA MKH 3586 and iPr-2-OH DA MKH 3586 were the major residues in all rotational crops. Two

additional residues, glucose conjugates DA OH Glu I MKH 3586 and DA OH Glu II MKH 3586 were the major residues in wheat hay and straw only. Triazolinone MKH 3586, tBu-OH DA MKH 3586, N-Me DA MKH 3586, and a conjugate of triazolinone MKH 3586 were found in smaller amounts. The metabolite profiles in rotational crops from the 47-, 138- and 364-day PBIs were similar, however, in 364-DAT crops, amicarbazone was generally found at lower levels and iPr-2-OH DA MKH 3586 at higher levels than in the respective crop matrices from the other PBIs.

The petitioner did not propose a metabolic profile for rotational crops. The petitioner noted that the metabolites identified in rotational crop were similar to those found in the primary crop metabolism study (corn; see DER for MRID 45121633) and stated that the major metabolites were those in which the tertiary carbon of the isopropyl moiety had undergone deamination and oxidation. We note that two metabolites identified in rotational crops, triazolinone MKH 3586 and N-Me DA MKH 3586 were not found in the corn metabolism study.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: PP#0F6131.
DP Barcode: D288216
PC Code: 114004

Template Version September 2003

19

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.2.1, 7.2.2, and 7.2.3/OPPTS 860.1340/OECD IIA 4.2.5, 4.2.6 and 4.3
Residue Analytical Method - Livestock Commodities

Primary Evaluator	Manying Xue, Chemist RAB3/HED (7509C)	Date: 03/23/05
Approved by	Leung Cheng, Senior Chemist RAB3/HED (7509C)	Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 09/21/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

45121705 Haan, R. (2000) An Analytical Method for the Determination of MKH 3586 Residues in Animal Matrices: Lab Project Number: 108111: M6120201. Unpublished study prepared by Bayer Corp. 78 p.

45121706 Bauer, M. (2000) Independent Laboratory Validation for the Analytical Method for the Determination of MKH 3586 Residues in Animal Matrices: Lab Project Number: 109472: M6112301. Unpublished study prepared by Battelle. 67 p.

45121713 Haan, R. (1999) Extraction Efficiency of the Analytical Residue Method for the Determination of MKH 3586 Residues in Cattle Tissues: Lab Project Number: 109111: M6220201. Unpublished study prepared by Bayer Corp. 33 p.

EXECUTIVE SUMMARY:

Arvesta Corporation has proposed an LC/MS/MS method for the enforcement of tolerances for residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 in ruminant commodities. The method, entitled "An Analytical Method for the Determination of MKH 3586 Residues in Animal Matrices" (Bayer Report Number 108111), was used for the determination of residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in samples from the livestock feeding study associated with DP Barcode D288216.

The method includes instructions for the analysis of milk and tissue samples. Briefly, tissue samples are extracted using accelerated solvent extraction (ASE) with 0.05% phosphoric acid as the extraction solvent; milk samples are simply diluted with water. The extracts are oxidized using potassium permanganate to convert amicarbazone residues to a common moiety, iPr-2-OH DA MKH 3586. The oxidized extracts are then cleaned up by solid phase extraction (SPE) and dissolved in water/methanol for LC/MS/MS analysis. The validated limit of quantitation (LOQ) is 0.01 ppm for each analyte in all matrices. The reported limit of detection (LOD) was 0.005 ppm for each analyte. All results are reported as parent equivalents.

The petitioner has not proposed any separate confirmatory analytical procedures for the method, stating that confirmation of analyte identity is made using two criteria: (1) co-chromatography of the sample peak with the internal standard; and (2) detection of the two daughter ions of the target analyte (iPr-2-OH MKH 3586) at the same retention time.

Method validation data for the LC/MS/MS method demonstrated adequate method recoveries at the LOQ for residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 from milk and liver, and at the LOQ and 10x the LOQ for a mixture of amicarbazone, DA MKH 3586 and iPr-2-OH DA MKH 3586 from milk, liver, kidney, muscle, and fat. Overall recovery ranges (and CVs) from these matrices were 79-112% (11%) for amicarbazone, 97-116% (7%) for DA MKH 3586, 86-122% (15%) for iPr-2-OH DA MKH 3586, and 62-103% (12%) for the mixture of amicarbazone, DA MKH 3586 and iPr-2-OH DA MKH 3586. The fortification levels and samples used in method validation are adequate to bracket expected residue levels for milk, muscle, fat, and kidney but are not adequate for liver.

Adequate independent laboratory validation data have been submitted for this method using cattle liver samples. The submitted radiovalidation data are adequate to demonstrate that the method adequately extracts incurred residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 from goat liver.

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 proposed enforcement method will be forwarded to ACB for petition method validation. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D288216.

COMPLIANCE:

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

A. BACKGROUND INFORMATION

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis.

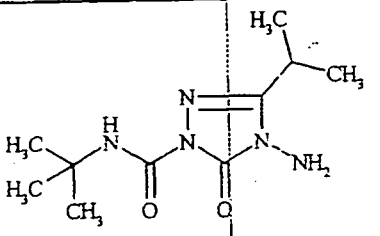
TABLE A.1. Amicarbazone Nomenclature.	
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-*tert*-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS registry number	129909-90-6
End-use formulation (EP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.		
Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-4} Pa @ 25°C 1.30×10^{-4} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, Log(K_{ow})	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

An LC/MS/MS method, entitled "An Analytical Method for the Determination of MKH 3586 Residues in Animal Matrices" (Bayer Report Number 108111), was used for the determination of

residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in samples from the livestock feeding study associated with DP Barcode D288216.

B.1.1. Principle of the Method:

The method includes instructions for the analysis of milk and tissue samples. Briefly, tissue samples are extracted using ASE (accelerated solvent extraction) with 0.05% phosphoric acid as the extraction solvent; milk samples are simply diluted with water. The extracts are oxidized using potassium permanganate to convert amicarbazone residues to a common moiety, iPr-2-OH DA MKH 3586. Following addition of the deuterated internal standard, the oxidized extracts are then cleaned up by SPE (solid phase extraction) and dissolved in water/methanol for LC/MS/MS analysis.

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

Method ID	Bayer Report Number 108111
Analytes	Amicarbazone and its metabolites that are oxidized to iPr-2-OH DA MKH 3586 [amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586]
Extraction solvent/technique	Tissue samples: Samples are homogenized in the presence of dry ice and/or liquid nitrogen. The homogenized sample is mixed with Celite and extracted using ASE, using 0.05% (or 0.01 M) phosphoric acid as the extraction solvent and conducting extraction at 150 °C and 1500 psi. The extract is diluted with water and then oxidized by mixing with potassium permanganate and heating at 70 °C for 1.5 hours. Sodium bisulfite (10%; to react with remaining permanganate), 3 M phosphoric acid, and the internal standard [iPr-2-OH DA MKH 3586-d_6] are added. Milk samples: The sample is mixed with water and then oxidized by mixing with potassium permangante and heating at 70 °C for 1.5 hours. Sodium bisulfite (10%), 3 M phosphoric acid, and the internal standard [iPr-2-OH DA MKH 3586-d_6] are added.
Cleanup strategies	Extracts are cleaned up by C-18 SPE, using 1% acetic acid in methanol to elute residues. The eluate is evaporated to dryness and redissolved in water:methanol (70:30, v:v) for LC/MS/MS analysis.
Instrument/Detector	HPLC utilizing a reverse-phase C-18 column and a gradient mobile phase of 2mM ammonium acetate and 0.1% acetic acid in methanol, with tandem mass spectrometry (MS/MS) detection using electrospray ionization operating in the positive ion mode. The daughter ion transitions monitored are: m/z 243 to m/z 144 for iPr-2-OH DA MKH 3586 m/z 243 to m/z 126 for iPr-2-OH DA MKH 3586 m/z 144 to m/z 126 for iPr-2-OH DA MKH 3586 daughter ion m/z 249 to m/z 150 for iPr-2-OH DA MKH 3586-d_6 The ions m/z 144 (analyte) and m/z 150 (internal standard) are used for quantitation.
Standardization method	External and internals standards are used. Internal standard solution is added to samples and standards such that the internal standard concentration is the same in each. Standards are injected before and after each sample (maximum of 10 sample injections between standards), and the concentration of analyte in the sample is calculated by comparing the relative response of analyte and internal standard with the average relative response in the standard solutions. The concentrations of standards of DA MKH 3586 (used for fortification) and iPr-2-OH DA MKH 3586 are calculated to be equivalent to amicarbazone on a molar basis. Therefore, all residue results are reported in parent equivalents. Linearity of the detector response is to be determined by injecting a blank and 7 standards; the relative response of analyte and internal standard is calculated for each standard and plotted against concentration. The method specifies that the correlation coefficient should be ≥0.99.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Amicarbazone Residues in livestock Matrices.	
Stability of std solutions	Standard solutions are to be stored refrigerated (4 ± 3 °C); standards are reportedly stable under these conditions for at least 6 months
Retention times	Approximately 11 minutes for iPr-2-OH DA MKH 3586.

B.2. Enforcement Method

The proposed enforcement method for livestock commodities is the same as the data-gathering method.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

TABLE C.1.1. Recovery Results from Method Validation of Cattle Matrices using the Data-Gathering Analytical Method.[1]

Cattle Matrix	Fortified Analyte	Spiking Level (ppm)	Recoveries Obtained [2] (%)	Mean Recovery ± SD [CV] (%)
Liver	Amicarbazone	0.010	79, 92, 98	90 ± 9.7 [10.8]
	DA MKH 3586	0.010	101, 103, 116	107 ± 8.1 [7.6]
	iPr-2-OH DA MKH 3586	0.010	91, 99, 120	103 ± 15.0 [14.5]
Milk	Amicarbazone	0.010	97, 99, 112	103 ± 8.1 [7.9]
	DA MKH 3586	0.010	97, 98, 111	102 ± 7.8 [7.7]
	iPr-2-OH DA MKH 3586	0.010	86, 95, 122	101 ± 18.7 [18.5]
Liver	Mixed standard of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586	0.010	62, 63, 78, 80, 84, 85, 93	78 ± 10.2 [13.1]
		0.100	70, 75, 86	
Kidney	Mixed standard of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586	0.010	63, 69, 74, 78, 79, 84, 93	78 ± 8.6 [11.0]
		0.100	75, 80, 86	
Muscle	Mixed standard of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586	0.010	64, 67, 70, 74, 78, 81	73 ± 5.7 [7.8]
		0.100	73, 74, 80	
Fat	Mixed standard of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586	0.010	62, 69, 72, 73, 74, 82	75 ± 7.7 [10.2]
		0.100	76, 83, 87	

TABLE C.1.1. Recovery Results from Method Validation of Cattle Matrices using the Data-Gathering Analytical Method.[1]				
Cattle Matrix	Fortified Analyte	Spiking Level (ppm)	Recoveries Obtained [2] (%)	Mean Recovery ± SD [CV] (%)
Milk	Mixed standard of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586	0.010	79, 80, 80, 81, 83, 83, 83, 86, 87, 87, 88, 90, 91, 95, 98	89 ± 7.9 [8.9]
		0.100	101, 101, 103	

[1] Standards were prepared in acetonitrile:water (approximately 1:10, v:v). HED notes that certain validation data from the 0.010-ppm fortification level (mixed standard) are from the livestock feeding study (refer to the DER for MRID 45121707).
[2] Recoveries were corrected for residues in the control samples.

The fortification levels and samples used in method validation are adequate to bracket expected residue levels for milk, muscle, fat, and kidney but are not adequate for liver. Method validation data be submitted for liver reflecting fortification levels up to 1.5 ppm to bracket the residue levels observed in the feeding study.

The petitioner has not proposed any separate confirmatory analytical procedures for this method, stating that confirmation of analyte identity is made using two criteria: (1) co-chromatography of the sample peak with the internal standard; and (2) detection of the two daughter ions (m/z 144 and m/z 126) at the same retention time. In addition, the petitioner referenced the interference study submitted with the plant commodity method (refer to the DER for MRID 45121708), which addressed the issue of interference from pesticides identified by the petitioner as registered for use on corn. We note that to fully demonstrate the lack of interference for the current method, the petitioner would have to investigate all pesticides with tolerances for livestock commodities. However, because of the specificity of MS/MS detection, and the confirmatory criteria detailed in the method, no interference study is needed to support this method.

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Amicarbazone Residues in Cattle Matrices.	
Analytes	Amicarbazone and its metabolites that are oxidized to iPr-2-OH DA MKH 3586 [amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586]
Equipment ID	Thermoseparation Products model 3500/3200 HPLC; Finnigan MAT TSQ 7000 MS; Alltech Altima 5 µm C-18 or Phenomenex Prodigy ODS3 5 µm column
Limit of quantitation (LOQ)	0.010 ppm The LOQ was defined as the lowest fortification level at which adequate recoveries were obtained.
Limit of detection (LOD)	0.005 ppm The LOD was defined as the lowest standard concentration readily detected on the linearity curve.

TABLE C.1.2.	Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Amicarbazone Residues in Cattle Matrices.
Accuracy/Precision	Percent recoveries and coefficients of variance (CVs) indicate acceptable accuracy/precision at the LOQ for residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 from milk and liver, and at the LOQ and 10xLOQ for a mixture of amicarbazone, DA MKH 3586 and iPr-2-OH DA MKH 3586 from milk, liver, kidney, muscle, and fat. Overall recovery ranges (and CVs) from these matrices were 79-112% (11%) for amicarbazone, 97-116% (7%) for DA MKH 3586, 86-122% (15%) for iPr-2-OH DA MKH 3586, and 62-103% (12%) for the mixture of amicarbazone, DA MKH 3586 and iPr-2-OH DA MKH 3586.
Reliability of the Method/ [ILV]	An independent laboratory method validation [ILV] of the proposed enforcement method was conducted to verify the reliability of the method for the determination of residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 in cattle liver. The values obtained are indicative that the method is reliable. See Section C.3 below.
Linearity	The method/detector response was linear (coefficient of determination, $r^2 = 0.9996$) within the range of 0.005-1.00 ppm in solvent blank.
Specificity	Peaks were well defined and symmetrical. There appeared to be no carryover to the following chromatograms.

The extraction solvents used in the proposed enforcement method are not similar to those used in the goat metabolism study (refer to the DER for MRID 45121630). In the goat metabolism study, the majority of the TRR (~85-99% TRR) was extracted from goat tissues using ACN (milk and fat) or 0.1% acetic acid in methanol and then acetone (liver, kidney, and muscle). This differs from the ASE extraction procedures of the proposed enforcement method, in which 0.05% phosphoric acid is used as the extraction solvent for tissues; milk samples are not subjected to extraction procedures.

Arvesta has submitted radiovalidation data for the proposed enforcement method (MRID 45121713) using samples of goat liver from the metabolism study. No radiovalidation data were generated for milk samples since milk samples are not subjected to extraction procedures in the proposed enforcement method. The liver sample, which had been stored frozen at -20 ± 5 °C since collection, was reextracted and analyzed according to the procedures of the metabolism study, as follows. A subsample of liver was extracted three times with methanol containing 0.1% acetic acid and then extracted once with acetone. The combined extracts were concentrated, diluted with methanol, evaporated to dryness, and redissolved in acetonitrile:water (1:1, v:v). The extract was applied to a C-18 SPE column which was eluted with ACN:water (1:1, v:v); the load and rinse fractions were combined, evaporated to dryness, and redissolved in methanol:water (3:7, v:v) containing 0.1% acetic acid for HPLC analysis.

[We note that this extraction procedure differs slightly from the procedure used in the metabolism study, in which the combined methanol and acetone liver extracts were concentrated, diluted with water, and applied to a strong anion exchange column which was eluted with water and methanol. The methanol eluate was evaporated to dryness and redissolved in methanol:water (1:9, v:v) containing 0.1% acetic acid for HPLC analysis.]

Samples of goat liver were also extracted according to the procedures of the proposed enforcement method, as described above in Table B.1.1.

Extracts were analyzed by HPLC using a system equipped with a radioactivity detector and a C-18 column, using a gradient mobile phase of water and methanol, each containing 0.1% acetic acid. Metabolites were identified by comparison of retention times with those of reference standards of [^{14}C]amicarbazone, [^{14}C]DA MKH 3586, and [^{14}C]iPr-2-OH DA MKH 3586. The results of the study are reported below in Table C.1.3.

TABLE C.1.3. Extraction Efficiency of the Enforcement Analytical Method Using Radiolabeled Samples from the Goat Metabolism Study.

Matrix	Original Metabolism Study Results (% TRR)	Radiovalidation Study Results (% TRR)			
		Extraction using procedures of metabolism study	Extraction using procedures of enforcement method [1]	Extraction efficiency (%), using current metabolism study results [2]	Extraction efficiency (%), using results from original metabolism study [3]
Liver					
Extracted residues	94	90	96	107	102
Amicarbazone	11	9	8	89	73
DA MKH 3586	60	58	49	84	82
iPr-2-OH DA MKH 3586	5	4	3	75	60
Total toxic residue	76	71	60	85	79

[1] Average of three replicate samples.
[2] Extraction efficiency = (ppm, extraction using procedures of enforcement method) ÷ (ppm, extraction using procedures of metabolism study) x 100.
[3] Extraction efficiency = (ppm, extraction using procedures of enforcement method) ÷ (ppm, original metabolism study results) x 100.

The submitted extraction efficiency data demonstrate that the extraction procedures of the proposed enforcement method adequately extract aged residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 from goat liver samples.

The petitioner stated that the goat liver sample that was used for the radiovalidation study had been stored frozen for 45 months prior to extraction; no actual dates of extraction/analysis were provided. Based on a comparison of the results of the original metabolism study with those of the radiovalidation study, residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 were relatively stable in goat liver during 45 months of frozen storage.

C.2. Enforcement Method

The proposed enforcement method for livestock commodities is the same as the data-gathering method.

C.3. Independent Laboratory Validation

An independent laboratory validation (ILV) of the proposed enforcement method was conducted by Battelle (Columbus, OH) using samples of cattle liver (MRID 45121706).

Samples of homogenized cattle liver (obtained by Battelle from the petitioner) were separately fortified with amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 at 0.01 ppm (LOQ) and 0.20 ppm. Fortified and unfortified (control) samples were analyzed using the proposed enforcement method as described in Table B.1.1. Cattle liver samples were chosen for the ILV study because this sample was considered to be a difficult matrix to analyze. It was noted that the method was modified slightly to use separate fortification standards for each analyte tested (instead of a mixed fortification standard).

The first ILV trial failed; high recoveries were observed for DA MKH 3586 at the 0.01ppm fortification level and peaks were observed in the control sample chromatograms at levels >20% of the LOQ. Based on consultation with the petitioner, the ILV laboratory modified the HPLC mobile phase gradient times (to lengthen the column rinse and re-equilibration times). The second ILV trial was successful. Recoveries of amicarbazone, DA MKH 3586, and iPr-2OH DA MKH 3586 from cattle liver samples are reported in Table C.3.1. Combined residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 were reportedly 0.0012 and 0.0015 ppm in/on two samples of unfortified cattle liver.

The laboratory reported that a set of 12 samples required one person approximately 16 hours over the course of 3 days, with both the extractions (in the ASE extractor) and the LC/MS/MS analyses run unattended overnight. No critical steps were identified by the ILV laboratory and no method modifications were recommended.

TABLE C.3.1: **Recovery Results Obtained by an Independent Laboratory Validation of the Enforcement Method for the Determination of Amicarbazone Residues in Animal Matrices.**

Matrix	Analyte	Spiking Level (ppm)	Recoveries Obtained [1]	Mean Recovery ± SD [CV]
Cattle liver	Amicarbazone	0.01	75, 89	76 ± 8.8 [11.5]
		0.20	70, 71	
	DA MKH 3586	0.01	81, 81	79 ± 2.1 [2.6]
		0.20	77, 78	
	iPr-2-OH DA MKH 3586	0.01	77, 90	82 ± 6.2 [7.6]
		0.20	77, 83	

[1] Recoveries were corrected for residues in control samples.

D. CONCLUSION

Adequate method validation data have been submitted for the proposed LC/MS/MS enforcement method for the determination of residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in ruminant commodities. The fortification levels and samples

used in method validation are adequate to bracket expected residue levels for milk, muscle, fat, and kidney but are not adequate for liver; additional validation data are needed for liver.

Adequate independent laboratory validation data have been submitted for this method using cattle liver samples. The submitted radiovalidation data are adequate to demonstrate that the method adequately extracts incurred residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 from goat liver. The proposed enforcement method will be forwarded to ACB for petition method validation.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: PP#0F6131
DP Barcode: D288216
PC Code: 114004

Template Version September 2003

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Field Corn

Primary Evaluator: Manying Xue, Chemist, RAB3/HED (7509C) — Date: 03/23/05

Approved by: Leung Cheng, Senior Chemist, RAB3/HED (7509C) — Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; updated draft submitted 09/21/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

45121710 Krolski, M. (2000) MKH 3586 70 WG--Magnitude of the Residues in Corn: Lab Project Number: 109558: M619CO01: M619CO02. Unpublished study prepared by Bayer Corp. 311 p.

EXECUTIVE SUMMARY:

Arvesta Corporation has submitted field trial data depicting the magnitude of the residue of amicarbazone in/on field corn forage, fodder (stover), and grain. The petitioner conducted a total of 23 field corn field trials in Regions 1 (NY and PA, 2 trials), 2 (GA; 1 trial), 5 (IA, IL, IN, KS, MI, MN, MO, NE, and OH; 19 trials), and 6 (TX; 1 trial), during the 1997 and 1998 growing seasons. The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 for field corn.

The 23 field corn trials each consisted of three plots treated according to different treatment regimens; a fourth plot was not treated to generate control samples. At the first plot, a single broadcast preemergence (PRE) application of the 70% DF formulation was made at ~0.45 lb ai/A on the day of planting field corn seed. At the second plot, a single broadcast preplant incorporated (PPI) application was made to the soil at ~0.45 lb ai/A on the day of planting; the formulated solution was incorporated into the soil to a depth of ~2 inches prior to planting seed. At the third plot, a single early postemergence (POST) broadcast application was made at ~0.25 lb ai/A, approximately 42 days after planting. Applications were made in 9.9-20.2 gal/A of water using ground equipment. Only limited information pertaining to weather conditions and soil characteristics was included in the submission.

Samples from all trials were collected at the following growth stages: forage was collected at the late dough to early dent stage, 74-118 days posttreatment from the PRE and PPI plots and 30-78 days posttreatment from the POST plot; and stover and grain were collected at crop maturity, 110-167 days posttreatment from the PRE and PPI plots and 67-125 days posttreatment from the POST plot. To evaluate residue decline, additional samples of field corn forage, stover, and

grain were collected at various posttreatment intervals from each treatment plot of two 1997 field trials.

Residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 were quantitated in/on field corn forage, stover, and grain using the proposed LC/MS/MS enforcement method with a validated limit of quantitation of 0.01 ppm for each analyte. This method is adequate for data collection based on acceptable concurrent method recovery data.

The maximum storage intervals of crop samples from harvest to analysis were 811-821 days (~27 months) for field corn forage, stover, and grain. In support of the field corn field trials, the petitioner cited storage stability data submitted in conjunction with a corn metabolism study which demonstrated that the extraction profile and metabolite profiles were generally stable in corn forage, stover, and grain for 8, 7, and 11 months, respectively; these data are insufficient to support the current field trial study. The petitioner additionally stated that reanalysis of the 1997 field trial samples after up to 26 months of frozen storage demonstrated similar residue levels; however, no data to support this statement were included in the submission. The petitioner should submit data demonstrating the stability of residues of amicarbazone, DA MKH 3586, and iPr-2OH DA MKH 3586 in/on field corn forage, stover, and grain during up to 27 months of frozen storage.

The maximum individual and combined residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on field corn matrices from the submitted field corn field trials are reported in the table below.

Commodity	PHI (days)	Maximum Residue Levels (ppm)			
		Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total
Preemergence application at 0.44-0.46 lb ai/A					
Forage	74-118	0.1197	0.0634	0.0387	0.211
Stover	110-167	0.0589	0.0423	0.0946	0.150
Grain	110-167	<0.010	<0.010	0.0122	<0.032
Preplant incorporated application at 0.44-0.46 lb ai/A					
Forage	74-118	0.0919	0.0575	0.0304	0.172
Stover	110-167	0.1332	0.0987	0.2001	0.432
Grain	110-167	<0.010	<0.010	0.0138	<0.034
Early postemergence application at 0.24-0.26 lb ai/A					
Forage	30-78	0.3956	0.1285	0.0343	0.557
Stover	67-125	0.1690	0.0615	0.0796	0.240
Grain	67-125	0.0250	<0.010	<0.010	<0.045

In two field corn field trials (in KS and IN), samples of forage, stover, and grain were collected at four intervals following the last application to evaluate residue decline. Residue decline data from the IN trial indicated that combined residues of amicarbazone and its metabolites DA MKH

3586 and iPr-2-OH DA MKH 3586 did not significantly increase or decrease with increasing PHI's. All residues were below the method LOQ at each sampling interval in/on all samples (except one sample of forage) from the KS decline trial; therefore, decline could not be evaluated.

The petitioner reported the dry matter content for all samples collected in these trials. Dry matter contents were 19-43% for forage, 29-71% for stover, and 63-95% for grain. The values for stover were all lower than the value reported in Table 1 of OPPTS 860.1000 (83%); the forage and grain ranges were closer to expected values (40% and 88%, respectively).

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

Under the conditions and parameters used in the study, the field corn field trial residue data are classified as scientifically acceptable. Pending data demonstrating the stability of residues of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 in/on field corn forage, stover, and grain during up to 27 months of frozen storage. In addition, information pertaining to the weather conditions (including temperatures) over the course of the study, with comparison to historical averages (for both temperature and rainfall), should be submitted. A discussion of any unusual weather conditions should be included. Because the proposed use pattern of amicarbazone on field corn is dependent on soil type, soil pH, and soil organic matter, information pertaining to these characteristics should be submitted for all field trials included in this submission.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D288216.

COMPLIANCE:

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

A. BACKGROUND INFORMATION

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis.

TABLE A.1. Amicarbazone Nomenclature.

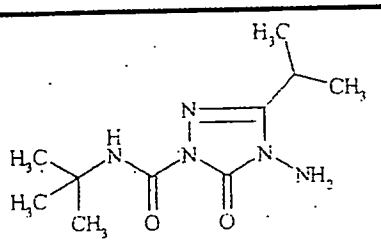
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-*tert*-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS #	129909-90-6
End-use formulation (EUP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.

Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L.	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-6} Pa @ 25°C 1.30×10^{-6} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, Log(K_{ow})	pKa = 17(log P_{ow} =1 23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

TABLE B.1.1 Trial Site Conditions.

Trial Identification: City, State; Year	Soil characteristics *			
	Type	%OM	pH	CEC
Oxford, IN; 1997	Silt loam	3.9	5.7	Not reported (NR)
Tifton, GA; 1997	Sandy loam	1.1	5.9	NR
Howe, IN; 1997	Not provided			
Springfield, NE; 1997	Not provided			
Stilwell, KS; 1997	Silt loam	2.4	6.8	NR
Germansville, PA; 1998	Soil characteristics were not provided for the 1998 trials			
North Rose, NY; 1998				
Springfield, NE; 1998				
Stilwell, KS; 1998				
Oxford, IN; 1998				
Dexter, MO; 1998				
Bagley, IA; 1998				
Richland, IA; 1998				
New Holland, OH; 1998				
Carlyle, IL; 1998				
Dow, IL; 1998				
Sheridan, IN; 1998				
Noblesville, IN; 1998				
Conklin, MI; 1998				
Campbell, MN; 1998				
York, NE; 1998				
Richland, IA; 1998				
Uvalde, TX; 1998				

* Soil characteristics data are required because the use pattern of amicarbazone is based on soil type, pH and organic matter [see OPPTS 860.1500(h)(3)(vi)(A)(10)].

The petitioner did not include any weather information except total rainfall/irrigation from first application to last sampling for each site. Historical weather data were not presented, and no discussion was provided concerning weather conditions during the conduct of the studies. This information should be submitted [see OPPTS 860.1500(h)(3)(vi)(A)(5)].

TABLE B.1.2. Study Use Pattern.

Location: City, State; Year	EP[a]	Application					Tank Mix Adjuvants
		Treat. No. and Crop Stage at Application[b]	Rate (lb ai/A)	RTI[c] (days)	Method (gal/A)	Total Rate (lb ai/A)	
Oxford, IN; 1997	70% DF	PRE; seed	0.45	NA	Broadcast (14.8)	0.45	None
		PPI; seed	0.45	NA	Broadcast (14.8)	0.45	None
		POST; 10 leaves	0.25	NA	Broadcast (14.8)	0.25	None
Tifton, GA; 1997	70% DF	PRE	0.45	NA	Broadcast (11.5)	0.45	None
		PPI	0.45	NA	Broadcast (11.5)	0.45	None
		POST; BBCH 39	0.25	NA	Broadcast (12.7)	0.25	None
Howe, IN; 1997 (decline study)	70% DF	PRE	0.45	NA	Broadcast (16.8)	0.45	None
		PPI	0.45	NA	Broadcast (16.8)	0.45	None
		POST	0.25	NA	Broadcast (16.8)	0.25	None
Springfield, NE; 1997	70% DF	PRE	0.46	NA	Broadcast (14.3)	0.46	None
		PPI	0.45	NA	Broadcast (14.1)	0.45	None
		POST	0.25	NA	Broadcast (14.3)	0.25	None
Stilwell, KS; 1997 (decline study)	70% DF	PRE	0.45	NA	Broadcast (10.2)	0.45	None
		PPI	0.45	NA	Broadcast (10.1)	0.45	None
		POST; 9 leaves	0.25	NA	Broadcast (10)	0.25	None
Germansville, PA; 1998	70% DF	PRE; dry seed	0.46	NA	Broadcast (19.1)	0.46	None
		PPI	0.46	NA	Broadcast (19.3)	0.46	None
		POST; 6 leaves	0.25	NA	Broadcast (18.9)	0.25	None
North Rose, NY; 1998	70% DF	PRE; dry seed	0.45	NA	Broadcast (18)	0.45	None
		PPI, dry seed	0.46	NA	Broadcast (18.5)	0.46	None
		POST; ≥9 leaves, unfolded	0.25	NA	Broadcast (18.3)	0.25	None
Springfield, NE; 1998	70% DF	PRE; dry seed	0.46	NA	Broadcast (17.6)	0.46	None
		PPI; dry seed	0.46	NA	Broadcast (19)	0.46	None
		POST; 7 leaves, unfolded	0.25	NA	Broadcast (18.5)	0.25	None
Stilwell, KS; 1998	70% DF	PRE; dry seed	0.45	NA	Broadcast (10)	0.45	None
		PPI; dry seed	0.45	NA	Broadcast (10)	0.45	None
		POST; 6 leaves, unfolded	0.25	NA	Broadcast (9.9)	0.25	None
Oxford, IN; 1998	70% DF	PRE; dry seed	0.45	NA	Broadcast (13.0)	0.45	None
		PPI; dry seed	0.45	NA	Broadcast (13.1)	0.45	None
		POST; 6 nodes detectable	0.24	NA	Broadcast (16.2)	0.24	None
Dexter, MO; 1998	70% DF	PRE; dry seed	0.45	NA	Broadcast (17.5)	0.45	None
		PPI; dry seed	0.45	NA	Broadcast (17.5)	0.45	None
		POST; 7 nodes detectable	0.25	NA	Broadcast (12.7)	0.25	None
Bagley, IA; 1998	70% DF	PRE; dry seed	0.44	NA	Broadcast (18.6)	0.44	None
		PPI; dry seed	0.44	NA	Broadcast (18.6)	0.44	None
		POST; 6 nodes detectable	0.25	NA	Broadcast (20.2)	0.25	None

TABLE B.1.2 Study Use Pattern.

Location: City, State; Year	EP[a]	Application					Tank Mix Adjuvants
		Treat. No. and Crop Stage at Application[b]	Rate (lb ai/A)	RTI[c] (days)	Method (gal/A)	Total Rate (lb ai/A)	
Richland, IA; 1998	70% DF	PRE; dry seed	0.45	NA	Broadcast (16.9)	0.45	None
		PPI; dry seed	0.45	NA	Broadcast (16.6)	0.45	None
		POST; 8 leaves, unfolded	0.24	NA	Broadcast (15.8)	0.24	None
New Holland, OH; 1998	70% DF	PRE	0.44	NA	Broadcast (17.5)	0.44	None
		PPI	0.44	NA	Broadcast (17.3)	0.44	None
		POST; 5 leaves, unfolded	0.25	NA	Broadcast (19.8)	0.25	None
Carlyle, IL; 1998	70% DF	PRE; dry seed	0.46	NA	Broadcast (15.3)	0.46	None
		PPI	0.45	NA	Broadcast (15.2)	0.45	None
		POST; ≥9 leaves, unfolded	0.25	NA	Broadcast (17.3)	0.25	None
Dow, IL; 1998	70% DF	PRE; dry seed	0.44	NA	Broadcast (18)	0.44	None
		PPI; dry seed	0.45	NA	Broadcast (18.2)	0.45	None
		POST; ≥9 leaves, unfolded	0.25	NA	Broadcast (17.9)	0.25	None
Sheridan, IN; 1998	70% DF	PRE; dry seed	0.45	NA	Broadcast (18.1)	0.45	None
		PPI; dry seed	0.45	NA	Broadcast (17.7)	0.45	None
		POST; 6 leaves, unfolded	0.26	NA	Broadcast (18.7)	0.26	None
Noblesville, IN; 1998	70% DF	PRE; dry seed	0.45	NA	Broadcast (18.7)	0.45	None
		PPI	0.45	NA	Broadcast (18.8)	0.45	None
		POST; ≥9 leaves, unfolded	0.26	NA	Broadcast (18.5)	0.26	None
Conklin, MI; 1998	70% DF	PRE; dry seed	0.45	NA	Broadcast (17.9)	0.45	None
		PPI	0.46	NA	Broadcast (18.1)	0.46	None
		POST; 8 leaves, unfolded	0.25	NA	Broadcast (16.5)	0.25	None
Campbell, MN; 1998	70% DF	PRE; dry seed	0.45	NA	Broadcast (17.9)	0.45	None
		PPI	0.45	NA	Broadcast (18)	0.45	None
		POST; 7 leaves, unfolded	0.25	NA	Broadcast (15.2)	0.25	None
York, NE; 1998	70% DF	PRE; dry seed	0.45	NA	Broadcast (15.1)	0.45	None
		PPI; dry seed	0.45	NA	Broadcast (15)	0.45	None
		POST; 6 leaves, unfolded	0.25	NA	Broadcast (15)	0.25	None
Richland, IA; 1998	70% DF	PRE; dry seed	0.46	NA	Broadcast (16.4)	0.46	None
		PPI; dry seed	0.45	NA	Broadcast (16.1)	0.45	None
		POST; ≥9 leaves, unfolded	0.25	NA	Broadcast (16.2)	0.25	None

TABLE B.1.2. Study Use Pattern.

Location: City, State; Year	EP[a]	Application					Tank Mix Adjuvants
		Treat. No. and Crop Stage at Application[b]	Rate (lb ai/A)	RTI[c] (days)	Method (gal/A)	Total Rate (lb ai/A)	
Uvalde, TX; 1998	70% DF	PRE; dry seed	0.46	NA	Broadcast (15.1)	0.46	None
		PPI	0.45	NA	Broadcast (14.9)	0.45	None
		POST; 8 nodes detectable	0.25	NA	Broadcast (14.7)	0.25	None

[a] EP = End-use Product; 70% dry flowable (DF).
[b] Three separate plots were treated at each trial site; one plot received a single preemergence (PRE) application, one plot received a single preplant incorporated (PPI) application, and one plot received a single early postemergence (POST) application. Preemergence and preplant incorporated applications were made on the day of planting at all sites except one (Springfield, NE, 1997) in which applications were made three days prior to planting.
[c] RTI = Retreatment Interval not applicable (NA) because only one application was made at each test plot.

TABLE B.1.3. Trial Numbers and Geographical Locations.

NAFTA Growing Region	Field corn		
	Submitted	Requested	
		Canada	US
1	2		1
2	1		1
5	19		17
6	1		1
Total	23		20

B.2. Sample Handling and Preparation

Single samples were collected from the PRE and PPI plots, and duplicate samples were collected from the POST plot. Samples were frozen at the field within 3 hours of sampling. Frozen samples were shipped within 62 days of collection to the analytical laboratory, where samples were stored at <-15 °C until analysis.

B.3. Analytical Methodology

Samples of field corn forage, stover, and grain were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 by Bayer Corporation (Stilwell, KS) using the LC/MS/MS method proposed as the enforcement method for plant commodities. Only a brief description of the method was included in the submission. For a complete description of the method, refer to the DER for MRID 45121708.

Briefly, homogenized samples of field corn forage, stover, and grain were extracted using an accelerated solvent extractor. The extract, following centrifugation, was cleaned up by solid phase extraction. Residues were quantitated by LC/MS/MS, using deuterated internal reference standards of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586. Residues of metabolites were reported as parent equivalents. The validated LOQ was 0.01 ppm

each for amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on all field corn matrices. The limits of detection (LODs) were calculated to be 0.001, 0.006, and 0.001 ppm for each analyte in/on field corn forage, stover, and grain, respectively (three times the standard deviation of the average control response).

C. RESULTS AND DISCUSSION

The petitioner conducted a total of 23 field corn field trials in Regions 1 (NY and PA, 2 trials), 2 (GA; 1 trial), 5 (IA, IL, IN, KS, MI, MN, MO, NE, and OH; 19 trials), and 6 (TX; 1 trial), during the 1997 and 1998 growing seasons. The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 for field corn. The 23 field corn field trials each consisted of three plots treated according to different treatment regimens; a fourth plot was not treated to generate control samples. At the first plot, a single broadcast preemergence (PRE) application of the 70% DF formulation was made at ~0.45 lb ai/A on the day of planting field corn seed. At the second plot, a single broadcast preplant incorporated (PPI) application was made to the soil at ~0.45 lb ai/A on the day of planting; the formulated solution was incorporated into the soil to a depth of ~2 inches prior to planting. At the third plot, a single early postemergence (POST) broadcast application was made at ~0.25 lb ai/A, approximately 42 days after planting. Applications were made in 9.9-20.2 gal/A of water using ground equipment.

Samples from all trials were collected at the following growth stages: forage was collected at the late dough to early dent stage, 74-118 days posttreatment from the PRE and PPI plots and 30-78 days posttreatment from the POST plot; and stover and grain were collected at crop maturity, 110-167 days posttreatment from the PRE and PPI plots and 67-125 days posttreatment from the POST plot. To evaluate residue decline, additional samples of field corn forage, stover, and grain were collected at various posttreatment intervals from each treatment plot of two 1997 field trials (Howe, IN and Stilwell, KS).

Residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on field corn forage, stover, and grain were quantitated using the proposed LC/MS/MS enforcement method. Method validation and concurrent method recovery data are presented in Table C.1. The study submission did not distinguish between recovery data generated for method validation and concurrent validation data. The validated limit of quantitation (LOQ) was 0.01 ppm for each analyte. This method is adequate for data collection based on acceptable concurrent method recovery data.

Residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on field corn commodities are presented in Table C.3; a summary of residue data in field corn commodities is presented in Table C.4. Following a single preemergence application to field corn at 0.44-0.46 lb ai/A, combined residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 were <0.030-0.211 ppm in/on forage samples, <0.030-0.150 ppm in/on stover samples, and <0.030-<0.032 ppm in/on grain samples.

Following a single preplant incorporated application to field corn at 0.44-0.46 lb ai/A, combined residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 were <0.030-0.172 ppm in/on forage samples, <0.030-0.432 ppm in/on stover samples, and <0.030-<0.034 ppm in/on grain samples.

Following a single early postemergence application to field corn at 0.24-0.26 lb ai/A, combined residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 were <0.030-0.557 ppm in/on forage samples, <0.030-0.240 ppm in/on stover samples, and <0.030-<0.045 ppm in/on grain samples.

Apparent residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 were each less than the method LOQ (<0.01 ppm) in/on 24 samples each of untreated field corn forage, stover, and grain.

In two field corn field trials (KS and IN), samples of forage, stover, and grain were collected at four intervals following the last application to evaluate residue decline. Residue decline data from the IN trial indicated that combined residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 did not significantly increase or decrease with increasing PHI's. All residues were below the method LOQ at each sampling interval in/on all samples (except one sample of forage) from the KS decline trial; therefore, decline could not be evaluated.

The petitioner reported the dry matter content for all samples collected in these trials. Dry matter contents were 19-43% for forage, 29-71% for stover, and 63-95% for grain. The values for stover are all lower than the value reported in Table 1 of OPPTS 860.1000 (83%); the forage and grain ranges are closer to expected values (40% and 88%, respectively).

Sample storage conditions and intervals are summarized in Table C.2. The maximum storage intervals of crop samples from harvest to analysis were 811-821 days (~27 months) for field corn forage, stover, and grain. In support of the field corn field trials, the petitioner cited storage stability data (refer to the DER for MRID 45121633) submitted in conjunction with a corn metabolism study which demonstrated that the extraction profile and metabolite profiles were generally stable in corn forage, stover, and grain for 8, 7, and 11 months, respectively. These data are insufficient to support the current field trial study because only very limited quantitative data were included in MRID 45121633 and because they do not reflect the storage intervals of the field trial samples. The petitioner also stated that reanalysis of the 1997 field trial samples after up to 26 months of frozen storage demonstrated similar residue levels; however, no data to support this statement were included in the submission. The petitioner submit data demonstrating the stability of residues of amicarbazone, DA MKH 3586, and iPr-2OH DA MKH 3586 in/on field corn forage, stover, and grain during up to 27 months of frozen storage.

TABLE C.1. Summary of Concurrent Recoveries of Amicarbazone and Its Metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 from Field Corn Matrices.

Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev (%)
Field corn forage	Amicarbazone	0.010	14	73, 74, 82, 82, 84, 85, 86, 87, 88, 88, 90, 91, 94, 98	84 ± 7
		0.050	3	80, 84, 85	
		0.600	3	71, 73, 75	
	DA MKH 3586	0.010	14	80, 85, 88, 90, 90, 91, 92, 92, 95, 99, 100, 102, 107, 111	92 ± 9
		0.050	3	91, 94, 94	
		0.600	3	80, 80, 84	
	iPr-2-OH DA MKH 3586	0.010	14	79, 83, 83, 85, 87, 89, 89, 91, 91, 96, 94, 98, 100, 119	90 ± 9
		0.050	3	85, 94, 95	
		0.600	3	79, 82, 83	
Field corn stover	Amicarbazone	0.010	12	70, 71, 73, 75, 78, 78, 79, 82, 84, 88, 96, 106	80 ± 10
		0.050	3	82, 83, 86	
		0.500	3	70, 70, 71	
	DA MKH 3586	0.010	12	77, 87, 90, 90, 91, 91, 92, 93, 93, 94, 98, 115	91 ± 8
		0.050	3	92, 94, 95	
		0.500	3	79, 82, 84	
	iPr-2-OH DA MKH 3586	0.010	12	68, 76, 78, 80, 84, 85, 85, 85, 89, 90, 95, 102	85 ± 8
		0.050	3	85, 90, 96	
		0.500	3	78, 81, 86	
Field corn grain	Amicarbazone	0.010	16	70, 70, 71, 72, 76, 76, 76, 85, 85, 87, 89, 90, 91, 91, 92, 95	82 ± 9
		0.050	6	70, 74, 83, 85, 90, 93	
	DA MKH 3586	0.010	16	71, 74, 76, 76, 77, 79, 79, 82, 84, 86, 86, 88, 93, 96, 98, 100	85 ± 9
		0.050	6	72, 79, 87, 89, 93, 94	
	iPr-2-OH DA MKH 3586	0.010	16	69, 70, 78, 80, 80, 87, 87, 88, 90, 93, 95, 97, 105, 105, 110, 105	89 ± 11
		0.050	6	77, 78, 91, 91, 95, 97	

TABLE C.2. Summary of Storage Conditions.

Matrix	Storage Temp. (°C)	Actual Storage Duration from Harvest to Analysis *	Interval of Demonstrated Storage Stability
Field corn forage	<-15	364-811 days (12.0-26.6 months)	None available
Field corn stover		336-818 days (11.0-26.9 months)	None available
Field corn grain		336-821 days (11.0-27.0 months)	None available

* Samples were analyzed within 29 days of extraction.

TABLE C.3. Residue Data from Field Corn Field Trials with Amicarbazone.

Trial ID: City, State; Year	Region	Field Corn Variety	Matrix	Treatment[a]	Total Rate (lb ai/A)	PHI (days)	Residues (ppm)[b]			
							Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total
Field corn forage										
Oxford, IN; 1997	5	S 696 LT	Forage	PRE	0.45	109	*0.0051*	*0.0044*	*0.0043*	<0.030
			Forage	PPI	0.45	109	*0.0045*	*0.0050*	*0.0064*	<0.030
			Forage	POST	0.25	66	*0.0058, 0.0080*	*0.0020, 0.0018*	*0.0017, 0.0014*	<0.030, <0.030
Tifton, GA; 1997	2	DPL 5750	Forage	PRE	0.45	91	0.0289	0.0178	0.0236	0.070
			Forage	PPI	0.45	91	0.0404	0.0226	0.0303	0.093
			Forage	POST	0.25	48	0.0476, 0.0567	0.0170, 0.0181	0.0168, 0.0172	0.081, 0.092
Howe, IN; 1997 (decline study)	5	GL 283	Forage	PRE	0.45	91	0.0188	0.0372	0.0480	0.104
			Forage	PRE	0.45	96	0.0214	0.0322	0.0387	0.092
			Forage	PRE	0.45	101	0.0173	0.0252	0.0282	0.071
			Forage	PRE	0.45	105	0.0227	0.0442	0.0334	0.100
			Forage	PPI	0.45	91	0.0290	0.0285	0.0327	0.090
			Forage	PPI	0.45	96	0.0341	0.0256	0.0304	0.090
			Forage	PPI	0.45	101	0.0265	0.0229	0.0250	0.074
			Forage	PPI	0.45	105	0.0277	0.0295	0.0263	0.084
			Forage	POST	0.25	50	0.0334, 0.0361	0.0114, 0.0113	*0.0095, 0.0098*	<0.055, <0.057
			Forage	POST	0.25	55	0.0275, 0.0338	0.0100, 0.0102	*0.0084, 0.0088*	<0.048, <0.054
			Forage	POST	0.25	60	0.0294, 0.0315	0.0112, 0.0114	0.0110, *0.0094*	0.052, <0.053
			Forage	POST	0.25	64	0.0272, 0.0345	0.0111, 0.0162	*0.0096*, 0.0113	<0.048, 0.062
Springfield, NE; 1997	5	DeKalb 574GR	Forage	PRE	0.46	99	*0.0098*	*0.0067*	*0.0098*	<0.030
			Forage	PPI	0.45	99	0.0221	*0.0061*	0.0104	<0.043
			Forage	POST	0.25	57	0.0333, 0.0466	*0.0074, 0.0076*	*0.0095, 0.0099*	<0.053, <0.067

TABLE C.3. Residue Data from Field Corn Field Trials with Amicarbazone.

Trial ID: City, State; Year	Region	Field Corn Variety	Matrix	Treatment[a]	Total Rate (lb ai/A)	PHI (days)	Residues (ppm)[b]			
							Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total
Stilwell, KS; 1997 (decline study)	5	Pioneer 3349	Forage	PRE	0.45	93	*0.0022*	*0.0031*	*0.0032*	<0.030
			Forage	PRE	0.45	98	*0.0014*	*0.0016*	*0.0022*	<0.030
			Forage	PRE	0.45	104	*0.0013*	*0.0023*	*0.0016*	<0.030
			Forage	PRE	0.45	107	*0.0017*	*0.0024*	*0.0017*	<0.030
			Forage	PPI	0.45	93	*0.0056*	*0.0027*	*0.0023*	<0.030
			Forage	PPI	0.45	98	*0.0038*	*0.0019*	*0.0017*	<0.030
			Forage	PPI	0.45	104	*0.0029*	*0.0019*	*0.0013*	<0.030
			Forage	PPI	0.45	107	*0.0019*	*0.0021*	ND	<0.030
			Forage	POST	0.25	49	*0.0082, 0.0083*	*0.0022, 0.0018*	*0.0012, 0.0014*	<0.030, <0.030
			Forage	POST	0.25	54	*0.0068*, 0.0127	*0.0016, 0.0039*	*0.0011, 0.0021*	<0.030, <0.033
			Forage	POST	0.25	60	*0.0048, 0.0052*	*0.0023, 0.0027*	ND, *0.0015*	<0.030, <0.030
			Forage	POST	0.25	63	*0.0035, 0.0036*	*0.0025, 0.0024*	*0.0014*, ND	<0.030, <0.030
Germansville, PA; 1998	1	Mycogen 2598	Forage	PRE	0.46	118	*0.0032*	*0.0035*	*0.0074*	<0.030
			Forage	PPI	0.46	118	*0.0063*	*0.0064*	*0.0098*	<0.030
			Forage	POST	0.25	78	*0.0070*, 0.0190	*0.0020, 0.0054*	*0.0018, 0.0049*	<0.030, <0.039
North Rose, NY; 1998	1	Agway 257	Forage	PRE	0.45	105	0.0158	*0.0069*	0.0106	<0.036
			Forage	PPI	0.46	105	0.0397	0.0141	0.0169	0.071
			Forage	POST	0.25	63	0.0518, 0.0506	0.0112, 0.0118	0.0114, 0.0134	0.074, 0.076
Springfield, NE; 1998	5	Dekalb 580 RR	Forage	PRE	0.46	88	0.0164	*0.0074*	*0.0079*	<0.036
			Forage	PPI	0.46	88	0.0122	*0.0069*	*0.0065*	<0.032
			Forage	POST	0.25	45	0.0314, 0.0393	*0.0086, 0.0091*	*0.0078, 0.0078*	<0.051, <0.059
Stilwell, KS; 1998	5	NK N7590	Forage	PRE	0.45	79	*0.0057*	*0.0058*	*0.0048*	<0.030
			Forage	PPI	0.45	79	*0.0068*	*0.0093*	*0.0082*	<0.030
			Forage	POST	0.25	30	0.0104, 0.0184	*0.0036, 0.0064*	*0.0025, 0.0049*	<0.030, <0.038

TABLE C.3. Residue Data from Field Corn Field Trials with Amicarbazone.

Trial ID: City, State; Year	Region	Field Corn Variety	Matrix	Treatment[a]	Total Rate (lb ai/A)	PHI (days)	Residues (ppm)[b]			
							Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total
Oxford, IN; 1998	5	Dekalb 566 RR	Forage	PRE	0.45	96	0.0171	*0.0047*	*0.0081*	<0.037
			Forage	PPI	0.45	96	0.0108	*0.0020*	*0.0026*	<0.031
			Forage	POST	0.24	48	0.0542, 0.0668	*0.0087, 0.0099*	*0.0094, 0.0088*	<0.074, <0.087
Dexter, MO; 1998	5	Pioneer 3394	Forage	PRE	0.45	74	0.0107	0.0268	0.0310	0.069
			Forage	PPI	0.45	74	*0.0078*	0.0295	0.0267	<0.066
			Forage	POST	0.25	33	0.0124, 0.0176	*0.0031, 0.0049*	*0.0022, 0.0040*	<0.032, <0.038
Bagley, IA; 1998	5	Dekalb 561 SR	Forage	PRE	0.44	116	*0.0021*	*0.0028*	*0.0051*	<0.030
			Forage	PPI	0.44	116	*0.0019*	*0.0029*	*0.0052*	<0.030
			Forage	POST	0.25	74	ND, ND	ND, ND	ND, ND	<0.030, <0.030
Richland, IA; 1998	5	NK 7070 BT	Forage	PRE	0.45	109	*0.0019*	*0.0017*	*0.0052*	<0.030
			Forage	PPI	0.45	109	*0.0016*	*0.0018*	*0.0057*	<0.030
			Forage	POST	0.24	69	*0.0046, 0.0051*	*0.0029, 0.0028*	*0.0080, 0.0049*	<0.030, <0.030
New Holland, OH; 1998	5	SC 1127 LL	Forage	PRE	0.44	117	*0.0034*	0.0127	0.0208	<0.044
			Forage	PPI	0.44	117	*0.0037*	*0.0088*	0.0129	<0.033
			Forage	POST	0.25	77	0.0120, 0.0120	0.0142, 0.0203	0.0137, 0.0124	0.040, 0.045
Carlyle, IL; 1998	5	Pioneer 33V08	Forage	PRE	0.46	90	*0.0060*	0.0189	0.0106	<0.040
			Forage	PPI	0.45	90	0.0104	0.0194	0.0183	0.048
			Forage	POST	0.25	48	0.0533, 0.0627	0.0115, 0.0130	0.0148, 0.0146	0.080, 0.090
Dow, IL; 1998	5	Garst 8541 IT	Forage	PRE	0.44	84	0.0161	0.0148	*0.0089*	<0.041
			Forage	PPI	0.45	84	0.0222	0.0158	0.0127	0.051
			Forage	POST	0.25	42	0.1007, 0.1425	0.0239, 0.0274	0.0168, 0.0163	0.141, 0.186
Sheridan, IN; 1998	5	Pioneer 3394	Forage	PRE	0.45	106	*0.0022*	*0.0028*	*0.0050*	<0.030
			Forage	PPI	0.45	106	*0.0037*	*0.0064*	0.0100	<0.030
			Forage	POST	0.26	62	0.0173, 0.0182	*0.0069, 0.0082*	*0.0073*, 0.0105	<0.037, <0.039

TABLE C.3. Residue Data from Field Corn Field Trials with Amicarbazone.

Trial ID: City, State; Year	Region	Field Corn Variety	Matrix	Treatment[a]	Total Rate (lb ai/A)	PHI (days)	Residues (ppm)[b]			
							Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total
Noblesville, IN; 1998	5	Dekalb 545 BTY	Forage	PRE	0.45	106	0.0271	0.0212	0.0327	0.081
			Forage	PPI	0.45	106	0.0175	0.0179	0.0219	0.057
			Forage	POST	0.26	62	0.0756, 0.1069	0.0205, 0.0247	0.0138, 0.0186	0.110, 0.150
Conklin, MI; 1998	5	Pioneer 3861	Forage	PRE	0.45	101	0.0116	*0.0085*	*0.0073*	<0.032
			Forage	PPI	0.46	101	0.0314	0.0110	*0.0091*	<0.052
			Forage	POST	0.25	58	0.0431, 0.0473	*0.0073, 0.0089*	*0.0041, 0.0050*	<0.063, <0.067
Campbell, MN; 1998	5	N 2555 BT	Forage	PRE	0.45	99	0.0218	*0.0036*	*0.0026*	<0.042
			Forage	PPI	0.45	99	0.0307	*0.0039*	*0.0021*	<0.051
			Forage	POST	0.25	56	0.0472, 0.0658	*0.0030, 0.0042*	*0.0015, 0.0021*	<0.067, <0.086
York, NE; 1998	5	Pioneer 34R06	Forage	PRE	0.45	109	0.0165	*0.0054*	*0.0087*	<0.037
			Forage	PPI	0.45	109	0.0162	*0.0084*	0.0117	<0.038
			Forage	POST	0.25	69	0.0172, 0.0188	*0.0055, 0.0060*	*0.0063, 0.0087*	<0.037, <0.039
Richland, IA; 1998	5	Pioneer 3335	Forage	PRE	0.46	109	*0.0044*	*0.0027*	*0.0023*	<0.030
			Forage	PPI	0.45	109	*0.0048*	*0.0016*	ND	<0.030
			Forage	POST	0.25	67	ND, *0.0034*	ND, ND	0.0109, 0.0124	<0.031, <0.032
Uvalde, TX; 1998	6	Pioneer 3245	Forage	PRE	0.46	84	0.1197	0.0634	0.0278	0.211
			Forage	PPI	0.45	84	0.0919	0.0575	0.0226	0.172
			Forage	POST	0.25	40	0.3956, 0.3942	0.1218, 0.1285	0.0322, 0.0343	0.550, 0.557
Field corn stover										
Oxford, IN; 1997	5	S 696 LT	Stover	PRE	0.45	165	ND	ND	ND	<0.030
			Stover	PPI	0.45	165	ND	ND	ND	<0.030
			Stover	POST	0.25	122	*0.0064*, ND	ND, 0.0110	ND, ND	<0.030, <0.031

TABLE C.3. Residue Data from Field Corn Field Trials with Amicarbazone.

Trial ID: City, State; Year	Region	Field Corn Variety	Matrix	Treatment[a]	Total Rate (lb ai/A)	PHI (days)	Residues (ppm)[b]			
							Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total
Tifton, GA; 1997	2	DPL 5750	Stover	PRE	0.45	110	0.0337	0.0278	0.0825	0.144
			Stover	PPI	0.45	110	0.1332	0.0987	0.2001	0.432
			Stover	POST	0.25	67	0.0839, 0.1002	0.0482, 0.0615	0.0796, 0.0784	0.212, 0.240
Howe, IN; 1997 (decline study)	5	GL 283	Stover	PRE	0.45	145	*0.0078*	0.0408	0.0312	<0.082
			Stover	PRE	0.45	152	*0.0078*	0.0438	0.0342	<0.088
			Stover	PRE	0.45	159	*0.0066*	0.0296	0.0188	<0.058
			Stover	PRE	0.45	166	*0.0074*	0.0328	0.0206	<0.063
			Stover	PPI	0.45	145	0.0143	0.0358	0.0324	0.083
			Stover	PPI	0.45	152	0.0101	0.0292	0.0299	0.069
			Stover	PPI	0.45	159	*0.0093*	0.0274	0.0176	<0.055
			Stover	PPI	0.45	166	*0.0080*	0.0196	0.0129	<0.043
			Stover	POST	0.25	104	*0.0080*, 0.0176	*0.0094*, 0.0163	0.0124, 0.0134	<0.032, 0.047
			Stover	POST	0.25	111	0.0160, 0.0167	0.0141, 0.0161	0.0132, 0.0125	0.043, 0.045
			Stover	POST	0.25	118	0.0138, 0.0164	0.0124, 0.0136	*0.0094*, *0.0077*	<0.036, <0.040
			Stover	POST	0.25	125	0.0204, 0.0224	0.0151, 0.0161	*0.0076*, 0.0108	<0.046, 0.049
Springfield, NE; 1997	5	DeKalb 574GR	Stover	PRE	0.46	167	*0.0065*	0.0114	0.0106	<0.032
			Stover	PPI	0.45	167	ND	*0.0089*	*0.0073*	<0.030
			Stover	POST	0.25	125	0.0102, 0.0163	*0.0076*, 0.0142	*0.0073*, *0.0098*	<0.030, <0.041

TABLE C.3. Residue Data from Field Corn Field Trials with Amicarbazone.

Trial ID: City, State; Year	Region	Field Corn Variety	Matrix	Treatment[a]	Total Rate (lb ai/A)	PHI (days)	Residues (ppm)[b]			
							Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total
Stilwell, KS; 1997 (decline study)	5	Pioneer 3349	Stover	PRE	0.45	148	ND	ND	ND	<0.030
			Stover	PRE	0.45	156	ND	*0.0071*	ND	<0.030
			Stover	PRE	0.45	162	ND	ND	ND	<0.030
			Stover	PRE	0.45	169	ND	ND	ND	<0.030
			Stover	PPI	0.45	148	ND	ND	ND	<0.030
			Stover	PPI	0.45	156	ND	ND	ND	<0.030
			Stover	PPI	0.45	162	*0.0069*	ND	ND	<0.030
			Stover	PPI	0.45	169	ND	ND	ND	<0.030
			Stover	POST	0.25	104	ND, *0.0092*	ND, ND	ND, ND	<0.030, <0.030
			Stover	POST	0.25	112	ND, *0.0083*	ND, ND	ND, ND	<0.030, <0.030
			Stover	POST	0.25	118	ND, 0.0104	ND, ND	ND, ND	<0.030, <0.030
			Stover	POST	0.25	125	*0.0061, 0.0078*	ND, *0.0065*	ND, ND	<0.030, <0.030
Germansville, PA; 1998	1	Mycogen 2598	Stover	PRE	0.46	154	ND	ND	ND	<0.030
			Stover	PPI	0.46	154	ND	*0.0061*	ND	<0.030
			Stover	POST	0.25	114	0.0101, 0.0117	ND, ND	ND, ND	<0.030, <0.032
North Rose, NY; 1998	1	Agway 257	Stover	PRE	0.45	133	0.0335	0.0123	0.0170	0.063
			Stover	PPI	0.46	133	0.0325	*0.0098*	*0.0069*	<0.053
			Stover	POST	0.25	91	0.0426, 0.0602	0.0104, 0.0126	*0.0061, 0.0071*	0.063, <0.083
Springfield, NE; 1998	5	Dekalb 580 RR	Stover	PRE	0.46	135	0.0110	0.0137	0.0270	0.052
			Stover	PPI	0.46	135	0.0123	0.0159	0.0446	0.073
			Stover	POST	0.25	92	0.0199, 0.0203	0.0128, 0.0143	0.0264, 0.0278	0.059, 0.062
Stilwell, KS; 1998	5	NK N7590	Stover	PRE	0.45	135	ND	0.0101	*0.0077*	<0.030
			Stover	PPI	0.45	135	*0.0074*	0.0118	0.0132	<0.035
			Stover	POST	0.25	86	0.0269, 0.0298	0.0114, 0.0134	*0.0074, 0.0085*	0.048, <0.053

TABLE C.3. Residue Data from Field Corn Field Trials with Amicarbazone.

Trial ID: City, State; Year	Region	Field Corn Variety	Matrix	Treatment[a]	Total Rate (lb ai/A)	PHI (days)	Residues (ppm)[b]			
							Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total
Oxford, IN; 1998	5	Dekalb 566 RR	Stover	PRE	0.45	136	0.0115	*0.0070*	ND	<0.032
			Stover	PPI	0.45	136	*0.0064*	ND	ND	<0.030
			Stover	POST	0.24	88	0.0408, 0.0691	0.0110, 0.0209	*0.0065*, 0.0118	<0.062, 0.102
Dexter, MO; 1998	5	Pioneer 3394	Stover	PRE	0.45	123	0.0134	0.0423	0.0946	0.150
			Stover	PPI	0.45	123	ND	0.0447	0.0816	<0.136
			Stover	POST	0.25	82	0.0177, 0.0306	0.0170, 0.0175	0.0186, 0.0175	0.053, 0.066
Bagley, IA; 1998	5	Dekalb 561 SR	Stover	PRE	0.44	160	ND	ND	ND	<0.030
			Stover	PPI	0.44	160	ND	ND	ND	<0.030
			Stover	POST	0.25	118	*0.0063, 0.0069*	ND, ND	ND, ND	<0.030, <0.030
Richland, IA; 1998	5	NK 7070 BT	Stover	PRE	0.45	159	ND	ND	ND	<0.030
			Stover	PPI	0.45	159	ND	ND	ND	<0.030
			Stover	POST	0.24	119	ND, ND	ND, ND	ND, ND	<0.030, <0.030
New Holland, OH; 1998	5	SC 1127 LL	Stover	PRE	0.44	146	ND	0.0111	*0.0077*	<0.031
			Stover	PPI	0.44	146	ND	*0.0088*	ND	<0.030
			Stover	POST	0.25	106	*0.0068, 0.0084*	*0.0091*, 0.0111	ND, ND	<0.030, <0.031
Carlyle, IL; 1998	5	Pioneer 33VO8	Stover	PRE	0.46	137	ND	0.0163	0.0211	<0.047
			Stover	PPI	0.45	137	*0.0057*	0.0167	0.0451	<0.072
			Stover	POST	0.25	95	0.0422, 0.0551	0.0197, 0.0239	0.0203, 0.0205	0.082, 0.100
Dow, IL; 1998	5	Garst 8541 IT	Stover	PRE	0.44	122	0.0121	0.0118	0.0139	0.038
			Stover	PPI	0.45	122	0.0183	0.0145	0.0201	0.053
			Stover	POST	0.25	80	0.1012, 0.1189	0.0276, 0.0306	0.0203, 0.0250	0.149, 0.174
Sheridan, IN; 1998	5	Pioneer 3394	Stover	PRE	0.45	152	ND	ND	ND	<0.030
			Stover	PPI	0.45	152	ND	0.0118	0.0111	<0.033
			Stover	POST	0.26	108	0.0112, 0.0198	0.0108, *0.0086*	*0.0098, 0.0068*	<0.032, <0.040

TABLE C.3. Residue Data from Field Corn Field Trials with Amicarbazone.

Trial ID: City, State; Year	Region	Field Corn Variety	Matrix	Treatment[a]	Total Rate (lb ai/A)	PHI (days)	Residues (ppm)[b]			
							Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total
Noblesville, IN; 1998	5	Dekalb 545 BTY	Stover	PRE	0.45	147	0.0266	0.0194	0.0147	0.061
			Stover	PPI	0.45	147	0.0223	0.0195	0.0135	0.055
			Stover	POST	0.26	103	0.1468, 0.1690	0.0390, 0.0442	0.0102, 0.0144	0.196, 0.228
Conklin, MI; 1998	5	Pioneer 3861	Stover	PRE	0.45	142	0.0152	0.0119	*0.0072*	<0.037
			Stover	PPI	0.46	142	0.0383	0.0115	*0.0088*	<0.060
			Stover	POST	0.25	99	0.0218, 0.0341	ND, *0.0090*	ND, ND	<0.042, <0.054
Campbell, MN; 1998	5	N 2555 BT	Stover	PRE	0.45	141	0.0179	ND	ND	<0.038
			Stover	PPI	0.45	141	0.0241	ND	ND	<0.044
			Stover	POST	0.25	98	0.0424, 0.0501	*0.0084, 0.0078*	ND, ND	<0.062, <0.070
York, NE; 1998	5	Pioneer 34R06	Stover	PRE	0.45	129	0.0143	0.0159	0.0303	0.060
			Stover	PPI	0.45	129	0.0249	0.0180	0.0304	0.073
			Stover	POST	0.25	89	0.0243, 0.0222	0.0188, 0.0193	0.0292, 0.0318	0.072, 0.073
Richland, IA; 1998	5	Pioneer 3335	Stover	PRE	0.46	144	ND	ND	ND	<0.030
			Stover	PPI	0.45	144	ND	ND	ND	<0.030
			Stover	POST	0.25	102	ND, ND	ND, ND	ND, ND	<0.030, <0.030
Uvalde, TX; 1998	6	Pioneer 3245	Stover	PRE	0.46	116	0.0589	0.0162	*0.0085*	<0.085
			Stover	PPI	0.45	116	0.0458	0.0130	*0.0072*	<0.069
			Stover	POST	0.25	72	0.0515, 0.0827	*0.0078*, 0.0166	ND, ND	<0.072, <0.109
Field corn grain										
Oxford, IN; 1997	5	S 696 LT	Grain	PRE	0.45	165	ND	ND	*0.0024*	<0.030
			Grain	PPI	0.45	165	ND	ND	*0.0014*	<0.030
			Grain	POST	0.25	122	ND, ND	ND, ND	ND, ND	<0.030, <0.030

TABLE C.3. Residue Data from Field Corn Field Trials with Amicarbazone.

Trial ID: City, State; Year	Region	Field Corn Variety	Matrix	Treatment[a]	Total Rate (lb ai/A)	PHI (days)	Residues (ppm)[b]			
							Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total
Tifton, GA; 1997	2	DPL 5750	Grain	PRE	0.45	110	ND	*0.0018*	0.0114	<0.031
			Grain	PPI	0.45	110	ND	*0.0033*	0.0136	<0.034
			Grain	POST	0.25	67	ND, ND	ND, ND	*0.0032, 0.0084*	<0.030, <0.030
Howe, IN; 1997 (decline study)	5	GL 283	Grain	PRE	0.45	145	ND	*0.0028*	0.0145	<0.035
			Grain	PRE	0.45	152	ND	*0.0024*	0.0142	<0.034
			Grain	PRE	0.45	159	ND	*0.0017*	0.0112	<0.031
			Grain	PRE	0.45	166	ND	ND	*0.0032*	<0.030
			Grain	PPI	0.45	145	ND	*0.0015*	0.0103	<0.030
			Grain	PPI	0.45	152	ND	*0.0021*	0.0138	<0.034
			Grain	PPI	0.45	159	ND	ND	*0.0040*	<0.030
			Grain	PPI	0.45	166	ND	*0.0020*	0.0136	<0.034
			Grain	POST	0.25	104	ND, ND	ND, *0.0025*	*0.0037*, 0.0132	<0.030, <0.033
			Grain	POST	0.25	111	ND, ND	ND, ND	*0.0034, 0.0052*	<0.030, <0.030
			Grain	POST	0.25	118	ND, ND	ND, ND	*0.0038, 0.0039*	<0.030, <0.030
			Grain	POST	0.25	125	ND, ND	ND, *0.0015*	*0.0042*, 0.0103	<0.030, <0.030
Springfield, NE; 1997	5	DeKalb 574GR	Grain	PRE	0.46	167	ND	ND	*0.0038*	<0.030
			Grain	PPI	0.45	167	ND	ND	*0.0023*	<0.030
			Grain	POST	0.25	125	ND, ND	ND, ND	*0.0025, 0.0032*	<0.030, <0.030

TABLE C.3. Residue Data from Field Corn Field Trials with Amicarbazone.

Trial ID; City, State; Year	Region	Field Corn Variety	Matrix	Treatment[a]	Total Rate (lb ai/A)	PHI (days)	Residues (ppm)[b]			
							Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total
Stilwell, KS; 1997 (decline study)	5	Pioneer 3349	Grain	PRE	0.45	148	ND	ND	ND	<0.030
			Grain	PRE	0.45	156	ND	ND	ND	<0.030
			Grain	PRE	0.45	162	ND	ND	ND	<0.030
			Grain	PRE	0.45	169	ND	ND	ND	<0.030
			Grain	PPI	0.45	148	ND	ND	ND	<0.030
			Grain	PPI	0.45	156	ND	ND	ND	<0.030
			Grain	PPI	0.45	162	ND	ND	ND	<0.030
			Grain	PPI	0.45	169	ND	ND	ND	<0.030
			Grain	POST	0.25	104	ND, ND	ND, ND	ND, ND	<0.030, <0.030
			Grain	POST	0.25	112	ND, ND	ND, ND	ND, ND	<0.030, <0.030
			Grain	POST	0.25	118	ND, ND	ND, ND	ND, ND	<0.030, <0.030
			Grain	POST	0.25	125	ND, ND	ND, ND	ND, ND	<0.030, <0.030
Germansville, PA; 1998	1	Mycogen 2598	Grain	PRE	0.46	154	ND	ND	*0.0012*	<0.030
			Grain	PPI	0.46	154	ND	ND	*0.0018*	<0.030
			Grain	POST	0.25	114	ND, ND	ND, ND	*0.0013, 0.0014*	<0.030, <0.030
North Rose, NY; 1998	1	Agway 257	Grain	PRE	0.45	133	ND	*0.0022*	*0.0077*	<0.030
			Grain	PPI	0.46	133	ND	*0.0020*	*0.0080*	<0.030
			Grain	POST	0.25	91	ND, ND	ND, *0.0015*	*0.0049, 0.0043*	<0.030, <0.030
Springfield, NE; 1998	5	Dekalb 580 RR	Grain	PRE	0.46	135	ND	ND	*0.0024*	<0.030
			Grain	PPI	0.46	135	ND	ND	*0.0032*	<0.030
			Grain	POST	0.25	92	ND, ND	ND, *0.0016*	*0.0022, 0.0028*	<0.030, <0.030
Stilwell, KS; 1998	5	NK N7590	Grain	PRE	0.45	135	ND	ND	*0.0028*	<0.030
			Grain	PPI	0.45	135	ND	ND	*0.0031*	<0.030
			Grain	POST	0.25	86	ND, ND	ND, ND	*0.0038, 0.0040*	<0.030, <0.030

TABLE C.3. Residue Data from Field Corn Field Trials with Amicarbazone.

Trial ID: City; State; Year	Region	Field Corn Variety	Matrix	Treatment[a]	Total Rate (lb ai/A)	PHI (days)	Residues (ppm)[b]			
							Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total
Oxford, IN; 1998	5	Dekalb 566 RR	Grain	PRE	0.45	136	ND	ND	ND	<0.030
			Grain	PPI	0.45	136	ND	ND	*0.0011*	<0.030
			Grain	POST	0.24	88	ND, ND	ND, ND	*0.0013, 0.0013*	<0.030, <0.030
Dexter, MO; 1998	5	Pioneer 3394	Grain	PRE	0.45	123	ND	*0.0028*	0.0122	<0.032
			Grain	PPI	0.45	123	ND	*0.0013*	*0.0084*	<0.030
			Grain	POST	0.25	82	ND, ND	ND, ND	*0.0026, 0.0034*	<0.030, <0.030
Bagley, IA; 1998	5	Dekalb 561 SR	Grain	PRE	0.44	160	ND	ND	*0.0020*	<0.030
			Grain	PPI	0.44	160	ND	ND	*0.0020*	<0.030
			Grain	POST	0.25	118	ND, ND	ND, ND	ND, *0.0012*	<0.030, <0.030
Richland, IA; 1998	5	NK 7070 BT	Grain	PRE	0.45	159	ND	ND	*0.0016*	<0.030
			Grain	PPI	0.45	159	ND	ND	*0.0017*	<0.030
			Grain	POST	0.24	119	ND, ND	ND, ND	ND, *0.0015*	<0.030, <0.030
New Holland, OH; 1998	5	SC 1127 LL	Grain	PRE	0.44	146	ND	ND	*0.0026*	<0.030
			Grain	PPI	0.44	146	ND	ND	*0.0017*	<0.030
			Grain	POST	0.25	106	ND, ND	ND, ND	*0.0020, 0.0034*	<0.030, <0.030
Carlyle, IL; 1998	5	Pioneer 33V08	Grain	PRE	0.46	137	ND	*0.0015*	*0.0060*	<0.030
			Grain	PPI	0.45	137	ND	ND	*0.0050*	<0.030
			Grain	POST	0.25	95	ND, ND	ND, ND	ND, *0.0021*	<0.030, <0.030
Dow, IL; 1998	5	Garst 8541 IT	Grain	PRE	0.44	122	ND	ND	*0.0048*	<0.030
			Grain	PPI	0.45	122	ND	ND	*0.0045*	<0.030
			Grain	POST	0.25	80	ND, ND	*0.0010, 0.0010*	*0.0042, 0.0047*	<0.030, <0.030
Sheridan, IN; 1998	5	Pioneer 3394	Grain	PRE	0.45	152	ND	ND	ND	<0.030
			Grain	PPI	0.45	152	ND	ND	*0.0033*	<0.030
			Grain	POST	0.26	108	ND, ND	ND, ND	*0.0026, 0.0029*	<0.030, <0.030

TABLE C.3. Residue Data from Field Corn Field Trials with Amicarbazone.

Trial ID: City, State; Year	Region	Field Corn Variety	Matrix	Treatment[a]	Total Rate (lb ai/A)	PHI (days)	Residues (ppm)[b]			
							Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total
Noblesville, IN; 1998	5	Dekalb 545 BTY	Grain	PRE	0.45	147	ND	ND	*0.0047*	<0.030
			Grain	PPI	0.45	147	ND	ND	*0.0031*	<0.030
			Grain	POST	0.26	103	ND, ND	ND, ND	*0.0026, 0.0030*	<0.030, <0.030
Conklin, MI; 1998	5	Pioneer 3861	Grain	PRE	0.45	142	ND	ND	*0.0023*	<0.030
			Grain	PPI	0.46	142	ND	ND	*0.0032*	<0.030
			Grain	POST	0.25	99	ND, ND	ND, ND	ND, *0.0019*	<0.030, <0.030
Campbell, MN; 1998	5	N 2555 BT	Grain	PRE	0.45	141	ND	ND	*0.0012*	<0.030
			Grain	PPI	0.45	141	ND	ND	*0.0013*	<0.030
			Grain	POST	0.25	98	ND, ND	ND, ND	ND, ND	<0.030, <0.030
York, NE; 1998	5	Pioneer 34R06	Grain	PRE	0.45	129	ND	*0.0010*	*0.0028*	<0.030
			Grain	PPI	0.45	129	ND	ND	*0.0028*	<0.030
			Grain	POST	0.25	89	ND, ND	ND, ND	*0.0018, 0.0025*	<0.030, <0.030
Richland, IA; 1998	5	Pioneer 3335	Grain	PRE	0.46	144	ND	ND	*0.0014*	<0.030
			Grain	PPI	0.45	144	ND	ND	ND	<0.030
			Grain	POST	0.25	102	ND, ND	ND, ND	*0.0013, 0.0013*	<0.030, <0.030
Uvalde, TX; 1998	6	Pioneer 3245	Grain	PRE	0.46	116	ND	*0.0021*	*0.0032*	<0.030
			Grain	PPI	0.45	116	*0.0037*	*0.0035*	*0.0044*	<0.030
			Grain	POST	0.25	72	ND, 0.0250	ND, *0.0034*	ND, *0.0020*	<0.030, <0.045

[a] PRE = Single preemergence application; PPI = Single preplant incorporated application; POST = Single early postemergence application.

[b] Nondetectable residues are reported as ND. Residues quantitated between the LOD and LOQ are italicized. The reported LODs for each analyte were 0.001 ppm for forage and grain and 0.006 ppm for stover, and the LOQ for each analyte was 0.01 ppm for all matrices. Total residues are the sum of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 residues in parent equivalents; the LOQ was used for individual residues reported at less than the LOQ in calculating total residues.

TABLE C.4. Summary of Residue Data from Field Corn Field Trials with Amicarbazone.

Commodity	Total Applic. Rate (lb ai/A)	PHI (days)	Analyte	Residue Levels (ppm)[a]					
				n	Min.	Max.	HAFT[b]	Median	Mean	Std. Dev.
Field corn treated preemergence (PRE)										
Forage	0.44-0.46	74-118	Amicarbazone	23	<0.010	0.1197	0.1197	0.0107	0.017	0.024
			DA MKH 3586		<0.010	0.0634	0.0634	0.0067	0.013	0.014
			iPr-2-OH DA MKH 3586		<0.010	0.0387	0.0387	0.0081	0.013	0.011
			Total		<0.030	0.211	0.211	0.031	0.042	0.043
Stover	0.44-0.46	110-167	Amicarbazone	23	<0.010	0.0589	0.0589	0.0078	0.014	0.013
			DA MKH 3586		<0.010	0.0423	0.0423	0.0114	0.014	0.011
			iPr-2-OH DA MKH 3586		<0.010	0.0946	0.0946	0.0077	0.019	0.024
			Total		<0.030	0.150	0.150	0.030	0.047	0.038
Grain	0.44-0.46	110-167	Amicarbazone	23	<0.010	<0.010	<0.010	0.005	0.005	0
			DA MKH 3586		<0.010	<0.010	<0.010	0.005	0.005	0
			iPr-2-OH DA MKH 3586		<0.010	0.0122	0.0122	0.005	0.006	0.003
			Total		<0.030	<0.032	<0.032	0.015	0.016	0.003
Field corn treated preplant incorporated (PPI)										
Forage	0.44-0.46	74-118	Amicarbazone	23	<0.010	0.0919	0.0919	0.0108	0.019	0.020
			DA MKH 3586		<0.010	0.0575	0.0575	0.0084	0.013	0.012
			iPr-2-OH DA MKH 3586		<0.010	0.0304	0.0304	0.0100	0.013	0.008
			Total		<0.030	0.172	0.172	0.036	0.045	0.036
Stover	0.44-0.46	110-167	Amicarbazone	23	<0.010	0.1332	0.1332	0.0064	0.019	0.028
			DA MKH 3586		<0.010	0.0987	0.0987	0.0115	0.017	0.020
			iPr-2-OH DA MKH 3586		<0.010	0.2001	0.2001	0.0073	0.025	0.043
			Total		<0.030	0.432	0.432	0.036	0.061	0.086
Grain	0.44-0.46	110-167	Amicarbazone	23	<0.010	<0.010	<0.010	0.005	0.005	0
			DA MKH 3586		<0.010	<0.010	<0.010	0.005	0.005	0
			iPr-2-OH DA MKH 3586		<0.010	0.0138	0.0138	0.005	0.006	0.003
			Total		<0.030	<0.034	<0.034	0.015	0.016	0.003
Field corn treated early postemergence										
Forage	0.24-0.26	19-78	Amicarbazone	46	<0.010	0.3956	0.3949	0.0295	0.050	0.080
			DA MKH 3586		<0.010	0.1285	0.1252	0.0075	0.015	0.025
			iPr-2-OH DA MKH 3586		<0.010	0.0343	0.0333	0.0086	0.010	0.007
			Total		<0.030	0.557	0.553	0.047	0.075	0.110

TABLE C.4. Summary of Residue Data from Field Corn Field Trials with Amicarbazone.

Commodity	Total Applic. Rate (lb ai/A)	PHI (days)	Analyte	Residue Levels (ppm)[a]						
				n	Min.	Max.	HAFT [b]	Median	Mean	Std. Dev.
Stover	0.24-0.26	67-125	Amicarbazone	46	<0.010	0.1690	0.1579	0.0211	0.037	0.039
			DA MKH 3586		<0.010	0.0615	0.0549	0.0111	0.015	0.012
			iPr-2-OH DA MKH 3586		<0.010	0.0796	0.0790	0.0070	0.014	0.016
			Total		<0.030	0.240	0.226	0.050	0.066	0.059
Grain	0.24-0.26	67-125	Amicarbazone	46	<0.010	0.0250	0.0175	0.005	0.005	0.003
			DA MKH 3586		<0.010	<0.010	<0.010	0.005	0.005	0
			iPr-2-OH DA MKH 3586		<0.010	<0.010	<0.010	0.005	0.005	0.001
			Total		<0.030	<0.045	<0.038	0.015	0.016	0.003

[a] For the calculation of minimum, maximum, and HAFT values, the LOQ value (<0.01 ppm) was used for residues reported between the LOQ and LOD and as nondetectable (ND) in Table C.4. For calculation of the mean and standard deviation, ½ the LOQ (0.005 ppm) was used for forage and grain residues reported below half the LOQ, and the LOD value (0.006 ppm) was used for stover values reported as ND (½ the LOQ was less than the LOD for stover); the reported residue value was used for all forage and grain residues greater than 0.005 ppm and for all stover values greater than 0.006 ppm. For the decline trials, residue data from the normal harvest interval only were included in the summary calculations.
[b] HAFT = Highest Average Field Trial.

D. CONCLUSION

The submitted field corn field trial data reflect the use of amicarbazone as a single broadcast application to field corn either as a preemergence or preplant incorporated application at 0.45 lb ai/A, or an early postemergence application at 0.25 lb ai/A. The residue decline data indicate that combined residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 did not significantly increase or decrease with increasing PHI's. An acceptable method was used for quantitation of residues in/on field corn commodities. Additional storage stability data, as well as information pertaining to weather conditions and soil characteristics, should be submitted to support this study.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: PP#0F6131
DP Barcode: D288216
PC Code: 114004

Template Version September 2003

2

Amicarbazone/MKH 3586/PC Code 114004/Arvesta Corporation
DACO 7.4.5/OPPTS 860.1520/OECD IIA 6.5.4 and IIIA 8.5
Processed Food and Feed - Field Corn

Primary Evaluator: Manying Xue, Chemist
RAB3/HED (7509C) Date: 03/23/05

Approved by: Leung Cheng, Senior Chemist
RAB3/HED (7509C) Date: 03/23/05

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; updated draft submitted 09/21/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

45121711 Krolski, M. (2000) MKH 3586 70 WG--Magnitude of the Residues in Corn Aspirated Grain Fractions and Corn Processed Commodities: Lab Project Number: 109559: M619CO03. Unpublished study prepared by Bayer Corp. 239 p.

EXECUTIVE SUMMARY:

Arvesta Corporation has submitted a processing study with field corn grain. Residues of amicarbazone were nondetectable (<0.001 ppm) in/on field corn grain collected 113 days following a single postemergence foliar broadcast application of the 70% DF formulation at 1.24 (5x) lb ai/A; detectable residues of metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 were below the method limit of quantitation (LOQ; <0.01 ppm) in corn grain, at 0.0011-0.0014 and 0.0037-0.0096 ppm, respectively. The average combined residues of amicarbazone and its metabolites, reported as amicarbazone equivalents, were <0.0097 ppm.

The processing data for amicarbazone indicate that residues of amicarbazone, parent are not likely to concentrate in any processed corn commodity; residues of amicarbazone were nondetectable (<0.001 ppm) in/on all subsamples of starch, meal, grits, flour, and refined oil (dry- and wet-milled) processed from treated corn grain.

The processing data for DA MKH 3586 indicate that residues of DA MKH 3586 may concentrate slightly in corn meal and grits (processing factors of 1.1x) but do not concentrate in starch, flour, or refined oil (<0.001 ppm in all subsamples).

The processing data for iPr-2-OH DA MKH 3586 indicate that residues of iPr-2-OH DA MKH 3586 may concentrate in corn meal (processing factor of 1.3x), are not likely to concentrate in corn flour (processing factor of 1.0x) or grits (processing factor of 0.8x), and reduce in starch and refined oil (processing factors of <0.2x).

Total combined residues of amicarbazone and its metabolites concentrated slightly in corn meal (processing factor of 1.2x) but did not concentrate in starch (0.3x), grits (0.9x), flour (1.0x), or refined,oil (0.3x).

Residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on field corn grain and its processed commodities were quantitated using the proposed LC/MS/MS enforcement method. The validated LOQ was 0.01 ppm for each analyte, and the limit of detection (LOD) was 0.001 ppm. This method is adequate for data collection based on acceptable concurrent method recovery data.

The maximum storage intervals for processing study samples from harvest/processing to analysis were 355 days (11.7 months) for corn grain, 21-37 days (~1 month) for starch, grits, and refined oil (wet-milled), 62 days (2 months) for meal, and 112 days (3.7 months) for refined oil (dry-milled). Samples of corn grain were processed ~1 year after harvest. In support of this study, the petitioner referenced storage stability data generated in conjunction with the field corn field trials (refer to the DER for MRID 45121710), reflecting reanalysis of 1997 field trial samples after up to 26 months of frozen storage; however, we note that no supporting data were included in MRID 45121710 to support the petitioner's statement that reanalysis of samples demonstrated similar residue levels. Additional data are required to support the storage stability study reported in MRID 45121710.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the processing study residue data are classified as scientifically acceptable, pending submission of the additional storage stability data required for the field corn field trial study (refer to the DER for MRID 45121710).

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D288216.

COMPLIANCE:

Signed and dated 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

Amicarbazone is a selective herbicide intended for preplant, preemergence, or early postemergence application to field corn for control of annual broadleaf weeds. The mode of action for amicarbazone is inhibition of photosynthesis.

TABLE A.1. Amicarbazone Nomenclature.

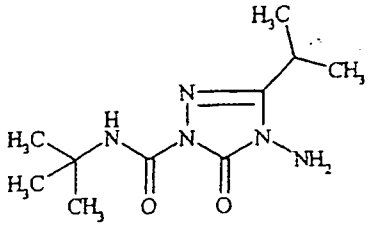
Chemical structure	
Common name	Amicarbazone
Company experimental name	MKH 3586
IUPAC name	4-amino-*N*-*tert*-butyl-4,5-dihydro-3-isopropyl-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS name	4-amino-*N*-(1,1-dimethylethyl)-4,5-dihydro-3-(1-methylethyl)-5-oxo-1*H*-1,2,4-triazole-1-carboxamide
CAS #	129909-90-6
End-use formulation (EUP)	70% dry flowable (DF) formulation (EPA Reg. No. 66330)

TABLE A.2. Physicochemical Properties of Technical Grade Amicarbazone.

Parameter	Value	Reference*
Melting point/range	137.5°C	MRID 45121501
pH	7.06 (2.5% slurry)	MRID 45121501
Density	1.12 g/mL @ 20°C	MRID 45121501
Water solubility	4.6 g/L	MRID 45121501
Solvent solubility (g/L)	n-Heptane = 0.07, xylene = 9.2, 1-octanol = 43, 2-propanol = 110, ethyl acetate = 140, acetone >250, polyethylene glycol = 79, acetonitrile >250, dimethylsulfoxide = 250, dichloromethane >250	MRID 45121502
Vapor pressure	3.00×10^{-6} Pa @ 25°C 1.30×10^{-6} Pa @ 20°C	MRID 45121501
Dissociation constant, pK_a	Does not dissociate. No acidic or basic properties.	MRID 45121501
Octanol/water partition coefficient, $Log(K_{ow})$	pKa = 17(log P_{ow} =1.23 @ pH 7 (20°C)	MRID 45121502
UV/visible absorption spectrum	Peak @221 nm; molar absorptivity (1000 cm^2/mol)	MRID 45121501

* D288202, MRIDs 45121501 & 45121502, S.B. Mathur, 07/07/2003.

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

TABLE B.1.2. Study Use Pattern.

Location: City, State; Year	EP[a]	Application						Tank Mix Adjuvants
		Timing	Application Rate (lb ai/A)	RTI[b] (days)	Treat. No.	Method (gal/A)	Total Rate (lb ai/A)	
Oxford, IN; 1998	70% DF	Postemergence; 8 nodes detectable	1.242	NA	1	Broadcast (11.9)	1.242	None

[a] EP = End-use Product; 70% dry flowable (DF).
[b] RTI = Retreatment Interval; not applicable (NA) because only one application was made.

The petitioner noted that a total of six separate plots at the Oxford, IN trial site were treated: three plots received a single preplant incorporated application of the 70% DF formulation at ~0.45, 1.35, or 2.25 lb ai/A, and three other plots received a single postemergence application of the 70% DF formulation at ~0.25, 0.75, or 1.24 lb ai/A. A seventh plot was not treated to generate control samples. Analysis of the resulting corn grain samples indicated that grain from the plot treated postemergence at 1.24 lb ai/A contained the highest residues, and these samples were chosen for processing. The petitioner only provided application data for this field trial.

B.2. Sample Handling and Processing Procedures

Bulk corn grain samples were frozen at the field and shipped on the day of harvest to the Food Protein Research and Development Center at Texas A&M University (Bryan, TX) for processing. The bulk corn grain sample was stored frozen (≤-12 °C) for ~1 year prior to processing. Corn grain was processed into grits, meal, flour, starch, and refined oil using small-scale milling procedures. Aspirated grain fractions were also generated. Adequate descriptions of the processing procedures and material balance flowcharts were provided.

Briefly, grain was dried to 10-13% moisture, then circulated through a dust-generating apparatus, and light impurities were removed by aspiration. The aspirated light impurities (grain dust) were classified by sieving and were separated using a Kice aspiration unit. The sample remaining after aspiration was screened to remove large and small foreign particles and then divided into two subsamples for dry milling and wet milling.

For dry milling, the cleaned grain was moisture-adjusted (20-22%), "tempered" for 2-2.5 hours, and impact milled. After milling, the cornstock was dried and shaker screened to isolate hull, germ, large, medium, and small grits, coarse meal, meal, and flour. The germ was oven-dried, heat-conditioned, flaked, and solvent extracted to remove crude oil from the flaked germ. The solvent (hexane) was removed from the crude oil with heating, and the oil was refined. After refining, the oil and soapstock were separated, and the refined oil was bleached and deodorized.

For wet-milling, the cleaned grain was steeped in water with sulfurous acid and then milled to recover germ, hull, gluten, and starch. After drying, the germ was heat-conditioned, flaked, and

pressed through an expeller to liberate crude oil. The presscake was solvent extracted as previously described for the dry-milling procedure for crude and refined oil. Corn grain, grain dust, and processed commodities were frozen following processing.

B.3. Analytical Methodology

Samples of corn grain and its processed commodities were analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 by Bayer Corporation (Stilwell, KS) using the LC/MS/MS method proposed as the enforcement method for plant commodities. Only a brief description of the method was included with the corn processing study; for a complete description of the method, refer to the DER for MRID 45121708.

Briefly, homogenized samples of corn grain matrices were extracted using an accelerated solvent extractor. The extract, following centrifugation, was cleaned up by solid phase extraction. Residues were quantitated by LC/MS/MS, using deuterated internal reference standards of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586. Residues of metabolites were reported as parent equivalents. The validated LOQ was 0.01 ppm each for amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on all corn matrices. The estimated method LOD previously determined for corn grain (0.001 ppm) from the field trial studies (refer to the DER for MRID 45121710) was used for all corn grain processed commodities.

Prior to analysis of treated samples, Bayer conducted method verification using corn starch, grits, meal, flour, and refined oil. These samples were fortified with amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586, each at the method LOQ (0.01 ppm). Method verification recoveries of all analytes were 82-106% for starch, 78-94% for grits, 76-94% for meal, 74-85% for flour, and 91-119% for refined oil.

C. RESULTS AND DISCUSSION

In a field corn study conducted in IN (Region 5), mature corn grain samples were collected 113 days following a single postemergence foliar broadcast application of the 70% DF formulation at 1.24 lb ai/A using ground equipment. Application was made to corn prior to ear formation. Field corn grain was processed using small-scale milling procedures into meal, grits, flour, and refined oil (dry milling) and starch and refined oil (wet milling). In addition, aspirated grain fractions were generated; however, because application was made prior to ear formation, grain surface residues were not expected, and aspirated grain fractions samples were not analyzed. The petitioner provided detailed descriptions and material balance flowcharts for corn grain processing (not available electronically).

The results of the processing study are presented in Table C.3; ten subsamples of field corn grain and three subsamples each of processed field corn commodities were analyzed. Residues of amicarbazone were nondetectable (<0.001 ppm) in/on treated corn grain prior to processing;

detectable residues of metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 below the method LOQ (<0.01 ppm) were observed in corn grain subsamples, at 0.0011-0.0014 and 0.0037-0.0096 ppm, respectively. The average combined residues of amicarbazone and its metabolites, reported as amicarbazone equivalents, were <0.0097 ppm.

The processing data for amicarbazone indicate that residues of amicarbazone, parent are not likely to concentrate in any processed corn commodity; residues of amicarbazone were nondetectable (<0.001 ppm) in/on all subsamples of starch, meal, grits, flour, and refined oil (dry- and wet-milled) processed from treated corn grain.

The processing data for DA MKH 3586 indicate that residues of this metabolite may concentrate slightly in corn meal and grits (<0.001-0.0016 ppm; processing factors of 1.1x) but do not concentrate in starch, flour, or refined oil (<0.001 ppm in all subsamples).

The processing data for iPr-2-OH DA MKH 3586 indicate that residues of this metabolite may concentrate in corn meal (0.0072-0.0112 ppm; processing factor of 1.3x), are not likely to concentrate in corn flour (0.0055-0.0090 ppm; processing factor of 1.0x) or grits (0.0048-0.0078 ppm; processing factor of 0.8x), and reduce in starch (<0.001-0.0016 ppm; processing factor of <0.2x) and refined oil (<0.001 ppm; processing factor of <0.2x).

Total combined residues of amicarbazone and its metabolites concentrated slightly in corn meal (processing factor of 1.2x) but did not concentrate in starch (0.3x), grits (0.9x), flour (1.0x), or refined oil (0.3x).

Apparent residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 were each less than the method LOD (<0.001 ppm) in all untreated corn grain (RAC), starch, meal, grits, flour, and refined oil samples, except for one corn grain control sample which bore detectable residues at the method LOD (0.0010 ppm).

The reported concentration factors do not exceed the theoretical concentration factors. According to Table 3 of OPPTS 860.1520, the maximum theoretical concentration factor for corn is 25x for corn oil. Concentration factors for other corn processed commodities are not listed in OPPTS 860.1520.

Residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 in/on field corn grain and its processed commodities were quantitated using the proposed LC/MS/MS enforcement method. Concurrent method recovery data are presented in Table C.1. The validated LOQ was 0.01 ppm for each analyte. This method is adequate for data collection based on acceptable concurrent method recovery data.

Table C.2 lists the storage conditions and intervals for samples used in the field corn processing study. The maximum storage intervals of processing study samples from harvest/processing to analysis were 355 days (11.7 months) for corn grain, 21-37 days (~1 month) for starch, grits, and refined oil (wet-milled), 62 days (2 months) for meal, and 112 days (3.7 months) for refined oil

(dry-milled). Samples of corn grain were processed ~1 year after harvest. In support of this study, the petitioner referenced storage stability data generated in conjunction with the field corn field trials (refer to the DER for MRID 45121710) reflecting reanalysis of 1997 field trial samples after up to 26 months of frozen storage; however, we note that no supporting data were included in MRID 45121710 to support the petitioner's statement that reanalysis of samples demonstrated similar residue levels. Additional data are required to support the storage stability study reported in MRID 45121710.

All processed commodities were initially analyzed within 41 days of processing, except for the dry-milled refined oil which was stored almost 4 months from processing to analysis. The petitioner stated that, because no residues were observed in the wet-milled refined oil, which was analyzed within 19 days of processing, the lack of detectable residues in dry-milled refined oil is not due to degradation upon storage. HED will not require storage stability data for corn processed commodities to support this study, provided that the required additional storage stability data are submitted for field corn grain. When submitted, the storage stability data for field corn grain may be used to support the storage conditions and intervals for corn meal samples.

TABLE C.1. Summary of Concurrent Recoveries of Amicarbazone from Field Corn Matrices.

Matrix	Analyte	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev (%)
Corn, grain	Amicarbazone	0.01	12	55, 60, 61, 62, 63, 65, 66, 66, 71, 71, 76, 81	66 ± 7
	DA MKH 3586	0.01		65, 66, 66, 66, 68, 68, 70, 72, 76, 77, 86, 87	72 ± 8
	iPr-2-OH DA MKH 3586	0.01		61, 63, 64, 67, 71, 72, 72, 76, 81, 89, 91, 92	75 ± 11
Corn, starch	Amicarbazone	0.01	1	89	89
	DA MKH 3586	0.01		78	78
	iPr-2-OH DA MKH 3586	0.01		98	98
Corn, grits	Amicarbazone	0.01	1	75	75
	DA MKH 3586	0.01		77	77
	iPr-2-OH DA MKH 3586	0.01		86	86
Corn, meal	Amicarbazone	0.01	1	70	70
	DA MKH 3586	0.01		73	73
	iPr-2-OH DA MKH 3586	0.01		96	96
Corn, flour	Amicarbazone	0.01	1	108	108
	DA MKH 3586	0.01		97	97
	iPr-2-OH DA MKH 3586	0.01		87	87
Refined corn oil	Amicarbazone	0.01	3	76, 81, 92	83 ± 8
	DA MKH 3586	0.01		100, 104, 107	104 ± 4
	iPr-2-OH DA MKH 3586	0.01		89, 93, 93	92 ± 2

TABLE C.2. Summary of Storage Conditions.

Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Storage Duration from Collection to Analysis	Interval of Demonstrated Storage Stability
Corn, grain	≤-12	337-355 days (11.1-11.7 months)	None available
Starch		28-29 days (0.9-1.0 months)	None available
Meal		41-62 days (1.3-2.0 months)	
Grits		36-37 days (1.2 months)	
Flour		30-35 days (1-1.1 months)	
Refined oil (dry-milled)		111-113 days (3.7 months)	
Refined oil (wet-milled)		19-21 days (0.6-0.7 months)	

TABLE C.3. Residue Data from Field Corn Processing Study with Amicarbazone.

RAC	Processed Commodity	Total Rate (lb ai/A)	PHI (days)	Residues (ppm) *				Processing Factor			
				Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total	Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total
Corn, grain	RAC	1.24	113	ND	*ND*	*0.0074*	<0.0094	–	–	–	–
				ND	*0.0014*	*0.0096*	<0.0120				
				ND	*0.0011*	*0.0086*	<0.0107				
				ND	*ND*	*0.0037*	<0.0057				
				ND	*0.0012*	*0.0077*	<0.0099				
				ND	*ND*	*0.0077*	<0.0097				
				ND	*0.0012*	*0.0080*	<0.0102				
				ND	*0.0013*	*0.0077*	<0.0100				
				ND	*0.0012*	*0.0077*	<0.0099				
				ND	*0.0013*	*0.0071*	<0.0094				
			average:	ND	*<0.0012*	*0.0075*	<0.0097				
	Starch	1.24	113	ND	ND	ND	<0.003	Not calculated (NC)	<1x	<0.2x	0.3x
				ND	ND	ND	<0.003				
				ND	ND	*0.0016*	<0.0036				
			average:	ND	ND	*<0.0012*	<0.0032				
	Meal	1.24	113	ND	*0.0013*	*0.0072*	<0.0095	NC	1.1x	1.3x	1.2x
				ND	*0.0016*	0.0100	<0.0126				
				ND	ND	0.0112	<0.0132				
			average:	ND	*<0.0013*	*0.0095*	0.0118				
	Grits	1.24	113	ND	*0.0016*	*0.0048*	<0.0074	NC	1.1x	0.8x	0.9x
				ND	ND	*0.0062*	<0.0082				
				ND	*0.0012*	*0.0078*	<0.0100				
			average:	ND	*<0.0013*	*0.0063*	<0.0085				
	Flour	1.24	113	ND	ND	*0.0080*	<0.0100	NC	<1x	1.0x	1.0x
				ND	ND	*0.0090*	<0.0110				
				ND	ND	*0.0055*	<0.0075				
			average:	ND	ND	*0.0075*	<0.0095				

TABLE C.3. Residue Data from Field Corn Processing Study with Amicarbazone.

RAC	Processed Commodity	Total Rate (lb ai/A)	PHI (days)	Residues (ppm) *				Processing Factor			
				Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total	Amicarbazone	DA MKH 3586	iPr-2-OH DA MKH 3586	Total
	Refined oil (dry-milled)	1.24	113	ND	ND	ND	<0.003	NC	<1x	<0.2x	0.3x
				ND	ND	ND	<0.003				
				ND	ND	ND	<0.003				
	Refined oil (wet-milled)	1.24	113	ND	ND	ND	<0.003	NC	<1x	<0.2x	0.3x
				ND	ND	ND	<0.003				
				ND	ND	ND	<0.003				

* Nondetectable residues are reported as ND and residues quantitated between the LOD and LOQ are italicized; the reported LOD and LOQ for each analyte were 0.001 ppm and 0.01 ppm, respectively, for all corn matrices. Total residues are the sum of amicarbazone, DA MKH 3586, and iPr-2-OH DA MKH 3586 residues in parent equivalents; in calculating total residues the LOD (0.001 ppm) was used for individual residues reported as ND.

D. CONCLUSION

The field corn processing data indicate that total residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586 may concentrate slightly in meal (1.2x average processing factor). Total residues do not appear to concentrate in corn starch, grits, flour and refined oil.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: ChemTeam: 02/24/05; ChemSAC: 03/16/05
Petition Number: PP#0F6131
DP Barcode: D288216
PC Code: 114004

Template Version September 2003

13544

R110166

Chemical:	Amicarbazone
PC Code:	114004
HED File Code	11500 Petition Files Chemistry
Memo Date:	06/06/2005
File ID:	DPD288216; DPD309766
Accession Number:	412-05-0096

HED Records Reference Center
06/27/2005