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U1NITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

Date: August 10, 2005

MEMORANDUM

SUBJECT: Amicarbazone: HED Human Health Risk Assessment for New Food Use Herbicide on Field Corn. PC Code:114004, Petition #: 0F6131, DP Barcode: D288216.

Regulatory Action: Section 3 Registration
Risk Assessment Type: Single Chemical/Aggregate

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1.0 Executive Summary

Amicarbazone, 4-amino-4,5-dihydro-N-(1,1-dimethylethyl)-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide, is a new triazolinone herbicide related to carfentrazone, flucarbazone, propoxycarbazone, and sulfentrazone. Section 3 registration of a dry flowable formulation is requested by Arvesta Corporation for use on field corn with the application timings including preplant, preeemergence, and early postemergence. In addition to tolerances on field corn commodities, an import tolerance is proposed for sugarcane, but HED is recommending against the latter due to the lack of field trials in the countries of use. Tolerances are also requested on a number of rotational crops due to the significant uptake of residues in those commodities. Tolerances are also needed on most livestock commodities.

The toxicology database is complete and adequate for purposes of risk assessment. Amicarbazone exhibits low acute toxicity. The major effects observed across species in multiple dose studies were body weight changes and liver effects. Amicarabazone is not mutagenic and has been classified as "not likely to be a human carcinogen". Although the acute neurotoxicity study in rats showed signs of neurotoxicity, no such effects were seen in the subchronic and developmental neurotoxicity studies. The database is complete with respect to pre- and post-natal toxicity and shows no evidence of increased qualitative or quantitative susceptibility. There are no residual uncertainties. HED recommends that the special FQPA safety factor be reduced to 1X and that no database uncertainty factor is needed.

The acute reference dose (RfD) and population adjusted dose (PAD) have been selected at 0.10 mg/kg/day for all populations based on neurotoxic effects in the acute rat neurotoxicity study. The chronic RfD and PAD have been selected at 0.023 mg/kg/day based on body weight effects in the chronic rat study and liver effects in the chronic dog study. No dermal endpoint was selected for occupational risk assessment based on the lack of systemic toxicity in a dermal rat study. The inhalation endpoint was based on a 90-day oral dog study in which assorted effects were observed in the thyroid, liver, gall bladder, and blood.

Although there are some residue chemistry data deficiencies, the database is adequate for conditional registration. The residues of concern in plants and livestock for tolerances and risk assessment consist of the parent, a desamino metabolite, and a hydroxylated derivative of the latter. The desamino metabolite is also included in the drinking water assessment along with its N-methyl derivative. Additional metabolites are included in livestock commodities for risk assessment purposes.

The acute and chronic dietary assessments were conducted using DEEM-FCID and are very conservative in that 100% crop treated and tolerance level residues were used in all commodities. Modeled drinking water levels provided by EFED were also included in the assessments. The risks for all populations are well below HED's level of concern. Since no residential uses are proposed for amicarbazone, these dietary risk estimates also represent the aggregate risks. Occupational risks are also below HED's level of concern.

Provided that revised Section B/proposed label, Section F/proposed tolerances, and analytical

reference standards are submitted, HED recommends for conditional registration of amicarbazone on field corn along with establishment of the tolerances listed below.

Tolerance Summary for Amicarbazone.	
Commodity	Recommended Tolerance (ppm)
Primary Crop and Livestock Tolerances	
Corn, field, grain	0.05
Corn, field, forage	0.8
Corn, field, stover	1.0
Sugarcane	None (additional data required)
Sugarcane, molasses	
Cattle, meat	0.01
Goat, meat	0.01
Hog, meat	0.01
Horse, meat	0.01
Sheep, meat	0.01
Cattle, meat byproducts, except liver	0.10
Goat, meat byproducts, except liver	0.10
Hog,, meat byproducts, except liver	0.01
Horse, meat byproducts, except liver	0.10
Sheep, meat byproducts, except liver	0.10
Milk	0.01
Cattle, fat	0.01
Goat, fat	0.01
Hog, fat	0.01
Horse, fat	0.01
Sheep, fat	0.01
Cattle, liver	1.0
Goat, liver	1.0
Hog, liver	0.1
Horse, liver	1.0
Sheep, liver	1.0
Poultry, liver	0.01
Rotational Crop Tolerances	
Alfalfa, forage	0.05
Alfalfa, hay	0.10
Cotton, undelinted seed	0.07
Cotton, gin byproducts	0.30
Soybean, forage	1.50
Soybean, hay	5.0
Soybean, seed	0.80

Tolerance Summary for Amicarbazone.	
Commodity	Recommended Tolerance (ppm)
Wheat, forage	0.50
Wheat, hay	1.0
Wheat, grain	0.10
Wheat, straw	0.50
Wheat, grain, milled byproducts	0.15

The residue chemistry data which HED recommends be required as a condition of registration are detailed in Section 10.2.

2.0 Ingredient Profile

2.1 Summary of Registered/Proposed Uses

Amicarbazone, 4-amino-4,5-dihydro-N-(1,1-dimethylethyl)-3-(1-methylethyl)-5-oxo-1H-1,2,4-triazole-1-carboxamide, is a new triazolinone compound proposed for the control of annual broad leaf weeds. Amicarbazone selectively inhibits acetolactase synthase, an enzyme involved in photosystem II of plants. Related compounds include carfentrazone, flucarbazone, propoxycarbazone, and sulfentrazone.

The petitioner, Arvesta Corporation, is currently seeking use of amicarbazone on field corn. Amicarbazone is formulated as a 70% dry, flowable powder. It is intended for use in either conventional, conservation or no-tillage crop management systems and is for preplant surface, preemergence incorporation (mixed into the top 1-2 inch layer of soil), preemergence and post emergence applications. This herbicide works best when applied and subsequently moved into the soil by rainfall, sprinkler irrigation or mechanical tillage prior to weed emergence. Applications are to be made with ground equipment only. The product is not to be applied by aerial equipment or through irrigation systems.

Although an import tolerance is being sought on sugarcane, HED is recommending against such a tolerance due to the lack of field trial data on that crop.

Table 2.1. Summary of Directions for Use of Amicarbazone.[1]

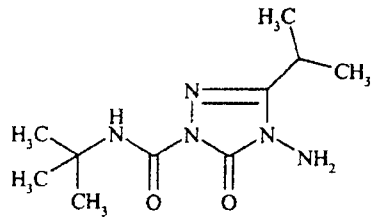
Applic. Timing; Type; Equip.	Applic. Rate (lb ai/A)	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ai/A)	PHI (days)	Use Directions and Limitations
Field Corn					
Early preplant (≥30 days prior to planting); Broadcast; Ground	0.448	NS	0.448	N/A	Use limited to Eastern Corn Belt: MN, MO, AR, 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%)		0.219-0.328 (soil OM <2%) 0.219-0.448 (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 IA 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	0.219		0.448		

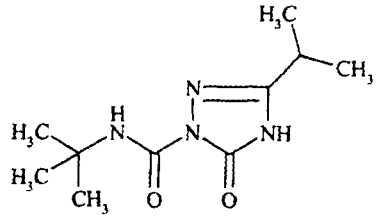
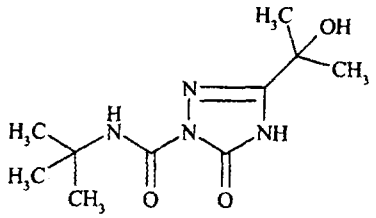
Field Corn					
Early Postemergence (emergence up to 10-leaf collar stage, V10); Broadcast or directed; Ground	0.098-0.153	NS	0.448	N/A	Application is to be made as a tank mix with recommended herbicides. Use of an adjuvant is recommended only when required for the tank mix herbicide; use of COC, MSO, or any adjuvant containing vegetable or petroleum oils is prohibited.
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.

2.2 Structure and Nomenclature

TABLE 2.2. Test Compound 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
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

2.3 Physical and Chemical Properties

TABLE 2.3. Physicochemical Properties

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})	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

3.0 Metabolism Assessment

3.1 Comparative Metabolic Profile

In an acceptable rat metabolism study using amicarbazone, 95% of the radioactive dose was recovered within 72 hours post dosing. Urinary excretion accounted for 64% of the dose, indicating substantial absorption, and fecal excretion accounted for 27% of the dose within 24 hours of dosing. The parent compound only accounted for approximately 3% of the radioactive dose, indicating rapid metabolism. Three major metabolites were identified in the excreta, iPr-2-OH DA MKH, tBu-OH DA MKH, and glucuronic acid conjugates. Based on the metabolic profile, the metabolism of MKH 3586 in rats primarily involves deamination followed by hydroxylation with elimination in the urine. The parent also undergoes glucuronic acid conjugation and elimination in the feces.

A second rat metabolism study was performed with an environmental degradate of amicarbazone, 4-methyl MKH 3586. The routes of excretion for this compound are similar to that of the parent. Over 90% of the radioactive dose was eliminated within 96 hours, the majority eliminated through the urine and feces within 24 hours of administration. Hydroxylation of the soil metabolite at the isopropyl moiety formed the major metabolite, 4-Me-i-Pr-2-OH DA MKH 3586, which was found primarily in urine. Further hydroxylation at the tertiary butyl group resulted in the formation of 4-Me-t-Bu-iPr-2-di-OH DA MKH 3586. Alternatively, additional hydroxylation of the isopropyl moiety formed 4-Me-iPr-1,2-di-OH DA MKH 3586. The pathway for the metabolism of 4-methyl DA MKH 3586 in rats primarily involves a series of hydroxylation reactions. No conjugates were detected.

Comparing studies in different species, the major plant and livestock metabolites DA MKH 3586 and iPr-2-OH DA MKH3586 are also observed in the rat. DA MKH 3585 is also a major environmental degradate. However, the other significant environmental degradate, N-methyl-desamino amicarbazone, is not seen as a rat metabolite.

3.2 Nature of the Residue in Foods

3.2.1. Description of Primary Crop Metabolism

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 corn metabolism study, the petitioner proposed that the major metabolic pathway in corn involves the deamination of the triazole amino group to form the desamino metabolite (DA MKH 3586) 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.

3.2.2 Description of Livestock Metabolism

The proposed mechanism of metabolism in livestock is 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.

3.2.3 Description of Rotational Crop Metabolism, including identification of major metabolites and specific routes of biotransformation

Radiolabeled amicarbazone was applied directly to silt loam soil at 0.9x the maximum proposed seasonal rate, and rotational kale, turnip, and wheat were planted 47, 138, and 364 days after treatment (DAT). Although a 30-day plant back interval was proposed, crops planted 30 DAT failed due to phytotoxicity.

Data indicate that uptake by rotational crops is likely to occur, with total radioactive residues (TRR) accumulating at ≥0.01 ppm in all rotated crops from all PBIs. The metabolites identified in the rotational crops were similar to those found in the primary crop corn metabolism study and the major metabolites were those in which the tertiary carbon of the isopropyl moiety had undergone deamination and oxidation. The total level of the glucose conjugates were higher in rotated wheat commodities than in corn commodities. Two minor metabolites identified in the rotational crops, triazolinone MKH 3586 and N-Me DA amicarbazone, were not found in the corn metabolism study.

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.

3.3 Environmental Degradation

Amicarbazone has a low vapor pressure (9.75×10^{-9} mm Hg at 30 °C) and is highly soluble in water (solubility 4,600 ppm at 20 °C). Based on submitted environmental fate studies, the chemical is moderately persistent in aerobic soil

($t_{1/2}$= 87 days determined for only one soil, shorter half-lives were determined under field conditions: $t_{1/2}$= 19 to 29 days). It appears to slowly degrade into a number of degradation products under different environmental conditions. Amicarbazone is stable to direct photolysis (indirect photolysis in natural pond water: $t_{1/2}$≈73 days); hydrolysis (except at pH 9: $t_{1/2}$= 66 days); and anaerobic aquatic metabolism ($t_{1/2}$= >4 years in the solid phase and 533 days in the water phase). With the exception of natural clear water bodies subjected to sunlight, the major route of dissipation of amicarbazone appears to be bio-transformation. In clear water bodies subjected to sunlight, indirect photolysis may contribute to amicarbazone dissipation. Although photolysis might occur on soil surfaces ($t_{1/2}$= 54 days), label recommended incorporation of the pesticide just after application may render this process unimportant.

Laboratory and field studies identified only three major degradates for amicarbazone: Des-amino (DA MKH 3586), N-methyl Des-amino and Decarboxamide. Des-amino and N-methyl Des-amino were both the result of biodegradation in aerobic soil in the laboratory and in the field, while Des-amino alone also resulted from indirect photolysis in aqueous systems. Decarboxamide resulted only from hydrolysis in alkaline aqueous systems (29% of applied dose after 30 hours at pH 9). Mobility studies were the only submitted studies for the major three degradates of amicarbazone; however, data from the single aerobic soil study on the parent suggests that both Des-amino and N-methyl Des-amino are highly persistent (did not show decline within a period of a year).

Available data suggest that amicarbazone is very highly mobile (K_{oc} range= 16.7 to 37.0 L kg^{-1}, from adsorption/desorption and aged leaching studies). Additionally, adsorption/desorption and aged leaching studies suggest that all of the three major degradates of amicarbazone are very highly mobile (ASTM 1996). Determined K_{oc} values were: Des-amino (K_{oc} range= 26.4 to 42.3 L kg^{-1}), N-methyl Des-amino (K_{oc} range= 34.3 to 56.4 L kg^{-1}) and Decarboxamide (K_{oc} range= 9.4 to 16.2 L kg^{-1}).

Given the moderate persistence/high mobility and solubility of the parent compound and the apparent high persistence/high mobility of its two degradates, amicarbazone is expected to dissipate slowly and at the same time be vulnerable to leaching/run-off. The Des-amino and N-methyl Des-amino degradates are also expected to behave similarly to the parent and become the major terminal degradates of this chemical in surface and ground water.

When amicarbazone is transported into alkaline ground water, surface water bodies and/or soils, the third degradate (Decarboxamide) is expected to become an important contaminant of surface and/or ground water for two reasons: it is the major hydrolysis degradate of parent in alkaline conditions and is highly mobile. The potential contamination of surface and ground water with this degradate is expected to be correlated to its persistence. Unfortunately, the only studies submitted on the degradates were adsorption/desorption, so that the persistence of decarboxamide is unknown.

3.4 Tabular Summary of Metabolites and Degradates

Table 3.4. Tabular Summary of Metabolites and Degradates

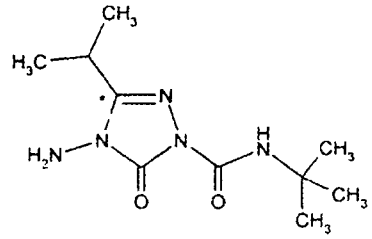
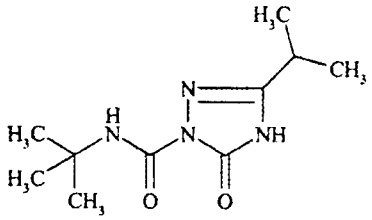
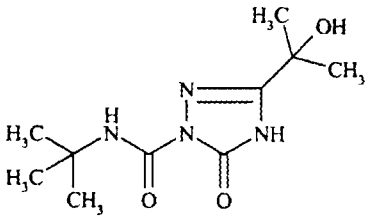
Chemical Name (other names in parenthesis)	Commodity	Matrices - Major Residue (>10%TRR)	Matrices - Minor Residue (<10%TRR)	Structure
Amicarbazone	Corn	forage, fodder, grain	none	
	Rotational Crops (47-day PBI)	kale, turnip top, wheat forage, wheat hay, wheat straw	turnip root	
	Ruminant	muscle, fat, liver, milk (day 1&2)	kidney	
	Poultry	liver	muscle, fat, eggs (day 1-3)	
	Rat	none	excreta	
	Water	major residue	none	
DA MKH 3586	Corn	grain, fodder	forage	
	Rotational Crops (47-day PBI)	kale, turnip top, wheat forage	turnip root, wheat hay, wheat straw	
	Ruminant	muscle, fat, kidney, liver, milk (day 1&2)	none	
	Poultry	eggs (days 1-3)	muscle, liver, fat	
	Rat	none	excreta	
	Water	aerobic soil	none	
iPr-2-OH DA MKH 3586	Corn	forage, fodder, grain	none	
	Rotational Crops (47-day PBI)	kale, turnip top, wheat forage, wheat hay, wheat straw	turnip root	
	Ruminant	kidney	muscle, fat, liver, milk (day 1&2)	
	Poultry	muscle, fat , eggs (days 1-3)	liver	
	Rat	excreta	none	
	Water	none	none	

Table 3.4. Tabular Summary of Metabolites and Degradates

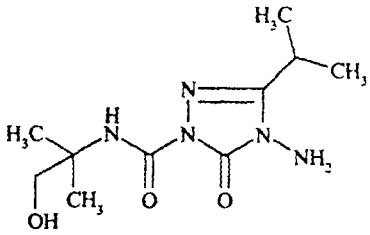
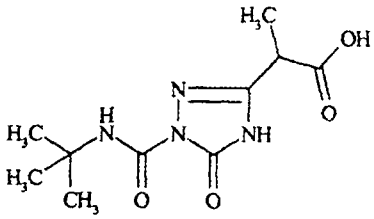
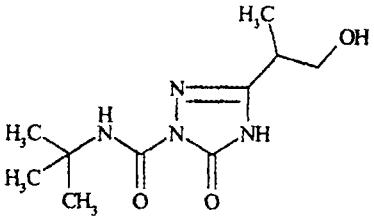
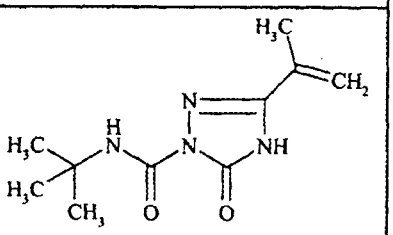
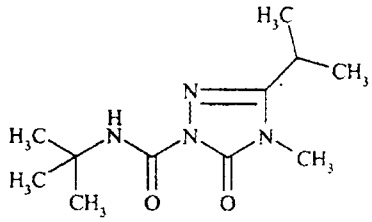
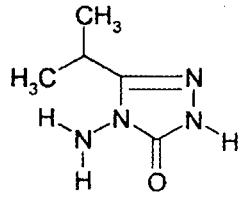
Chemical Name (other names in parenthesis)	Commodity	Matrices - Major Residue (>10%TRR)	Matrices - Minor Residue (<10%TRR)	Structure
tBu OH MKH 3586	Corn	NR	NR	
	Rotational Crops (47-day PBI)	NR	NR	
	Ruminant	muscle, kidney, milk (day 1-2)	fat, liver	
	Poultry	none	muscle, fat, eggs (days 1-3)	
	Rat	none	none	
	Water	none	none	
iPr-Acid DA MKH 3586	Corn	none	none	
	Rotational Crops (47-day PBI)	none	none	
	Ruminant	none	none	
	Poultry	liver	none	
	Rat	none	none	
	Water	none	none	
iPr-1-OH DA MKH 3586	Corn	none	none	
	Rotational Crops (47-day PBI)	none	none	
	Ruminant	none	none	
	Poultry	liver	muscle	
	Rat	none	none	
	Water	none	none	
iPr-ene-DA MKH 3586	Corn	none	none	
	Rotational Crops (47-day PBI)	none	none	
	Ruminant	liver	muscle, fat, kidney, milk (days 1-2)	
	Poultry	fat, eggs (days 1-3)	muscle, liver	
	Rat	none	none	
	Water	none	none	

Table 3.4. Tabular Summary of Metabolites and Degradates

Chemical Name (other names in parenthesis)	Commodity	Matrices - Major Residue (>10%TRR)	Matrices - Minor Residue (<10%TRR)	Structure
N-methyl DA MKH 3586	Corn	none	none	
	Rotational Crops (47-day PBI)	none	turnip top, wheat hay	
	Ruminant	none	none	
	Poultry	none	none	
	Rat	none	none	
	Water	indirect aqueous photolysis, aerobic soil, TFD	none	
Decarboxamide	Corn	none	none	
	Rotational Crops (47-day PBI)	none	none	
	Ruminant	none	none	
	Poultry	none	none	
	Rat	none	none	
	Water	hydrolysis, at pH = 9 only	none	

Corn; 45121633; 0.72 lbs ai/A; 1.6X label rate; 4 hours after planting; harvested 104-126 days post-application..
Goat; 45121630; 101 ppm;42X MTDB; 3 day dosing period.
Hen; 45121631; 15.3 ppm; 49X MTDB, 3 day dosing period.
Rotational Crops; 45121704; kale, turnip, wheat; 0.42 lbs ai/A, 0.9X label rate; applied directly to soil; 30-364 days.
Rat Metabolism; 45121701; 5.25-5.99 mg/kg gavage dose; Fischer rats.

3.5 Summary of Residues for Tolerance Expression and Risk Assessment

3.5.1 Tabular Summary

Table 3.5.1 Summary of Metabolites and Degradates to be included in the Risk Assessment and Tolerance Expression

Matrix		Residues included in Risk Assessment	Residues included in Tolerance Expression
Plants	Primary Crop	amicarbazone, DA MHK 3586, iPr-2-OH DA MKH 3586	amicarbazone, DA MHK 3586, iPr-2-OH DA MKH 3586
	Rotational Crop	amicarbazone, DA MHK 3586, iPr-2-OH DA MKH 3586	amicarbazone, DA MHK 3586, iPr-2-OH DA MKH 3586
Livestock	Ruminant	amicarbazone, DA MHK 3586, iPr-2-OH DA MKH 3586, tBuOH MKH 3586	amicarbazone, DA MHK 3586, iPr-2-OH DA MKH 3586
	Poultry	amicarbazone, DA MHK 3586, iPr-2-OH DA MKH 3586, iPr-Acid DA MKH 3586, iPr-1-OH DA MKH 3586, iPr-Ene DA MKH 3586	amicarbazone, DA MHK 3586, iPr-2-OH DA MKH 3586
Drinking Water		amicarbazone, des-amino (DA MKH 3586) and N-methyl-des-amino amicarbazone	Not Applicable

3.5.2 Rationale for Inclusion of Metabolites and Degradates

The residues of concern in field corn consist of the parent amicarbazone and the metabolites DA MKH 3586 (des-amino) and iPr-2-OH DA MKH. Based on the triazolinone ring still being present, HED can not rule out these metabolites as possessing similar toxicity as parent amicarbazone. They are also major metabolites (≥ 10% TRR) in at least two corn matrices (grain, fodder) and are determined along with the parent by the proposed tolerance enforcement method. Therefore, the risk assessment team concludes that these metabolites need to be included along with the parent as residues in the field corn tolerances and dietary risk assessment.

For livestock commodities the same residues as above are to be included in tolerances for enforcement purposes. The proposed livestock enforcement method measures all three compounds as iPr-2-OH DA MKH by oxidizing the parent and DA MKH 3586 with potassium permanganate. Each of the three compounds is

also a major residue in multiple matrices across the ruminant and poultry metabolism studies. For risk assessment purposes, the team concludes that additional metabolites need to be included with the three residues to be used for tolerance enforcement. In ruminants, the metabolite tBuOH MKH 3586 is to be included based on it being the highest level residue in milk (28% TRR) as well as a major metabolite ($\geq$ 10% TRR) in muscle and kidney. For poultry it is recommended that the metabolites iPr-Acid DA MKH 3586, iPr-1-OH DA MKH 3586, and iPr-Ene DA MKH 3586 be considered in the risk assessment due to their presence as major residues in liver, fat, or eggs. These additional metabolites to be included in the risk assessment all contain the triazolinone ring and therefore, as noted above, will be considered toxicologically equivalent to the parent.

In rotational crops the residues 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. These compounds are determined by the proposed plant enforcement method and were major residues in many of the samples analyzed in the confined study. In addition, quantifiable levels of these residues were observed in all matrices except turnip roots in the field rotational crop studies. For risk assessment purposes in rotational crops, the same three residues should be considered. In addition, for assessing human dietary exposure to residues in wheat grain (or any other rotated cereal grains), the two glucose conjugates of DA OH MKH 3586 should be included. These conjugates constituted about 30% of the grain TRR at the 138 and 364 day plant back intervals. Although these two conjugates were also major residues in the feed items wheat forage, hay and straw, the combined levels of the parent, DA MKH 3586, and iPr-2-OH DA MKH 3586 exceed the total levels of the conjugates in forage and hay, the feed items contributing the most to the livestock dietary burdens. Therefore, the risk assessment team has concluded that the use of the parent, DA MKH 3586, and iPr-2-OH DA MKH 3586 provides a reasonable estimate of livestock dietary burden and the conjugates do not need to be considered in the livestock component of the exposure assessment.

The risk assessment team has determined that the residues of concern in drinking water for dietary risk assessment are the parent amicarbazone and the des-amino (DA MKH 3586) and N-methyl-des-amino degradates. The team's rationale is presented below.

Amicarbazone is highly soluble in water, moderately persistent in aerobic soil ($t_{1/2}$= 87 days for one soil under laboratory conditions with shorter half-lives determined under field conditions: $t_{1/2}$= 19 to 29 days), and highly mobile. Amicarbazone is stable to direct photolysis (indirect photolysis in natural pond water: $t_{1/2}$=73 days); hydrolysis (except at pH 9: $t_{1/2}$= 66 days); and anaerobic aquatic metabolism. The three major degradates observed in the fate studies are the des-amino (DA MKH 3586), N-methyl-des-amino, and decarboxamide (see Table 3.6.2). All three of these degradates are very highly mobile. Data from the single aerobic soil study on the parent suggest that both des-amino (DA MKH 3586) and N-methyl-des-amino

are highly persistent (did not show decline within a period of a year). The decarboxamide was observed only in the alkaline hydrolysis study.

Given the moderate persistence/high mobility and solubility of the parent and the apparent high persistence/high mobility of its two degradates, parent is expected to dissipate slowly and at the same time be vulnerable to leaching/run-off. The resultant des-amino and N-methyl-des-amino degradates are also expected to behave similarly to the parent and become the major terminal residues of this chemical in surface and ground water. Only in cases where amicarbazone is transported into alkaline ground water, surface water bodies and/or soils, the third degradate (decarboxamide) is expected to become important. Amicarbazone is not recommended to be applied on calcareous soils with pH values substantially higher than 7.

With respect to the toxicity of the three degradates, HED concludes that they can not be ruled out from having similar toxicity as the parent based on the presence of the triazolinone moiety in all their structures.

Using the above information, the parent plus the des-amino and N-methyl-des-amino degradates need to be included in the drinking water component of the risk assessment based on the persistence and high mobility of these degradates along with their assumed similar toxicities to the parent. The decarboxamide does not need to be included in drinking water assessments due to the limited conditions ($pH \geq 9$) under which it could be a major degradate.

For modeling purposes, the acute exposure from water was calculated using only the parent compound. Although acute exposures at time points well after application may include the degradates, the total exposure to parent and degradates at any point in time is not expected to exceed the maximum modeled concentration of the parent. In this modeling scenario, the half-life of the parent in the aerobic soil metabolism study (87 days) was multiplied by three. Therefore, the parent was treated as being practically stable for this scenario.

Although parent plus its two main degradates (des-amino and N-methyl-des-amino) are present at the time-line of chronic exposure, only the two degradates were considered for modeling purposes. The decision was based on three reasons. The first reason is that the half-life calculated for residues (which include parent plus degradates) was nearly stable and in modeling of individual degradates, their half-lives were assumed to be stable. The second reason is that molecular conversion ratios from parent to degradates are 0.938 and 0.996 (i.e. application rate will not be reduced significantly by including just the degradates). The third reason is that degradates are expected to be the prominent species (rather than the parent) in long-term exposure (i.e. chronic exposure).

Table 3.5.2 Maximum concentrations of degradation products found in submitted fate studies.

	Max Degradate Concentration (% of applied) and Time (days) to Max Concentration					*Analyzed Degradates*	
Degradate	***Hydrolysis*** [2]	***Indirect Aqueous Photolysis*** [1]	***Aerobic Soil***	***Anaerobic Aquatic***	***Aerobic Aquatic***	***TFD***	***Ground Water***
Des-amino		**16% (35 days)**	**34% (180-270 days)**			**26-27% (59-61days)**	
N-methyl Des-amino			**11% (360 days)**		**No Data**		**Study is underway**
Decarboxamide	**29% (30 days) Only @ pH 9**						

[1] In natural pond water, pH 8.4, ***note*** that parent is stable to direct aqueous photolysis.

4.0 Hazard Characterization/Assessment

4.1 Hazard Characterization

The toxicology database for amicarbazone is complete and there are no data gaps. The scientific quality of the database for amicarbazone is adequate and the toxicity profile can be characterized for all effects, including potential developmental, reproductive, and neurotoxic.

The acute toxicity data indicate that amicarbazone has low toxicity via the oral (category III), dermal and inhalation (both category IV) routes of exposure. Amicarbazone was slightly irritating to the eyes of rabbits (category III), but the irritation cleared by day 7 post exposure. This chemical is neither a skin irritant nor sensitizer.

The database on amicarbazone indicates that general toxicity and liver changes are the major effects of this chemical. Decreased body weight and body weight gain were observed in studies with rats, mice, and rabbits. In many studies, decreased body weight and body weight gain were the most sensitive endpoints. In chronic dog and subchronic rat and dog studies, liver effects were seen, including increased absolute and relative liver weights, slight liver hypertrophy, dilation of sinusoids, and increased liver enzymes.

Slight effects on the thyroid gland were also seen in the subchronic rat and dog studies. These effects included such changes as increased T3, T4, and hyperplasia. However, these thyroid effects were not observed in the chronic rat nad dog studies. Mechanistic studies were performed in rats to further characterize amicarbazone's effects on the thyroid. The mechanistic studies showed that there were no treatment related effects on thyroid weight or microscopic changes in the thyroid at any dose. Thyroid to blood ratios of ^{125}I in treated groups were comparable to negative controls, indicating the increase in thyroid hormones is most likely not due to increased synthesis; thus, differences in thyroid hormones may be due to metabolism at another site. The liver is implicated as the extra-thyroidal site based on increased liver weights and UDP-glucuronosyltransferase activity.

In developmental studies with rats and rabbits where amicarbazone was administered by gavage, maternal toxicity was observed in the form of decreased body weight (rats), body weight gain (rats and rabbits), and food consumption (rats). The development LOAEL in the rat study was based on multiple skeletal developmental retardations (incomplete ossification/unossification was observed in parietal bones, interparietal bones, supraoccipital bones, squamosal bones, zygoma, pubis, xiphoid, and fontanelle). However, delayed ossification in the absence of decreased fetal viability (as observed in the 2-generation rat study), is generally not considered to be more severe or qualitatively more significant than decreased body weight and body weight gain in treated maternal animals. In the developmental

rabbit study, maternal animals were more sensitive than the developing fetuses to amicarbazone. The maternal NOAEL was 5 mg/kg/day, while the developmental NOAEL was 20 mg/kg/day. Fetal rabbit effects were decreased body weights and increased incidences of incomplete ossification. In the 2-generation reproduction study, both maternal and developmental endpoints were based on decreased body weight and body weight gain, indicating that there were no differences in qualitative or quantitative susceptibility for the 2-generation reproduction study in rats.

Neurotoxic effects were seen in an acute neurotoxicity study in rats. The clinical signs included eyelid ptosis, decreased approach response in both sexes, and red nasal staining in males. In a non-guideline study looking at the central nervous system (CNS) response in mice, a single oral dose (100 mg/kg) was found to cause minimal functional impairment in males. This impairment was characterized by increased reaction times to nociceptive stimuli, decreased traction force, impaired motor coordination, sedation, partial ptosis, and mild anticonvulsive effects. In another non-guideline behavioral study in rats, the following clinical signs were observed: sedation, ptosis, salivation. Additionally, at the HDT, piloerection, Straub phenomenon, and prone position were observed. The effects were observed at 30 minutes post dose with apparent recovery by 150 minutes post dose; the higher dose groups showed greater persistence of effects. A dose- and time-dependent effect was demonstrated on motor activity: decreased travel distance, increased resting time, and decreased rearing. Maternal clonic convulsions were observed in 3/27 rats in the developmental rat study following exposure to 300 mg/kg/day amicarbazone. Contrary to these signs of neurotoxicity, the subchronic and developmental neurotoxicity studies in rats did not result in any signs of neurotoxicity up to 100 mg/kg/day.

There were no systemic effects observed in the 21-day dermal toxicity study in rats exposed to amicarbazone. No treatment related signs of dermal irritation were observed.

Both the rat and mouse carcinogenicity studies indicate that amicarbazone is not likely to be a human carcinogen. At the doses tested there was no treatment related increase in tumor incidence when compared to controls. Dosing was considered adequate based on body weight and body weight changes in both sexes.

The mutagenicity battery for this chemical is complete and indicates that amicarbazone is not a mutagen.

Metabolism studies in rats indicate that amicarbazone and 4-methyl amicarbazone are rapidly excreted from the body. The majority of the dose (91-95%) was excreted 72-96 hours post dosing. Urinary excretion within 24 hours accounted for 64-70% of the radioactive dose, indicating substantial absorption of the chemical. Fecal excretion of amicarbazone accounted for approximately a third of the radiolabeled dose after 24 hours. Fecal excretion accounted for approximately

10% of 4-methyl amicarbazone 24 hours post dosing.

Table 4.1a Acute Toxicity Profile - Test Substance				
Guideline No.	**Study Type**	**MRID(s)**	**Results**	**Toxicity Category**
870.1100	Acute oral - Wistar rats	45121504	Males LD_{50} > 2050 mg/kg Females LD_{50} = 1015 mg/kg	III
870.1200	Acute dermal - Wistar rats	45121503	LD_{50} > 5000 mg/kg	IV
870.1300	Acute inhalation - Wistar rats	45121506	LC_{50} > 2.030 mg/L	IV
870.2400	Acute eye irritation - New Zealand white rabbit	45121510	Eye irritation was present (including corneal opacity) at 24 hours, but had cleared by day 7.	III
870.2500	Acute dermal irritation - New Zealand white rabbit	45121509	Primary irritation indices were 0	IV
870.2600	Skin sensitization - Hartley guinea pig	45121628 45121505	All scores during induction and challenge periods were 0.	Not a dermal sensitizer

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.3100 90-Day oral toxicity in rats (Fischer-344)	45121523 (1997) Acceptable/Guideline 0, 100, 250, 500, 1000, 2500, 5000ppm M: 0, 6.9, 18, 33, 67, 182, 354 mg/kg/day F: 0, 7.9, 21, 38, 78, 201, 397mg/kg/day	NOAEL = 33/38 mg/kg/day LOAEL = 67/78 mg/kg/day based on decreased BW ♀and overall (weeks 1-13) BWG, decreased red cell indices, clinical chemistry (increased cholesterol, T4 and T3 ♂, O-demethylase ♀, N-demethylase ♂), increased relative liver weights ♀, and histopathology effects in males (minimal hepatocytomegaly and minimal pigmentation in the spleen)
870.3150 90-Day oral toxicity in dogs (beagle)	45121524 (1998) Acceptable/guideline 0, 200, 800, or 2000 ppm M: 0, 6.74, 27.03, or 57.40 mg/kg/day F: 0, 6.28, 24.99, or 62.11mg/kg/day	NOAEL = 6.28 mg/kg/day LOAEL = 24.99 mg/kg/day based on increased thyroid vacuolization and decreased food consumption and glucose in females; increased platelets, phosphate, bile acids, absolute and relative liver weights, and lymphoid hyperplasia of the gall bladder in males; and decreased albumin and increased triglycerides, N-demethylase, and O-demthylase in both sexes.
870.3200 21/28-Day dermal toxicity in rats (Sprague-Dawley)	45121525 (1998) Acceptable/Guildline 0, 200, 500, 1000 mg/kg/day	NOAEL = 1000 mg/kg/day LOAEL = Not Observed
870.3700a Prenatal developmental in rats (Sprague-Dawley)	45121530 (1999) Acceptable/Guideline 0, 15, 100, 300 mg/kg/day	**Maternal** NOAEL = 15 mg/kg/day LOAEL = 100 mg/kg/day based on decreased BW/BWG and food consumption, and increased incidences of hard stools. **Developmental** NOAEL = 15 mg/kg/day LOAEL = 100 mg/kg/day based on multiple skeletal development retardations (incomplete ossification/unossification was observed in parietal bones, interparietal bones, supraoccipital bones, squamosal bones, zygoma, pubis, xiphoid, and fontanelle
870.3700b Prenatal developmental in rabbits (Himalayan)	45121627 (1999) Acceptable/Guideline 0, 5, 20, 70 mg/kg/day	**Maternal** NOAEL = 5 mg/kg/day LOAEL = 20 mg/kg/day based on decreased BWG during treatment. **Developmental** NOAEL = 20 mg/kg/day LOAEL = 70 mg/kg/day based on decreased fetal BW, and increased incidences of incomplete ossification of the 5th medial phalanx (bilateral) and the 13th caudal vertebra, and slightly thick ribs.
870.3800 Reproduction and fertility effects (Sprague-Dawley)	45121625 (1998) Acceptable/Guideline 0, 100, 500, 1000 ppm M: 0, 6.4, 33.9, 73.2	**Parental/Systemic** NOAEL = 6.4/7.3 mg/kg/day LOAEL = 33.9/38.7 mg/kg/day based on decreased BW/BWG in both sexes. **Reproductive** NOAEL = 73.2/84.0 mg/kg/day LOAEL = Not Observed **Offspring** NOAEL = 6.4/7.3 mg/kg/day

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.4100a Chronic toxicity in rats (Fischer-344)	45121512 (1999) Acceptable/Guideline 0, 50, 500, 1250/1000 ppm M: 0, 2.3, 25.3, 67 mg/kg/day F: 0, 2.7, 29.5, 65 mg/kg/day	NOAEL = 2.3/2.7 mg/kg/day LOAEL = 25.3/29.5 mg/kg/day based on decreased BW in ♀ and BWG in both sexes. At the doses tested there was not a treatment related increase in tumor incidence when compared to control. Dosing was considered adequate based on decreased BW in ♀ and BWG in both sexes.
870.4100b Chronic toxicity dogs (beagle)	45121529 (1999) Acceptable/Guideline 0, 75, 100, 300, 1200 ppm M: 0, 1.6, 2.5, 8.9, 31.5 mg/kg/day F: 0, 1.8, 2.3, 8.7 34.6mg/kg/day	NOAEL =2.5/2.3 mg/kg/day LOAEL = 8.9/8.7 mg/kg/day based on effects on the liver, including increased absolute and relative liver weights, and O-demethylase in ♂ ; increased globulin and cytochrome P-450 in ♀; and increased triglycerides and cholesterol in both sexes.
870.4300 Carcinogenicity mice (CD-1)	45121604 (1999) Acceptable/Guideline 0, 100, 1500, 4000 ppm M: 0, 15.7, 244.7, 709.0 mg/kg/day F: 0, 17.9, 275.0, 806.3 mg/kg/day	NOAEL = 244.7/275.0 mg/kg/day LOAEL = 709.0/806.3 mg/kg/day based on decreased BW and BWG in both sexes, and subclinical anemia, and hemosiderin pigmentation of the spleen in ♂. **no evidence of carcinogenicity** At the doses tested there was not a treatment related increase in tumor incidence when compared to control. Dosing was considered adequate based on decreased BW and BWG in both sexes, and subclinical anemia, and hemosiderin pigmentation of the spleen in ♂.
870.7485 Metabolism study in rats (Fischer-344)	45121701 (1997) Acceptable/guideline MKH 3586	95% of the radioactive dose was recovered within 72 hours following dosing. The majority of the dose was recovered from the urine within 24 hours (64%), indicating substantial absorption. Fecal excretion accounted for 27% of the dose within 24 hours. Major metabolites were DA MKH, N-methyl DA MKH, and decarboxamide.
870.7485 Metabolism study in rats (Fischer-344)	45121634 (1999) Acceptable/Guideline 4-methyl MKH 3586	91% of the radioactive dose was recovered within 96 hours. Urinary excretion accounted for 70% of the radioactive dose within 12 hours, showing substantial absorption. Only 8% of the radioactive dose was excreted via the feces within 24 hours.

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.5100 Bacterial reverse mutation test	45121617 (1999) Acceptable/Guideline 0, 16, 50, 158, 500, 1581, or 5000 µg/plate in the presence and absence of S9-activation	There was no evidence of induced mutant colonies over background.
870.5100 Bacterial reverse mutation test	45121616 (1996) Acceptable/Guideline 0, 16, 50, 158, 500, 1581, or 5000 µg/plate in the presence and absence of S9-activation	There was no evidence of induced mutant colonies over background.
870.5100 Bacterial reverse mutation test	45121519 (1995) Acceptable/Guideline 0, 16, 50, 158, 500, 1581, or 5000 µg/plate in the presence and absence of S9-activation	There was no evidence of induced mutant colonies over background.
870.5300 *In vitro* mammalian cell gene mutation test	45121514 (1997) Acceptable/Guideline 0, 250, 500, 1000, 2000, or 4000 µg/mL in the presence and absence of S9 activation for 5 hours 4000 µg/mL was the limit of solubility	There was no evidence that MKH3586 induced mutant colonies over background in the presence or absence of S9-activation.
870.5375 *In vitro* mammalian chromosome aberration test	45121513 (1997) Acceptable/Guideline 0, 1000. 2000, 4000, or 5000 µg/mL for 4 hours in the presence and absence of S9-activation	There was no evidence of chromosome aberration induced over background in the presence or absence of S9-activation.
870.5395 Mammalian erythrocyte micronucleus test	45121515 (1997) Acceptable/Guideline 100 mg/kg harvest times 16, 24, and 48 hours post dosing	There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow at any treatment time.

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.6200a Acute neurotoxicity screening battery in rats (Fischer-344)	45121528 (1996) Acceptable/Guideline in conjunction with 45121527 M: 0, 20, 150, 600 mg/kg/day F: 0, 20, 100, 400 mg/kg/day	NOAEL = 10 mg/kg/day LOAEL = 20 mg/kg/day based on eyelid ptosis, decreased approach response (both sexes), and red nasal staining in ♂. A series of acute neurotoxicity studies were performed, the NOAEL for this study comes from 45121527.
870.6200b Subchronic neurotoxicity screening battery in rats (Fischer-344)	45121532 (1999) Acceptable/Guideline 0, 100, 500, 1000 ppm M: 0, 6.7, 33.4, 66.5 mg/kd/day F: 0, 7.8, 38.2, 75.8 mg/kg/day	**Female** NOAEL = 7.8 mg/kg/day LOAEL = 38.2 mg/kg/day based on decreased BW and overall BWG in ♀. Male NOAEL = 66.5 mg/kd/day LOAEL = was not observed for males.
870.6300 Developmental neurotoxicity in rats (Wistar)	45441301 (2001) 0, 100, 500, 1000 ppm (gest): 0, 8, 39, 91 mg/kg/day (lact): 0, 17, 84, 177 mg/kg/day	**Maternal** NOAEL = 8 mg/kg/day LOAEL = 39 mg/kg/day based primarily on decreased feed efficiency (combination of decreased BWG and increased food consumption) during lactation. **Offspring** NOAEL = 39 mg/kg/day LOAEL = 91 mg/kg/day based on decreased BWG.
Subchronic mechanistic feeding study in rats (Fischer-344)	45121603 (1999) Acceptable/Non-guideline 0, 50, 1250, or 2500 ppm for 10 weeks M:0, 0.8, 19.4, 40.0 mg/kg/day F: 0, 0.6, 19.4, 28.8 mg/kg/day	Thyroid hormones were increased in the ≥ 19.4 mg/kg/day females and 40.0 mg/kg/day males. However, thyroid to blood ratios of ^{125}I in treated groups were comparable to negative controls, indicating there was no impairment of thyroid hormone synthesis. Thus, the differences in thyroid hormones is probably due to metabolism at an extra-thyroidal site. The liver was implicated as this site because liver weights and UDP-glucuronosyltransferase activity were increased.
In vitro studies on enzymes of thyroid hormone regulation	45121618 (1998) Acceptable/Non-guideline	MKH 3586 does not affect the iodide organification step of thyroid hormone synthesis or the peripheral metabolism of thyroid hormones via Type I or Type II deiodinases *in vivo*. These findings support the subchronic mechanistic studies in rats which indicate that upregulation of UDP-glucuronosyl transferase in the liver may account for alterations in thyroid hormone profile.

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
Non-guideline behavioral study in rats (HsdCbp WU)	45121511, 45121521, 45121629 Acceptable/Non-guideline 0, 1.0, 2.5, 5, 10, 20, or 100 mg/kg	The following clinical signs were observed: sedation, ptosis, salivation. Additionally at the HDT, piloerection, Straub phenomenon, and prone position were observed. The effects were observed at 30 minutes post dose, and no effect was observed at 150 minutes post dose, with the higher dose groups showing greater persistence of effects. A dose- and time-dependent effect was demonstrated on motor activity - decreased travel distance, increased resting time, and decreased rearing.
Non-guideline study of central nervous system safety pharmacoogy in mice (HsdWin: NMRI)	45121522 Acceptable/Non-guideline 0, 1, 20, 100 mg/kg/day	The data indicate that a single dose of MKH 3586 at 100 mg/kg causes minimal CNS functional impairment, characterized by increased reaction times to nociceptive stimuli, reduced traction force, impaired motor coordination, sedation, partial ptosis, and a mild anticonvulsive effect.

4.2 FQPA Hazard Considerations

4.2.1 Adequacy of the Toxicity Data Base

The toxicology database for amicarbazone is complete and adequate for purposes of risk assessment; there are no data gaps. Acceptable developmental toxicity studies for both rat and rabbit were submitted, along with an acceptable 2-generation reproduction study in rats.

4.2.2 Evidence of Neurotoxicity

The acute neurotoxicity study in rats showed several signs of neurotoxicity including eyelid ptosis, decreased approach response (both sexes) and red nasal staining in males. Two non-guideline studies also yielded signs of neurotoxicity in both rats and mice. These signs included eyelid ptosis, sedation, decreased response to nociceptive stimuli, and decreased motor coordination. At the high dose in the rat developmental study, 3/27 maternal rats demonstrated clonic convulsions. However, no neurotoxic effects were seen in adults in the subchronic neurotoxicity or pups in the developmental neurotoxicity studies in rats.

Acute Neurotoxicity Screening Battery in Rats

EXECUTIVE SUMMARY - In an acute oral neurotoxicity study (MRIDs 45121528), MKH 3586 (amicarbazone; 98.2% a.i., Lot/Batch # 17004/93) in 0.5% methylcellulose-0.4% Tween 80 in deionized water was administered in a single dose by gavage (10 mL/kg) to fasted Fischer 344 rats (12/sex/dose) at doses of 0, 20, 150 or 600 mg/kg in males and 0, 20, 100 or 400 mg/kg in females. All animals were observed for up to 14 days post-dosing. Functional observational

battery (FOB) and motor activity were evaluated pretreatment and on Days 0 (at the time of peak effect, approximately 3 hours post-dosing), 7 and 14. At termination, 6 rats/sex/group were perfused *in situ* for neurohistological examination and tissues from the control and 600/400 mg/kg groups were examined microscopically (brain tissue sections at level 5 from the 150/100 mg/kg groups were also evaluated). Additionally, two non-guideline acute oral studies were submitted. The purpose of the first study (MRID 45121526) was to investigate neuropathology (in particular, the time of peak effect and the possible effects on other brain regions at other time points) and to establish if a dose of 400 mg/kg produces changes in quantitative electroencephalography (qEEG) at 8 hrs or 1, 3, 7, 15 or 28 days following acute exposure to seven sets of 6 female rats/dose group at 0 or 400 mg/kg. Groups were sacrificed at 8 hrs and 1, 3, 7, 15 or 28 days post-dosing for neuropathological evaluation. The purpose of the second study (MRID 45121527) was to establish a NOAEL for clinical signs and FOB (the most sensitive parameters) in 6 rats/sex/dose group at 0, 2.5 or 10.0 mg/kg, single gavage dose. Acceptable positive control data (MRIDs 42770301 and 43656301) were previously reviewed.

At 20 mg/kg, during the FOB, increased incidences (# affected/12 vs. 0 controls) of eyelid ptosis (1-3 of both sexes during home-cage and 7 males in the open-field) and decreased approach response (no reaction in 2-6, both sexes) were observed on Day 0 (approximately 3 hours post-dosing). Additionally, eyelid ptosis and red nasal stain were noted during daily clinical observations in the males on Day 0.

At ≥150/100 mg/kg, increased incidences of oral stain, red nasal stain and urine stain (females only) were noted as clinical signs beginning on Day 0 and persisting up to one week, except for urine stain (Day 14). The following treatment-related FOB effects (# affected/12 vs. 0/12 controls, unless otherwise indicated) were observed on Day 0 (approximately 3 hours post-dosing): **home cage**, eyelid ptosis in both sexes (9-12); **handling**, (i) palpebral closure (half-closed to completely closed) in the females (1-12), (ii) ease of removal (minimal resistance with vocalizations to avoiding the hand) in males (1-6), (iii) mild nasal stain (red or clear) in both sexes (2-11), and (iv) mild oral stain (clear or red) in both sexes (2-7); **open field**, eyelid ptosis in the males (9-12) and in the females (6-11 treated vs 1 control); and **reflex/physiologic**, decreased approach response (no reaction) in the males (7-11 treated vs 4 controls) and impaired righting reflex (slight incoordination to landing on side) in the females (2-4). Additionally, decreases ($p<=0.05$) in mean body temperature were observed in both sexes (34.5-36.4°C treated vs. 37.0-37.1°C controls). Total **locomotor activity** was dose-dependently decreased on Day 0 in the ≥100 mg/kg females (↓30-43%, not statistically significant) and both total motor/locomotor activities reduced in males at 150 mg/kg (↓56-66%, not significant).

At 600/400 mg/kg, treatment-related mortality was observed in the males (5/12) and females (1/12) on Days 0 or 1 post-dosing. Increased incidences of the following were noted at the daily clinical evaluation on Day 0 in both sexes: eyelid

ptosis (5-6), clear nasal discharge (4-5) and salivation (3-4). The following treatment-related effects were observed in the FOB evaluation on Day 0 in both sexes (unless otherwise indicated): **home cage**, (i) repetitive forepaw movements (1-3), (ii) decreased activity (1-3), (iii) mild tremors (3 males) and (iv) chewing movements (2 males); **handling**, palpebral closure (half-closed to completely closed) in the males (12) and rigid muscle tone (1-4); **open field**, (i) mild tremors (3 males), (ii) decreased arousal, some to minimal movement (6 treated females vs 1 control) and (iii) tail mutilation (2 females); and **reflex/physiologic**, decreased approach response (no reaction) in the females (11 treated vs 4 controls) and impaired righting reflex, slight incoordination to landing on side in males (3 treated vs 1 control). The only FOB effects that persisted were red nasal stain in one 600 mg/kg male and red oral stain in one 400 mg/kg female on Day 7 and tail lesions (due to self-mutilation) in two 400 mg/kg females on Days 7 and 14. Lack of **motor/locomotor** effects in males at 600 mg/kg may have been related to the excessive toxicity/mortality at that dose, based on extreme variability of activities in individual animals of that group. Slight neuronal necrosis in the midline of the thalamus (reuniens nucleus) was observed in 1/6 male and 1/6 female (both animals survived to Day 14). No compound-related effects on body weight, brain weight or gross pathology were observed at any dose.

The two non-guideline neurotoxicity studies satisfied the purposes for which they were intended. The first study (MRID 45121526) verified the neuropathological effects observed in the main study high-dose males and females and the time of peak effect at 400 mg/kg. One female sacrificed 24 hr post-dosing had slight neuronal necrosis of the thalamus in the area of the reuniens nucleus. Quantitative EEG demonstrated treatment-related electrophysiological changes, primarily increases in total power, absolute delta, theta and alpha, and total power within the theta-beta range at 3, 8 and 24 hr post-dosing (under normotonic, auditory, visual and somatosensory stimulus conditions). A NOAEL for neurotoxicity was not established in the guideline study; the supplemental study MRID 45121527 established a NOAEL for clinical signs and FOB parameters at 10 mg/kg.

The neurotoxicity LOAEL is 20 mg/kg based on eyelid ptosis and decreased approach response (both sexes) and red nasal stain(males). The NOAEL is 10 mg/kg.

The study is classified as **acceptable/guideline** in conjunction with MRID 45121527 and satisfies the guideline requirement (OPPTS 870.6200a; OECD 424) for an acute neurotoxicity screening battery in rats.

Subchronic Neurotoxicity Study in Rats

EXECUTIVE SUMMARY - In a subchronic neurotoxicity study (MRID 45121532), MKH 3586 (amicarbazone; ≥98.2% a.i., Lot/Batch # 17004/93) was administered in the diet for 13 weeks to 12 Fischer 344 rats/sex/dose at doses of 0, 100, 500 or 1000 ppm (equivalent to 0/0, 6.7/7.8, 33.4/38.2 or 66.5/75.8 mg/kg/day

[M/F]). Functional observational battery (FOB) and motor activity were evaluated during Weeks -1 (prior to dosing), 4, 8 and 13. At termination, 6 rats/sex/group were perfused *in situ*, and tissues from the control and 1000 ppm groups were examined microscopically. Acceptable positive control data (MRIDs 42770301 and 43656301) were previously reviewed by the Agency.

No compound-related effects on mortality, clinical signs, FOB, motor or locomotor activity, ophthalmology, brain weight or gross and neuropathology parameters were observed at any dose. Motor activity data indicated that habituation was unaffected by treatment.

In the ≥500 ppm females, decreases ($p<=0.05$) were observed in body weights (↓ 6-7%, Weeks 10-13), overall (Day 0-91) body weight gains (calculated by reviewers, decr 17-22%). Food consumption was reduced (↓ 6-12%) when expressed as g/animal/day but total mean food consumption for the entire study was comparable for all groups on a g/kg bw/day basis (91.0, 90.3, 88.5 and 87.8 g/kg bw/day, controls to high dose). Body weights, overall body weight gains and food consumption were similar to controls throughout the study in the males. The LOAEL is 500 ppm (equivalent to 38.2 mg/kg/day [females]) based on decreased body weight and overall body weight gain. The NOAEL is 100 ppm (equivalent to 7.8 mg/kg/day [females]). A NOAEL was not established for males.

No evidence of neurotoxicity was observed at any dose.

The submitted study is classified as acceptable/guideline and satisfies the guideline requirements (OPPTS 870.6200b) for a subchronic neurotoxicity screening battery in rats.

Developmental Neurotoxicity Study in Rats

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (2001, MRID 45441301), MKH 3586 (97.8-98.4% a.i., batch # 05362/0005) was administered to parent female Wistar rats in the diet at concentrations of 0, 100, 500 or 1000 ppm from gestation day 0 through postnatal day (PND) 21. The average daily intake of MKH 3586 was approximately 0, 8, 39 and 91 mg/kg/day during gestation and 0, 17, 84, and 177 mg/kg/day during lactation, for the 0, 100, 500, and 1000 ppm groups, respectively. A Functional Operational Battery (FOB) was performed on 10 dams/dose on gestation days 6, 13, and 20 and on and lactation days 4, 11, and 21. On postnatal day 4, litters were culled to yield four males and four females. Offspring, representing at least 20 litters/dose, were allocated for periodic detailed clinical observations including (abbreviated FOB), assessment of motor activity, assessment of auditory startle response habituation, assessment of learning and memory, and neuropathology at study termination (day 75 of age). Thyroid and neural tissues (10/sex/dose) were assessed histologically on PND 11 and at study termination. Pup physical development was assessed by bodyweight, and sexual

maturation of females (age at vaginal opening) and of males (age at completion of balano-preputial separation) was determined.

Maternal Toxicity. Treatment-related effects were limited to body weight and gain, increased food consumption and decreased food efficiency. During *gestation*, body weight at 1000 ppm was decreased ($p<0.05$) of 5% on GD 6, with differences persisting to GD 20 (4%, $p < 0.05$) associated with a 12% decrease ($p<0.05$) in body weight gain from GD 0 to GD 20. The high dose group was also associated with increased food consumption (13%) and associated decrease in feed efficiency (22%). During *lactation*, maternal body weight was decreased for high-dose rats; decreases averaged 4% on LD 0 to 9% on LD 14 and LD 21 ($p<0.05$ or 0.01). Non significantly lower body weights were noted for the 500 ppm dose group of 2-3% but body weight gain during lactation was reduced 28% at 500 ppm and 45% at 1000 ppm. Food consumption during lactation was increased 7% at 500 ppm and 8% at 1000 ppm. The combination of body weight decrease and increase food consumption resulted in a decrease in food efficiency or 33% at 500 ppm and 49% at 1000 ppm. The maternal LOAEL is 500 ppm (39 mg/kg/day) based primarily on decreased feed efficiency (combination of decreased body weight gain and increased feed consumption) during lactation. The maternal NOAEL is 100 ppm (8 mg/kg/day).

Offspring Toxicity. Treatment-related effects for offspring were also limited to decreased body weight and body weight gain at 500 ppm and above. At birth, the average body weight of treated offspring was not different from controls at any dose level. By PND 11, body weight was decreased 7-8% ($p<0.05$) for high-dose male and female offspring, this decrease averaged 11-12% ($p<0.01$) by weaning on PND 21. Body weight gain was decreased in males and females in the 500 (8-13 %; $p<0.05$ or $p<0.01$) and 1000 ppm (11-21 %; $p<0.05$ or $p<0.01$) groups. Offspring in the 500 ppm group had recovered by termination; however, decreased body weight gain persisted to study termination in high-dose animals. The offspring LOAEL is 500 ppm (91 mg/kg/day) based on decreased body weight gain. The offspring NOAEL is 39 mg/kg/day.

4.2.3 Developmental Toxicity Studies

The developmental rat study yielded maternal and developmental NOAELs = 15 mg/kg/day. The maternal NOAEL was based on decreased body weight, body weight gain, and food consumption and increased incidence of hard stool observed at the LOAEL. The developmental NOAEL was based on multiple skeletal development retardations including incomplete and unossified bones. The fetal effects may or may not be a lasting adverse effect. However, in the absence of decreased fetal viability in the 2-generation reproduction study, it is unlikely that the skeletal effects have a lasting adverse effect on the rat fetuses. Therefore, these effects are neither considered more severe than maternal effects nor interpreted as a sign increased fetal susceptibility. A developmental study in rabbits showed similar effects. The maternal NOAEL = 5 mg/kg/day based on decreased body

weight gain during treatment. The developing rabbit fetuses were less sensitive to amicarbazone exposure. The developmental NOAEL for rabbits is 20 mg/kg/day based on decreased fetal body weights and incomplete ossification of several bones at the LOAEL of 70 mg/kg/day . Neither study showed increased qualitative or quantitative susceptibility of the offspring.

Development Study in Rats

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 45121530), MKH 3586 Technical (Amicarbazone; 98.1-98.5% a.i.; Lot/Batch # 05362/0005) in 0.5% aqueous carboxymethylcellulose and 0.4% Tween 80 solution was administered daily by oral gavage at a dose volume of 10 mL/kg bw to 30 female Sprague Dawley rats/group at dose levels of 0, 15, 100 or 300 mg/kg/day on gestation days (GD) 6 through 19. All dams were sacrificed on GD 20; their fetuses were removed by cesarean and examined. In a supplemental developmental toxicity study (MRID 45121531), conducted to determine whether the skeletal findings observed in the definitive developmental toxicity study at 15 mg/kg/day were incidental and not compound-related, MKH 3586 Technical (Amicarbazone; 97.8-97.9% a.i.; Lot/batch #05362/0005) in 0.5% (w/v) aqueous carboxymethylcellulose and 0.4% (w/v) Tween 80 solution was administered daily by oral gavage at a dose volume of 10 mL/kg to 30 female Sprague-Dawley rats/group at nominal dose levels of 0, 5 or 15 mg/kg/day on gestation days (GD) 6-19. All dams were sacrificed on GD 20; their fetuses were removed by cesarean and examined.

In the main study, hard stools were observed in 9/20 dams at 100 mg/kg/day and 22/27 dams at 300 mg/kg/day beginning several days after dosing began. At 300 mg/kg/day, clonic convulsions were observed in 3/27 dams. The death of one pregnant female at 300 mg/kg/day was not clearly treatment-related. Body weights were dose-dependently decreased ($p<=0.05$) at 100 (↓7-8%) and 300 (↓4-13%) mg/kg/day on GD 7-20, resulting in decreased (statistics not performed) body weight gains for GD 6-20 both when corrected for (↓45-67%) and uncorrected for (↓19-44%) gravid uterine weights. Gravid uterine weights were slightly lower than controls (↓14%, not significant), due in part to lower fetal weights. Food consumption was decreased ($p<=0.05$) at 100 mg/kg/day on GD 6-9 (↓24-42%) and at 300 mg/kg/day on GD 6-12 and 14-15 (↓15-67%). Terminal body weights were decreased ($p<=0.05$) at 100 (↓8%) and 300 (↓13%) mg/kg/day. The 300 mg/kg/day dams demonstrated increased ($p<=0.05$) absolute (↑12%) and relative liver weights (incr. 28%). The maternal LOAEL is 100 mg/kg/day, based on decreased body weights/body weight gains and food consumption, and increased occurrence of hard stools. The maternal NOAEL is 15 mg/kg/day.

There were no abortions, premature deliveries, late resorptions or complete litter resorptions. No effects of treatment were noted on numbers of litters, live fetuses, dead fetuses, early resorptions, placental weight, sex ratio or post-implantation losses. Decreased ($p<=0.01$) body weights were observed in the 300 mg/kg/day

male and female fetuses (↓13-16%). There were no treatment-related external, visceral or skeletal variations or malformations. Multiple dose-related increases ($p<=0.05$) in retardations of fetal skeletal development were noted. At >=100 mg/kg/day, incomplete ossification/unossification was observed in: (i) parietal bones, (ii) interparietal bones; (iii) supraoccipital bones; (iv) squamosal bones; (v) zygoma; (vi) pubis; (vii) xiphoid; and (viii) metacarpals. Enlargement was observed in the sagittal and squamosal sutures and posterior fontanelle. Additionally at 300 mg/kg/day, incomplete ossification/unossification was observed in: (i) nasal bones; (ii) lumbar arches; (iii) sacral arches; (iv) sacral centra; (v) caudal centra; (vi) metatarsals; (vii) manubrium; (viii) sternebrae, segments 2, 3, 4 and 5; (ix) ribs; (x) hyoid body; and (xi) caudal arches. Enlargement was noted in the frontal, coronal and lamboidal sutures. No dose-related skeletal findings were noted in the supplemental developmental toxicity study, confirming that skeletal findings observed in the definitive developmental toxicity study at 15 mg/kg/day were probably not compound-related. The developmental LOAEL is 100 mg/kg/day, based on multiple skeletal developmental retardations. The developmental NOAEL is 15 mg/kg/day.

This study is classified acceptable/guideline (OPPTS 870.3700a) and satisfies the requirements for a developmental study in the rat. The Registrant is asked to provide (1) appropriately stained (Alizarin red S and Alcian blue) historical controls for skeletal variations and (2) breeding records for control animals as confirmatory data.

Developmental Study in Rabbits

EXECUTIVE SUMMARY: In an oral developmental toxicity study (MRID 45121627), MKH 3586 (Amicarbazone; Lot/batch # 05362/0005; 98.2% a.i.) was administered in 0.5% carboxymethylcellulose in demineralized water via gavage, in a dosing volume of 5 mL/kg, to 22-23 female Himalayan CHBB:HM rabbits/group at dose levels of 0, 5, 20 or 70 mg/kg/day on gestation days (GD) 6 through 28. All surviving does were sacrificed on GD 29 and their fetuses were removed by cesarean and examined. Because the control group exhibited an unusually low incidence of malformations, causing the MKH 3586-treated groups to appear to have increased malformation rates by comparison, a supplementary study (MRID 45121626) was conducted with 22 rabbits/group at dose levels of 0 and 70 mg/kg/day to determine whether MKH 3586 caused treatment-related malformations at that dose.

Maternal toxicity: At >=20 mg/kg/day in the main study, body weight gains were decreased during treatment ($p<=0.05$; decr. 29-30%) compared to concurrent controls (GD 6-29). The decreases during the treatment interval resulted in decreased overall (decr. 10-120%; GD 0-29) body weight gains either uncorrected or corrected for gravid uterine weight, but it is noted that due to a relatively large mean gain during pretreatment (GD 0-6) compared to the other groups, cumulative GD 0-29 gain at 70 mg/kg/day was less than at 20 mg/kg/day (mean body weight at

70 mg/kg/day was about 4% less than controls during treatment, but not significant). Mean body weights were slightly lower than controls (↓3-4%, not significant) throughout most of treatment. At 70 mg/kg/day, in the main study, one doe aborted on Day 21. This animal displayed multiple signs of maternal toxicity (ears cold to touch, reduced feces and urine output, discolored urine and decreased food and water consumption). Decreased urination in the supplementary study, decreased water consumption in the main and supplementary studies and ears cold to the touch in the supplementary study were observed (in a few animals on GD 6-7 but generally later). In the supplementary study, body weight gains were decreased (decr. 29%; not significant) during the treatment interval (GD 6-29), resulting in decreased (decr. 23-24%; not significant) overall (GD 0-29) uncorrected or corrected body weight gains. Body weights also were decreased ($p<=0.05$) from GD 11-29 (decr. 3-4%). Maternal food consumption was decreased during GD 6-21 in the main study ($p<=0.05$; decr. 14-43%) and during GD 6-21 and 24-27 in the supplementary study (decr. 11-48%). There was an increase in partially necrotic placentas (above historical control range) in the main and supplementary studies (2.2-2.4% fetuses; 11.1-15.0% litters) compared to controls (0.0-0.7% fetuses; 0.0-4.5% litters) and in coarse grained placentas in the main study (8.7% fetuses; 15.0% litters) compared to controls (0%). No effects on maternal gross pathology were reported. The maternal toxicity LOAEL is 20 mg/kg/day based on decreased body weight gains during treatment. The maternal toxicity NOAEL is 5 mg/kg/day.

Developmental toxicity: At 70 mg/kg/day, in the main study, a slight, non-significant decrease in fetal weights (↓5-6%) was seen. There was an increased ($p<=0.05$) incidence of incomplete ossification of the 5th medial phalanx digit, on both the right (39.4% fetuses; 70.0% litters) and left (40.9% fetuses; 70.0% litters) sides. In the supplementary study, fetal weights were decreased ($p<=0.01$; decr. 11-13%); Slightly thickened right 6th (2.9% fetuses; 16.7% litters) and left 7th (9.4% fetuses; 38.9% litters) ribs and incomplete ossification of the 13th caudal vertebra (6.5% fetuses; 38.9% litters) were observed. There were no effects of treatment the number of resorptions (early, late or complete litter), number of fetuses (live or dead), number of litters, post-implantation loss, fetal weights or sex ratio in either the main or the supplementary studies. The main study findings were supported by the supplementary study. The developmental toxicity LOAEL is 70 mg/kg/day based on decreased fetal weights and increased incidences of incomplete ossification of the 5th medial phalanx digit (bilateral) and the 13th caudal vertebra and slightly thickened ribs. The developmental toxicity NOAEL is 20 mg/kg/day.

This study is classified acceptable/guideline (OPPTS 870.3700b; OECD 414) and satisfies the requirements for a developmental study in the rabbit.

4.2.4 Reproductive Toxicity Study

The parental systemic NOAEL for the 2-generation reproduction study in rats was 6.4 mg/kg/day, based on decreased body weight and body weight gains seen at higher doses. There were no reproductive effects observed in this study; thus a reproductive LOAEL was not observed. The offspring NOAEL is 6.4 mg/kg/day, based on decreased pup body weights and overall decreased body weights throughout treatment observed at the LOAEL of 33.9 mg/kg/day. Neither quantitative nor qualitative susceptibility were observed in this study.

Two-Generation Reproduction Study in Rats

EXECUTIVE SUMMARY: In a two-generation reproduction toxicity study (MRID 45121625), MKH 3586 Technical (Amicarbazone; 98.2-99.1% a.i.; Lot/batch #05362/0005) was administered in the diet to 30 Sprague-Dawley rats/sex/dose at nominal dose levels of 0, 100, 500 or 1000 ppm (equivalent to 0/0, 6.4/7.3, 33.9/38.7 or 73.2/84.0 mg/kg bw/day in males/females). The P and F_1 parents were given test diets for 10 weeks before they were mated to produce the F_1 and F_2 litters. The F_1 pups were weaned at 21 days of age, and approximately 30 pups/sex/group (1 pup/sex/litter as nearly as possible) were randomly selected as parents of the F_2 generation. Sperm parameters were not evaluated in the parental males, and sexual maturation and developmental landmarks were not evaluated in the offspring.

Parental toxicity: At 500 ppm, P males and females had reduced cumulative body weight gain during the premating period (males ↓9%, days 0-105 and females ↓20%, days 0-70), although no significant decreases in mean body weight were observed due to higher mean body weights compared to controls. Food consumption was slightly but statistically significantly reduced only during the first week of treatment. F_1 males and females showed sporadic statistically significant decreases in mean body weight during premating (↓5-20%, males and ↓6%, females). Cumulative gain decreased 5% in males (days 0-98) and 8% in F_1 females (days 0-70), with no decreases in food consumption. Mean body weights during gestation were reduced in F_1 females only (↓6-11%). Statistically significant reductions in mean body weight of F_1 females during lactation (↓6-8%) were not accompanied by reduced gain. At 1000 ppm, P generation males showed reduced cumulative gain during premating (↓12%, days 0-105); statistically significant reductions in mean body weights were not observed due to higher initial mean body weight compared to controls. Significantly reduced mean body weights were observed in females during days 0-70 (↓4-6%) and cumulative gain was reduced by 29%. Significantly decreased body weight in F_1 males (↓6-12%, days 0-98) and females (↓10-14%, days 0-70) were observed for the entire premating period; this was due largely to lower initial weights in both sexes, with cumulative gain reduced by 4% in males and 13% in females. Statistically significant reductions in mean body weights were observed during gestation in both P (↓6-7%) and F_1 (↓11-12%) females, along with reduced gain (↓11-12%). Mean body

weights were significantly decreased throughout lactation for P females (↓7-18%) and F_1 females (↓10-13%) despite increased food consumption, although cumulative gain was not affected. Terminal body weights were decreased ($p \leq 0.05$) in F_1 males and females. Relative liver weights were slightly increased ($p \leq 0.05$) in both P and F_1 male and females (↑11-15%), due in part to reduced terminal body weights. Minimal to slight hepatocytomegaly was noted in F_1 males (10/30) compared to controls (0/30), but no microscopic pathology consistent with liver toxicity was observed. No treatment-related findings were noted at 100 ppm. The parental toxicity LOAEL is 500 ppm (equivalent to 33.9/38.7 mg/kg bw/day [M/F]), based on decreased body weight/weight gain in both sexes. The NOAEL is 100 ppm (6.4/7.3 mg/kg bw/day [M/F]).

Offspring toxicity: At 500 ppm, decreased body weights were observed on PND 14 and 21 in F_1 and F_2 pups; cumulative gains for these groups were decreased 10-13% ($p \leq 0.05$). At 1000 ppm, decreased ($p \leq 0.05$) body weights were noted on PND 4, 7, 14 and 21 in F_1 pups, and on PND 7, 14 and 21 in F_2 pups(↓10-23%). Cumulative gains for this period were decreased 20-25% ($p \leq 0.01$). There were no effects of treatment on the birth, live birth, viability or lactation indices, sex ratio or gross pathology in the F_1 or F_2 pups. No treatment-related findings were noted at 100 ppm. The LOAEL for offspring toxicity is 500 ppm (equivalent to 33.9/38.7 mg/kg bw/day [M/F]), based on decreased pup body weights and overall body weight gains. The NOAEL is 100 ppm (equivalent to 6.4/7.3 mg/kg bw/day [M/F]).

Reproductive effects: The reproductive toxicity LOAEL was not observed. The reproductive toxicity NOAEL is 1000 ppm (equivalent to 73.2/84.0 mg/kg bw/day [M/F]).

This study is classified as acceptable/guideline and satisfies the guideline requirements (OPPTS 870.3800; OECD 416) for a three-generation reproduction study in the rat.

4.2.5 Additional Information from Literature Sources

As this is a new active ingredient, there is no additional information from literature sources.

4.2.6 Pre-and/or Postnatal Toxicity

4.2.6.1 Determination of Susceptibility

There is no indication of increased qualitative nor quantitative susceptibility of the fetuses in the developmental studies with rats and rabbits, or a 2-gereration reproduction study in rats following exposure to amicarbazone. In the developmental rat study, the developing fetuses have multiple skeletal retardations. However, these effects are seen in the

presence of maternal toxicity, and the 2-generation reproduction study in rats does not indicate decreased fetal survival. Delayed skeletal ossification is most likely not a sign of increased qualitative susceptibility to amicarbazone in developing rat fetuses.

4.2.6.2 Degree of Concern Analysis and Residual Uncertainties for Pre and/or Post-natal Susceptibility

The toxicology database for amicarbazone is complete with respect to pre- and post-natal toxicity and without evidence of increased qualitative or quantitative susceptibility, therefore, HED has no residual uncertainty regarding this finding.

4.3 Hazard Identification and Toxicity Endpoint Selection

4.3.1 Acute Reference Dose (aRfD) - Females age 13-49

The developmental toxicity rat and rabbit studies and the 2-generation reproduction study did not demonstrate increased susceptibility of the fetus. All developmental effects were seen at the same dose or higher than the dose that caused maternal toxicity. A single aRfD was chosen for the general population and women of childbearing age. The NOAEL of 10 mg/kg/day in the acute neurotoxicity screening battery is protective of developmental effects seen in both rat and rabbit developmental studies.

The acute RfD is 0.10 mg/kg/day.

4.3.2 Acute Reference Dose (aRfD) - General Population

The acute neurotoxicity screening battery was selected for this exposure scenario, as clinical signs were seen following a single dose of amicarbazone.

The acute RfD is 0.10 mg/kg/day.

4.3.3 Chronic Reference Dose (cRfD)

The chronic dietary endpoint is based on decreased body weight and body weight gain in rats and liver effects in dogs following chronic exposure to amicarbazone. The NOAEL for this study is 2.3 mg/kg/day.

The chronic RfD is 0.023 mg/kg/day.

4.3.4 Incidental Oral Exposure (Short and Intermediate Term)

As there are no proposed residential uses at this time, an incidental oral risk assessment was not performed.

4.3.5 Dermal Absorption

There was no dermal absorption study submitted as part of the registration package for this chemical. However, a 21-day dermal toxicity study in rats was submitted. In this study, no systemic toxicity occurred at the limit dose, and no dermal irritation was seen. Using a ratio of the oral LOAELs from several studies in rats to the NOAEL from the 21-day dermal study in rats, an absorption factor of less than 5% was calculated. HED concludes that dermal absorption is not a major route of uptake for this chemical. Therefore, the dermal route of exposure was not considered as part of the risk assessment.

21-day Dermal Study in Rats - Executive Summary

In a 21-day dermal toxicity study (MRID 45121525), MKH 3586 (amicarbazone; 98.5% a.i., Lot/Batch # 05362/0005) was applied undiluted to the shaved intact skin of 10 Sprague-Dawley rats/sex/dose at 0, 200, 500, or 1000 mg/kg bw/day (limit dose) for 6 hours/day. The females received 17 applications and the males received 18 applications over a period of 21 and 22 days, respectively. Both sexes were dosed for 5 consecutive days during the first two weeks. During the third week, the females and males were dosed for 7 and 8 consecutive days, respectively, prior to terminal sacrifice.

No compound-related effects on mortality, clinical signs, body weight, body weight gain, food consumption, ophthalmoscopic examinations, hematology, clinical chemistry, organ weights, or gross or histopathological parameters were observed in either sex. No treatment-related signs of dermal irritation were observed.

The LOAEL was not observed. The NOAEL is 1000 mg/kg/day.

4.3.6 Inhalation Exposure

Because a subchronic inhalation study was not submitted for this chemical, the short- and intermediate-term inhalation endpoints were selected from the subchronic dog study (MRID 45121524). The NOAEL was 6.28 mg/kg/day based on increased thyroid vacuolization and decreased food consumption and glucose in females; increased platelets, phosphate, bile acids, absolute and relative liver weights, and lymphoid hyperplasia of the gall baldder in males; and decreased albumin and increased triglyercides, N-demethylase, and O-demethylase in both sexes that were seen at the LOAEL of 24.99 mg/kg/day. In the absence of data to generate an inhalation absorption value, a conservative default value of 100% absorption was used for risk assessment purposes.

Long-term occupational exposure was considered negligible for this chemical based on its proposed use pattern. Therefore a long-term inhalation risk assessment was not performed at this time.

4.3.7 Margins of Exposure

All margins of exposure for dietary and short- and intermediate-term exposures are 100. The default uncertainty factors of 10X for interspecies variation and 10X for intraspecies variation are the only uncertainty factors/safety factors for this chemical.

4.3.8 Recommendation for Aggregate Exposure Risk Assessments

As there are no proposed residential uses for this chemical at this time. Therefore, the aggregate risk assessment consists solely of dietary exposure through food and water.

4.3.9 Classification of Carcinogenic Potential

The carcinogenic classification of this chemical is "not likely to be a human carcinogen." Neither the mouse carcinogenicity or chronic/carcinogenicity rat studies produced a treatment related increased incidence of tumors when compared with controls. Both studies were determined to have adequate dosing based on other effects observed.

Table 4.3. Summary of Toxicological Doses and Endpoints for Chemical for Use in Human Risk Assessments

Exposure Scenario	Dose Used in Risk Assessment, UF	Special FQPA SF* and Level of Concern for Risk Assessment	Study and Toxicological Effects
Acute Dietary (females 13-49)	NOAEL = 10 mg/kg/day UF = 100X **Acute RfD = 0.10 mg/kg/day**	Special FQPA SF = 1X **aPAD = 0.10 mg/kg/day**	Acute Neurotoxicity Screening Battery LOAEL = 20 mg/kg/day, based on eyelid ptosis, decreased approach response, red nasal staining in ♂
Acute Dietary (general population)	NOAEL = 10 mg/kg/day UF = 100X **Acute RfD = 0.10 mg/kg/day**	Special FQPA SF = 1X **aPAD = 0.10 mg/kg/day**	Acute Neurotoxicity Screening Battery LOAEL = 20 mg/kg/day, based on eyelid ptosis, decreased approach response, red nasal staining in ♂

Table 4.3. Summary of Toxicological Doses and Endpoints for Chemical for Use in Human Risk Assessments			
Exposure Scenario	**Dose Used in Risk Assessment, UF**	**Special FQPA SF* and Level of Concern for Risk Assessment**	**Study and Toxicological Effects**
Chronic Dietary (all populations)	NOAEL = 2.3 mg/kg/day UF = 100X Chronic RfD = 0.023mg/kg/day	Special FQPA SF = 1X **cPAD = 0.023 mg/kg/day**	Chronic Rat and Chronic Dog LOAEL = 25.3 and 8.7, respectively, based on RAT - decreased BW and BWG DOG - liver effects, including increased absolute and relative liver weights, and O-demethylase in ♂; increased globulin and cytochrome p450 in ♀; and increased triglycerides and cholesterol in both sexes
Dermal (all durations)	Not required: No systemic toxicity by dermal route was seen at the limit dose. Evidence of low dermal absorption.		
Inhalation Short-Term (1 - 30 days)	NOAEL = 6.28 mg/kg/day	LOC for MOE = 100	90-Day Oral Toxicity in Dogs LOAEL = 24.99 mg/kg/day, based on increased thyroid vacuolization and decreased food consumption and glucose in females; increased platelets, phosphate, bile acids, absolute and relative liver weights, and lymphoid hyperplasia of the gall bladder in males; and decreased albumin and increased triglycerides, N-demethylase, and O-demethylase in both sexes

Table 4.3. Summary of Toxicological Doses and Endpoints for Chemical for Use in Human Risk Assessments			
Exposure Scenario	**Dose Used in Risk Assessment, UF**	**Special FQPA SF* and Level of Concern for Risk Assessment**	**Study and Toxicological Effects**
Inhalation Intermediate-term (1-6 months)	NOAEL = 6.28 mg/kg/day	LOC for MOE = 100	90-Day Oral Toxicity in Dogs LOAEL = 24.99 mg/kg/day, based on increased thyroid vacuolization and decreased food consumption and glucose in females; increased platelets, phosphate, bile acids, absolute and relative liver weights, and lymphoid hyperplasia of the gall bladder in males; and decreased albumin and increased triglycerides, N-demethylase, and O-demethylase in both sexes
Cancer (oral, dermal, inhalation)	**Classification:** There was no treatment related increase in tumor incidence when compared to control. Dosing was considered adequate. This chemical is not likely to be a carcinogen.		

UF = uncertainty factor, FQPA SF = Special FQPA safety factor, NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, PAD = population adjusted dose (a = acute, c = chronic) RfD = reference dose, MOE = margin of exposure, LOC = level of concern, NA = Not Applicable

* Refer to Section 4.5

4.4 Special FQPA Safety Factor

Based on the hazard database, HED recommends that the special FQPA safety factor be reduced to 1X because there are no/low concerns and no residual uncertainties with regard to pre- and/or post-natal toxicity. After evaluating the toxicological and exposure data, the HED recommends the 1X safety factor based upon the following:

- The toxicity database showed no increase in susceptibility in fetuses and pups with *in utero* and post-natal exposure.
- The dietary food exposure assessment is based on HED-recommended tolerance-level residues and assumes 100% crop treated for all commodities, which results in very high-end estimates of dietary exposure.
- The dietary drinking water assessment is based on values generated by model and associated modeling parameters which are designed to provide conservative, health-protective, high-end estimates of concentrations in water.
- There are no residential uses proposed for this chemical at this time.

4.5 Endocrine disruption

EPA is required under the FFDCA, as amended by FQPA, to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) "may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." Following recommendations of its Endocrine Disruptor and Testing Advisory Committee (EDSTAC), EPA determined that there was a scientific basis for including, as part of the program, the androgen and thyroid hormone systems, in addition to the estrogen hormone system. EPA also adopted EDSTAC's recommendation that the Program include evaluations of potential effects in wildlife. For pesticide chemicals, EPA will use FIFRA and, to the extent that effects in wildlife may help determine whether a substance may have an effect in humans, FFDCA authority to require the wildlife evaluations. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP).

In the available toxicity studies on amicarbazone, there was no estrogen, androgen, and/or thyroid mediated toxicity.

5.0 Public Health Data

This is a new active ingredient, thus there are no human health data at this time.

6.0 Exposure Characterization/Assessment

Acute and chronic dietary exposure analyses were performed for amicarbazone. The analyses were based on tolerance level residues (modified by DEEM default processing factors) and the assumption that 100% of the crop will be treated. Estimated drinking water concentrations were included in the analyses. HED considers these analyses to be conservative and unrefined. As a result, the assumptions made will considerably overestimate actual exposure. There are no uncertainties that will cause an underestimation of dietary risk to the general U.S. population or any population subgroups.

6.1 Dietary Exposure/Risk Pathway

6.1.1 Residue Profile

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 tertiary 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, although additional validation data are requested for cattle liver. Methods 108340 and 108111 appear to meet guideline requirements as acceptable enforcement methods (ACB/BEAD memo, 5/27/05, 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. Even though the early postemergence application rate was the lowest of the three

application types, it resulted in the highest residues. Residues following preplant incorporated application were slightly higher than those following preemergence treatment. Maximum combined residues were 0.56 ppm in forage, 0.43 ppm in stover, and 0.045 ppm in grain. 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 poultry for amicarbazone residues.

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 will be required on crops routinely rotated with corn, with the exceptions of 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.

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

6.1.2 Acute and Chronic Dietary (Food and Drinking Water) Exposure and Risk

D. Dotson, Dietary Exposure Memo, 4/19/05, D313745.

Acute and chronic dietary risk assessments were conducted for the new active ingredient amicarbazone using the Dietary Exposure Evaluation Model (DEEM-FCID, Version 2.03), which uses food consumption data from the USDA's Continuing Surveys of Food Intakes by Individuals (CSFII) from 1994-1996 and 1998.

Acute Dietary Exposure Results and Characterization

A conservative acute dietary exposure analysis was performed for amicarbazone. The analysis was based on tolerance level residues (modified by DEEM default processing factors) and the assumption that 100% of the crop will be treated. The estimated drinking water concentration was included in the analysis. The value used in the analysis (0.1052 ppm) is slightly higher than the final concentration of 0.1029 ppm recommended by EFED. The risk estimates for all population subgroups are below HED's level of concern. At the 95th percentile of exposure, the risk estimate for the general U.S. population is 7% of the acute population adjusted dose (aPAD). The most highly exposed population subgroup is All Infants, which utilizes 23% of the aPAD. For all population subgroups, the major contributor to the exposure estimates is drinking water.

Chronic Dietary Exposure Results and Characterization

A conservative chronic dietary exposure analysis was performed for amicarbazone. The analysis was based on tolerance level residues (modified by DEEM default processing factors) and the assumption that 100% of the crop will be treated. The estimated drinking water concentration was included in the analysis. The value used in the analysis (0.1052 ppm) is slightly higher than the final concentration of 0.1029 ppm recommended by EFED. The risk estimates for all population subgroups are below HED's level of concern. The risk estimate for the general U.S. population is 14% of the chronic population adjusted dose (cPAD). The most highly exposed population subgroup is All Infants, which utilizes 39% of the cPAD. For all population subgroups, the major contributor to the exposure estimates is drinking water.

Cancer Dietary Exposure Results and Characterization

A cancer dietary exposure analysis was not performed because it was determined that amicarbazone was not likely to cause cancer in humans.

Table 6.1 Summary of Dietary Exposure and Risk for Amicarbazone.								
Population Subgroup[a]	Acute Dietary (95th Percentile)			Chronic Dietary			Cancer Dietary	
	aPAD mg/kg	Exposure, mg/kg/day	% aPAD	cPAD, mg/kg/day	Exposure, mg/kg/day	% cPAD	Exposure mg/kg/day	Risk
General U.S. Population	0.10	0.007269	7	.023	0.003240	14	NA	NA
All Infants (< 1 year old)	**0.10**	0.023444	23	**.023**	**0.008859**	**39**	NA	
Children 1-2 years old	0.10	0.012278	12	.023	0.005957	26		
Children 3-5 years old	0.10	0.011188	11	.023	0.005572	24		
Children 6-12 years old	0.10	0.007855	8	.023	0.003846	17		
Youth 13-19 years old	0.10	0.005720	6	.023	0.002651	12		
Adults 20-49 years old	0.10	0.006141	6	.023	0.002866	13		
Adults 50+ years old	0.10	0.005370	5	.023	0.002803	12		
Females 13-49 years old	0.10	0.006099	6	.023	0.002827	12		

[a] The values for the population with the highest risk for each type of risk assessment are bolded.

Contribution of Drinking Water to Total Exposure

A critical element contribution (CEC) analysis was performed for both the acute and chronic analyses in order to determine the contribution of drinking water to the total risk estimates. Water was a major source of dietary exposure to amicarbazone. In the acute analysis, water contributed between 64 and 87% of the total exposure. In the chronic analysis, water contributed between 55 and 82% of the total exposure. The values for the various population subgroups are given in table 6.2 below.

Table 6.2 Contribution of Residues in Drinking Water to Total Dietary Exposure		
Population Subgroup	Acute Dietary (95th Percentile)	Chronic Dietary
General U.S. Population	67%	68%
All Infants (< 1 year old)	87%	82%
Children 1-2 years old	68%	55%
Children 3-5 years old	70%	55%
Children 6-12 years old	64%	55%
Youth 13-19 years old	80%	61%
Adults 20-49 years old	84%	72%
Adults 50+ years old	86%	78%
Females 13-49 years old	85%	73%

6.2 Water Exposure/Risk Pathway

M. Ruhman, Estimated Drinking Water Concentrations of Parent Amicarbazone and its Degradates for the Use in Human Health Risk Assessment. 2/03/05, D288178.

Table 6.3 Summary of Estimated Surface and Ground Water Concentrations for Amicarbazone.		
Exposure Duration	**Total Amicarbazone, Des-amino, and N-methyl des-amino amicarbazone**	
	Surface Water Conc., ppb[a]	**Ground Water Conc., ppb[b]**
Acute (peak)[c]	21.4	102.9
Chronic (annual average) (non-cancer)	13.4	102.9
Chronic (cancer)	NA	NA

[a] From the Tier I FIRST. Input parameters are based on one application of 0.45 lbs ai/acre (parent compound only for acute/peak exposure) and 0.4268 lbs ai/acre (des-amino + N-methyl-des-amino for chronic or annual average exposure); parent K_{oc}=16.7 L Kg^{-1}, des-amino K_{oc} = 26.4 L Kg^{-1}, N-methyl-des-amino K_{oc} = 34.3 L Kg^{-1}; and a parent half-life of 261 days (87 days multiplied by 3). The degradates were assumed to be stable. The % crop treated value used was the defualt value for corn (46%). The material was assumed to be applied by ground spray and wetted in as recommended. The total solubility for parents plus degradates was considered to be 4,600 mg/L as suggested in the data. The aerobic aquatic metabolism half-life was 522 days (aerobic soil entry x 2); the degradates were assumed to remain stable.

[b] From the SCI-GROW model assuming a maximum seasonal use rate 0.45 lbs ai/acre (parent compound only for acute/peak exposure) and 0.4268 lbs ai/acre (des-amino + N-methyl-des-amino for chronic or annual average exposure); parent K_{oc}=24.2 L Kg^{-1}, des-amino K_{oc} = 33.4 L Kg^{-1}, N-methyl-des-amino K_{oc} = 49.4 L Kg^{-1}, and a parent half-life of 87 days. The degradates were assumed to be stable.

[c] Although the acute estimated drinking water concentration is based on the parent only, it is a conservative surrogate for the parent plus degradates. This is based on the assumption that all three compounds are of equal toxicity and there will never be more combined parent plus degradates than the initial application of the parent only. In essence the maximum concentration of toxic equivalents is being considered in this risk assessment.

There are no residential uses under consideration at this time.

7.0 Aggregate Risk Assessments and Risk Characterization

There are no proposed residential uses for this chemical, thus aggregate risk estimates are equivalent to the dietary (food plus water) estimates and are below HED's level of concern for all population subgroups.

8.0 Cumulative Risk Characterization/Assessment

Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding for amicarbazone and any other substances. Furthermore, amicarbazone does not appear to have a toxic metabolite that is produced by other substances. For the purposes of this tolerance action, therefore, EPA has not assumed that amicarbazone has a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at http://www.epa.gov/pesticides/cumulative/.

9.0 Occupational Exposure/Risk Pathway

9.1 Short- and Intermediate-term Handler Risk

Exposure Scenarios

There are two handler scenarios that are expected to result in the highest exposure for the proposed uses:

- Mixing/Loading Dry Flowable for Ground Applications (Scenario 1)
- Applying Sprays with Groundboom Equipment (Scenario 2)

Application Rate

The maximum application rate listed on the proposed labels provided by the Registration Division was used for all exposure assessments. The maximum rate is 0.45 lb ai/A. This application rate was selected for risk assessment purposes because it represents both the highest single application rate (corn) and the maximum seasonal application rate.

Area Treated

Based on HED's Exposure Science Advisory Council Policy Number 9.1, 200 acres per day treated was assumed for application on corn using groundboom equipment.

Body Weight

The average body weight for general population (70 kg) was used for all exposure scenarios covered in this risk assessment.

Dermal Absorption

Although a dermal absorption rate of 5% was calculated for amicarbazone, HED has concluded that a dermal risk assessment is not needed.

Exposure Frequency

No data on the number of exposure days per year was provided For this risk assessment, it was assumed that handlers would be exposed for less than 6 months per year (i.e. short-/intermediate-term in duration).

Unit Exposures

The unit exposures used in this assessments are based on the PHED Version 1.1 as presented in the August 1998 PHED Surrogate Exposure Guide. PHED was designed by a task force of representatives from the U.S. EPA, Health Canada, the California Department of Pesticide Regulation, and member companies of the American Crop Protection Association. PHED is a software system consisting of two parts–a database of measured exposure values for workers involved in the handling of pesticides under actual field conditions and a set of computer algorithms used to subset and statistically summarize the selected data. Currently, the database contains values for over 1,700 monitored individuals (i.e., replicates).

Users select criteria to subset the PHED database to reflect the exposure scenario being evaluated. The subsetting algorithms in PHED are based on the central assumption that the magnitude of handler exposures to pesticides is primarily a function of activity (e.g., mixing/loading, applying), formulation type (e.g., wettable powders, granulars), application method (e.g., aerial, groundboom), and clothing scenarios (e.g., gloves, double layer clothing).

There are three basic risk mitigation approaches considered appropriate for controlling occupational exposures. These include administrative controls, the use of personal protective equipment or PPE, and the use of engineering controls. Occupational handler exposure assessments were completed by HED using baseline, PPE, and engineering controls. [Note: Administrative controls available generally involve altering application rates for handler exposure scenarios. These are typically not utilized for completing handler exposure assessments.] The baseline clothing level scenario for occupational exposure scenarios is generally an individual wearing long pants, a long-sleeved shirt, no chemical resistant gloves, and no respirator. The first

level of mitigation generally applied is PPE. As reflected in the calculations included herein, PPE may involve the use of an additional layer of clothing, chemical-resistant gloves, and a respirator. The next level of mitigation considered in the risk assessment process is the use of appropriate engineering controls which, by design, attempt to eliminate the possibility of human exposure. Examples of commonly used engineering controls include enclosed tractor cabs and cockpits, closed mixing/loading/transfer systems, and water-soluble packets.

Table 9.1. Intermediate Term Occupational Exposure and Risk Estimates for Amicarbazone. All estimates are at baseline mitigation (long sleeve shirt, long pants, shoes and sock, no respirator).

Exposure Scenario (Scenario #)	Crop	Inhalation Unit Exposure[a] (Ug/lb ai)	Daily Inhalation Dose[b], mg/kg/day	Intermediate-term MOE[c]
Mixer/Loader				
Mixing/Loading Dry Flowable for Ground application (1)	Corn	0.77	.00099	6,300
Applicator				
Applying Sprays with Groundboom (2)	Corn	0.74	.00095	6,600

a Baseline Inhalation Unit Exposure represents no respiratory protection, open mixing/loading, and open cab tractors, as appropriate. Eng. Cont. Inhalation Unit Exposure represents enclosed cockpit.

b Daily inhalation dose (mg/kg/d) = (unit exposure (µg/lb ai) * (1mg/1000 µg) conversion * appl. rate (lb ai/acre) * daily acres treated / body weight.

c Inhalation MOE = NOAEL (6.28 mg/kg/d) / daily inhalation dose (mg/kg/d). UF = 100.

9.2 Short/Intermediate/Long-Term Postapplication Risk

Post-application exposure assessments were not performed because no dermal endpoints were selected and inhalation exposures are expected to be neglible.

The technical material has a Category III for acute eye irritation, and a Category IV for acute dermal toxicity & acute dermal irritation. Per the Worker Protection Standard (WPS), a 12-hr restricted entry interval (REI) is required. Therefore, the 12 hour REI appearing on the label is appropriate for this chemical.

10.0 Data Needs and Label Requirements

10.1 Toxicology

None

10.2 Residue Chemistry

The following deficiencies should be satisfied prior to establishment of the proposed tolerances (on field corn, rotational crop, and livestock commodities) and approval of the conditional registration.

860.1200 Directions for Use

The proposed label should be 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.
- The plant back interval (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.

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_9
  - DA amicarbazone-d_9
  - iPr-2-OH DA amicarbazone-d_6

860.1550 Proposed Tolerances (Section F of PP#0F6131)

- 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.

- The proposed tolerances should be revised to reflect recommended tolerance levels and the correct commodity definitions as specified in the Tolerance Summary in Section 1.0 (Executive Summary).

HED has no objections to the following deficiencies being addressed as **conditions of registration**.

860.1300 Nature of the Residue - Plants

- The dates of initial and final sample extraction and analysis should be submitted in order to determine 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 in order to determine whether storage stability data are required to support the studies.

860.1340 Residue Analytical Methods

- 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.

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.

860.1480 Meat, Milk, Poultry, and Eggs

- Based on data from the poultry metabolism study and the calculated theoretical dietary burden for amicarbazone residues 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.

860.1900 Field Accumulation in Rotational Crops

- 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.

HED is recommending against the proposed import tolerance for sugarcane commodities. For further consideration of these tolerances, the petitioner should submit labels (and translations) bearing use directions for sugarcane from the countries where this use is allowed. In addition, sugarcane field trial data are required reflecting the approved uses of amicarbazone in those countries. In lieu of submitting the field trial data, sugarcane commodities can be withdrawn from the petition.

10.3 Occupational and Residential Exposure

None

References:

Dotson, D. (2005). Amicarbazone: Acute and Chronic Dietary Exposure Assessments of the Section 3 Registration Action for use of Amicarbazone on Field Corn, Cotton, Soybeans, Wheat, and Animal Commodities. 4/19/05. DP Barcode: D313745, PP# 0F6131.

Ruhman, M. (2005). Estimated Drinking Water Concentrations of Parent Amicarbazone and its Degradates for the Use in Human Health Risk Assessment. 2/03/05. DP Barcode: D288178.

Wang, S. (2005). Occupational and Residential Exposure/Risk Assessment for the Uses of Amicarbazone on Field Corn. 6/20/05. DP Barcode: D313746.

Xue, M. (2005) Petition for the Establishment of Permanent Tolerances for Use on Field Corn and Imported Sugarcane. Summary of Analytical Chemistry. 6/06/05. DP Barcode: D288216, D309766.

Appendices

1.0 TOXICOLOGY DATA REQUIREMENTS

The requirements (40 CFR 158.340) for food use for amicarbazone are in Table 1. Use of the new guideline numbers does not imply that the new (1998) guideline protocols were used.

Test		Technical	
		Required	Satisfied
870.1100	Acute Oral Toxicity	yes	yes
870.1200	Acute Dermal Toxicity	yes	yes
870.1300	Acute Inhalation Toxicity	yes	yes
870.2400	Primary Eye Irritation	yes	yes
870.2500	Primary Dermal Irritation	yes	yes
870.2600	Dermal Sensitization	yes	yes
870.3100	Oral Subchronic (rodent)	yes	yes
870.3150	Oral Subchronic (nonrodent)	yes	yes
870.3200	21-Day Dermal	no	yes
870.3250	90-Day Dermal	no	-
870.3465	90-Day Inhalation	no	-
870.3700a	Developmental Toxicity (rodent)	yes	yes
870.3700b	Developmental Toxicity (nonrodent)	yes	yes
870.3800	Reproduction	yes	yes
870.4100a	Chronic Toxicity (rodent)	yes	yes
870.4100b	Chronic Toxicity (nonrodent)	yes	yes
870.4200a	Oncogenicity (rat)	yes	yes
870.4200b	Oncogenicity (mouse)	yes	yes
870.4300	Chronic/Oncogenicity	yes	yes
870.5100	Mutagenicity—Gene Mutation - bacterial	yes	yes
870.5300	Mutagenicity—Gene Mutation - mammalian	yes	yes
870.5xxx	Mutagenicity—Structural Chromosomal Aberrations	yes	yes
870.5xxx	Mutagenicity—Other Genotoxic Effects	yes	yes
870.6100a	Acute Delayed Neurotox. (hen)	no	-
870.6100b	90-Day Neurotoxicity (hen)	no	-
870.6200a	Acute Neurotox. Screening Battery (rat)	yes	yes
870.6200b	90 Day Neuro. Screening Battery (rat)	yes	yes
870.6300	Develop. Neuro	yes	yes
870.7485	General Metabolism	yes	yes
870.7600	Dermal Penetration	no	-
Special Studies for Ocular Effects			
	Acute Oral (rat)	no	-
	Subchronic Oral (rat)	no	-
	Six-month Oral (dog)	no	-

2.0 NON-CRITICAL TOXICOLOGY STUDIES

Guideline Studies

870.3100. 90-Day Oral Toxicity Study in Rats. MRID 45121523.

EXECUTIVE SUMMARY - In a subchronic oral toxicity study (MRID 45121523), MKH 3586 (amicarbazone; 98.2% a.i., Lot/Batch #: 17004/93) was administered to 15 CDF[F-344]/BR rats/sex/group in the diet at dose levels of 0, 100, 250, 500, 1000, 2500, or 5000 ppm (equivalent to 0/0, 6.9/7.9, 18/21, 33/38, 67/78, 182/201, and 354/397 mg/kg/day [M/F], respectively) for 13 weeks. An additional 15 rats/sex/group were similarly treated at 0 and 5000 ppm and further observed during a 4-week recovery period following dosing.

No adverse treatment-related effects were noted in mortality, clinical signs, ophthalmoscopy, or gross pathology.

Body weight was decreased (p<=0.05) during Weeks 2-13 in the >=1000 ppm females (decr 4-21%) and in the >=2500 ppm males (decr 6-20%). Overall (Weeks 1-13) body weight gain (calculated by reviewers) was decreased at >=1000 ppm in both sexes (decr 7-62%). Food consumption was decreased (p<=0.05) throughout the study at >=2500 ppm in both sexes (decr 6-24%).

In the liver, increases (p<=0.05, unless otherwise noted) were observed in absolute weight in the >=2500 ppm females (incr 13-15%, not statistically significant) and in the 5000 ppm males (incr 18%) and in relative (to body) weight in the >=1000 ppm females (incr 11-41%) and in the ≥2500 ppm males (incr 21-43%). Cholesterol was increased at >=1000 ppm in both sexes (incr 27-298%). In the tissue samples collected at termination, O-demethylase activity was increased in the >=1000 ppm females (incr 33-89%) and in the >=2500 ppm males (incr 30 [not statistically significant]-60%), and N-demethylase activity was decreased in the >=1000 ppm males (decr 31-37%). Minimal to marked hepatocytomegaly was observed in the >=1000 ppm males (9-10 treated vs 0 controls) and in the >=2500 ppm females (8-10 treated vs 0 controls)

In the thyroid, increased incidence (# affected vs 0 controls) of minimal follicular cell hyperplasia was observed at >=2500 ppm in the males (2-7) and females (4-7). Increased T4 activity was observed in the >=1000 ppm males, and increased T3 activity was observed in the >=1000 ppm males and in the >=2500 ppm females.

In the spleen, increased incidence of hemosiderin pigmentation was observed in the >=1000 ppm males (4-5 treated vs 1 control) and in the >=2500 ppm females (3-10 treated vs 0 controls). The following differences (p<=0.05, unless otherwise noted) in hematology parameters were observed: (i) red blood cells, hemoglobin, and hematocrit were decreased in the >=1000 ppm males (decr 3-11%); (ii) hemoglobin, hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) were decreased in the >=1000 ppm females (decr 3-15%); (iii) reticulocytes were increased at >=2500 ppm in the males (incr 33 [not statistically significant]-144%) and in the females (incr 47 [not statistically significant]-200%); (iv) platelets were increased in the >=2500 ppm males (incr 14-32%); and (v) MCH and MCV were decreased in the >=2500 ppm males (decr 2-4%). Increased urinary pH was noted in the >=1000 ppm females, and increased urinary leukocytes were observed in the >=2500 ppm males and females.

In the >=2500 ppm females, increased incidence (# affected vs 0 controls) of minimal to moderate corticomedullary atrophy of the bone (3-10), and minimal to moderate atrophy of the bone marrow (3-8) were observed.

Additionally at 5000 ppm, red blood cells were decreased in the females, total protein was increased in the males and females, and globulins were increased in the males. The following histopathological effects were observed: minimal diffuse cytoplasmic vacuolization of the cortical epithelium cells of the adrenal glands in the males (10) and minimal cytoplasmic vacuolization of the pancreas in the males (10) and females (5).

The only finding below 1000 ppm was increased (p<=0.05) cholesterol at 250 (incr 20-31% in males) and 500 ppm (incr 23-47% in both sexes). In the 5000 ppm recovery group, all treatment-related effects showed signs of recovery; however, most parameters were still different (p<=0.05) from controls.

The LOAEL is 1000 ppm (equivalent to 67/78 mg/kg/day [M/F]) based on decreased body weight (females) and overall (Weeks 1-13) body weight gains, decreased red cell indices, clinical chemistry (increased cholesterol, T4 [males], T3 [males], o-demethylase [females], and n-demethylase [males]), increased relative (to body) liver weight in females, and histopathological effects in the males (minimal hepatocytomegaly and minimal pigmentation in the spleen). The NOAEL is 500 ppm (equivalent to 33/38 mg/kg/day [M/F]).

This study is classified as acceptable/guideline and satisfies the guideline requirements (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in the rat.

870.3150. 90-Day Oral Toxicity Study in Dogs. MRID 45121524

EXECUTIVE SUMMARY - In a subchronic oral study (MRID 45121524), MKH 3586 (Amicarbazone technical; 98.2-99.1% a.i., Lot/batch #: 05362/0005) was administered to 4 beagle dogs/sex/dose in the diet at doses of 0, 200, 800, or 2000 ppm (equivalent to 0/0, 6.74/6.28, 27.03/24.99, 57.40/62.11 mg/kg/day [M/F]) for up to 90 days. There were no treatment-related effects on survival, neurological evaluations, urinalysis, or ophthalmoscopy.

Food consumption was decreased by 22-43% (p<=0.05): in the 800 ppm females on Days 6, 12, and 28; in the 2000 ppm males on Day 15; and in the 2000 ppm females on Days 2, 6, 12, and 28. Additionally at 2000 ppm, one male was described as thin beginning on Day 21. Body weights were decreased by 6-15% (not significant [NS]) in females throughout treatment, and overall (Days 0-91) body weight gains (calculated by reviewers) were decreased by 29-40% in both sexes at this dose. Electrocardiography at the pre-terminal examination revealed the P-R interval was increased by 29% (p<=0.05) in the 2000 ppm males. In the males, platelets were increased (p<=0.05) at >=800 ppm; and, additionally at 2000 ppm, activated partial thromboplastin time and hemoglobin were decreased (p<=0.05).

Treatment-related effects were observed in the liver and gall bladder. In the liver tissue samples, N-demethylase and O-demethylase activities were increased (p<=0.05) over controls in both males of >= 800 ppm and females of >=200 ppm. N-demethylase and O-demethylase activities were increased (p<=0.05) by 62 and 42% over the controls,

respectively, in females of the 200 ppm group. N-demethylase and O-demethylase activities were increased ($p<=0.05$) over controls by 212 and 79%, respectively, in males and by 195 and 142 %, respectively, in females, of the 800 ppm group. At 2000 ppm, the increase ($p<=0.05$) in N-demethylase and O-demethylase activities was 260 and 129%, respectively, in males, and 239 and 83%, respectively, in females. Although terminal body weights were decreased by 13% (NS) in the 2000 ppm females, increases of 24-58% were noted in absolute (NS) and relative ($p<=0.05$) liver weights in the >=800 ppm males and 2000 ppm females. Additionally at 2000 ppm, increased incidence (NS, unless otherwise noted) of the following microscopic findings were observed in the liver vs 0/4 controls: (i) bile pigmentation in males (1/4); (ii) individual cell necrosis in males (3/4) and females (4/4; $p<=0.05$); and hypertrophy in females (1/4). Lymphoid hyperplasia of the gall bladder was increased (NS) in incidence and severity in the >=200 ppm males (2/4 to 3/4 treated vs 1/4 controls). Additionally at 2000 ppm, abnormal texture of the gall bladder was noted (vs 0 controls) in males (1/4 treated) and females (4/4 treated). Abnormal consistency of the gall bladder was observed in females (1/4 treated vs 0/4 controls). The following microscopic findings were increased (NS) vs 0/4 controls: (i) hyperplasia of the gall bladder in males (2/4) and females (3/4); (ii) bile pigmentation in the gall bladder in males (2/4) and females (2/4); and (iii) chronic inflammation of the gall bladder in females (2/4). The following clinical chemistry differences ($p<=0.05$) were noted: (i) increased phosphate in the >=200 ppm males; (ii) increased bile acids in the >=800 ppm males and 2000 ppm females; (iii) increased triglycerides and decreased albumin in the >=800 ppm animals; (iv) decreased glucose in the 800 ppm females and 2000 ppm animals; (v) decreased total protein and aspartate aminotransferase in the 2000 ppm males; and (vi) increased alkaline phosphatase in the 2000 ppm females.

Incidences of thyroid vacuolization were increased (NS; vs 0 controls) in the >=800 ppm females (1-3/4) and the 2000 ppm males (1/4). Additionally at 2000 ppm, absolute and relative thyroid weights were increased (NS) by 11-39% in both sexes, and incidences of cystic follicles and C-cell hyperplasia were increased (NS) in the thyroid in males (1/4 treated vs 0/4 controls). However, there were no effects of treatment on levels of T4, T3, or thyroid stimulating hormone. Kidney pigmentation was increased in the 2000 ppm males (2/4 treated vs 0/4 controls).

The only findings at 200 ppm were considered adaptive and not adverse. The increases in N-demethylase and O-demethylase in females (incr. 42-62%) and phosphate and bile acids in males (incr. 8-20%) were considered adaptive because, at this dose, they were not corroborated by gross or microscopic evidence of an adverse effect (such as the liver necrosis noted at 2000 ppm). Food consumption was decreased only on Day 12 and did not affect body weights or body weight gains at this dose. Finally, lymphoid hyperplasia was noted in the gall bladder in males, but was only marginally increased (2/4 treated vs 1/4 controls).

The LOAEL for this study was 800 ppm (equivalent to 27.03/24.99 mg/kg/day in M/F) based on: increased thyroid vacuolization and decreased food consumption and glucose in females; increased platelets, phosphate, bile acids, absolute and relative liver weights, and lymphoid hyperplasia of the gall bladder in males; and decreased albumin and increased triglycerides, N-demethylase, and O-demethylase in both sexes. The NOAEL is 200 ppm (equivalent to 6.74/6.28 mg/kg/day in M/F).

This study is classified acceptable/guideline and satisfies the guideline requirement (OPPTS 870.3150; OECD 409) for a 90-day oral toxicity study in the dog.

870.4100a. Chronic Toxicity in Rats. MRID 45121512.

EXECUTIVE SUMMARY - In a combined chronic toxicity/carcinogenicity study (MRID 45121512), 50 Fischer 344 rats/sex/dose were exposed to MKH 3586 (Amicarbazone; 98.1-98.6% a.i.; Lot/Batch #: 05362/0005) in the diet at concentrations of 0, 50, 500, or 1250/1000 (M/F) ppm (equivalent to 0/0, 2.3/2.7, 25/30, and 67/65 mg/kg/day in males/females) for up to 24 months. An additional group of 20 (control and high dose) or 10 (low and intermediate dose) rats/sex/dose were similarly treated and sacrificed at 12 months.

Mortality, clinical signs, ophthalmoscopic findings, food consumption, hematology, clinical chemistry, urinalysis, and gross and microscopic pathology for both sexes at all doses were unaffected by treatment. No treatment-related findings were noted at 50 ppm.

In the interim study at 1250/1000 ppm, decreases ($p<=0.05$) in body weight were noted throughout most of the 12 months (decr. 5-15%); decreases in body weight gain (calculated by the reviewers) were observed at Weeks 1-13 (decr. 13-25%), 13-50 (decr. 19-27%), and overall (Weeks 1-50, decr. 15-26%); and body weights were decreased ($p<=0.05$) at the interim sacrifice (decr. 10-16%). In the main study, decreases ($p<=0.05$) in body weight were generally observed throughout the 24 months in the 500 ppm females (decr. 3-11%) and in the 1250/1000 ppm groups (decr. 3-18%). Decreases in body weight gain were observed at >=500 ppm at Weeks 1-13 (decr. 5-24%; both sexes), 13-50 (decr. 26-32%; females only), 50-102 (decr. 43-60%; males only), and overall (Weeks 1-102, decr. 9-23%; both sexes). Body weights were also decreased (not significant) at the terminal sacrifice in the 1250/1000 ppm groups (decr. 8-9%).

The LOAEL is 500 ppm (equivalent to 25.3/29.5 mg/kg/day [M/F]) based on decreased body weights in females and body weight gains in both sexes. The NOAEL is 50 ppm (equivalent to 2.3/2.7 mg/kg/day [M/F]).

At the doses tested, there was not a treatment related increase in tumor incidence when compared to controls. Dosing was considered adequate, based on decreased body weights in females and body weight gains in both sexes.

This study is classified as acceptable/guideline and satisfies the guideline requirements (OPPTS 870.4300; OECD 453) for a combined chronic toxicity/carcinogenicity study in rats.

870.4100b. Chronic Toxicity in Dogs. MRID 45121529.

EXECUTIVE SUMMARY - In a chronic oral study (MRID 45121529), MKH 3586 (Amicarbazone technical; 98.1-98.6% a.i., Lot/batch #: 05362/0005) was administered to 4 beagle dogs/sex/dose in the diet at doses of 0, 75, 100, 300 or 1200 ppm (equivalent to 0/0, 1.6/1.8, 2.5/2.3, 8.9/8.7 or 31.5/34.6 mg/kg/day [M/F]) for one year. In addition to the typical parameters, the investigators evaluated heart rate, blood pressure, electrocardiography (ECG), cortisol, body temperature, circulating thyroid hormones, hepatic enzymes (o-demethylase, n-demethylase and P-450), neurological parameters (mental status/behavior, gait, postural status, postural reactions and spinal/cranial reflex tests) and immunological parameters (cell phenotyping via flow cytometry and immunoglobulin determination).

At ≥300 ppm, absolute and relative liver weights were dose-dependently increased by 26-41% in males. O-demethylase activity was dose-dependently increased by 83-158% in males, and cytochrome P-450 activity was increased by 67-73% in females. Increases in triglycerides (incr. 8-137%) and cholesterol (incr. 16-93%) were observed throughout treatment in both sexes. Globulin was increased by 18% in females at Day 363.

Additionally at 1200 ppm, absolute and relative liver weights were increased by 13-16% in the females. Slight liver hypertrophy and dilatation of the sinusoids were observed in males (1/4 each treated vs 0/4 each controls). In the liver tissue samples collected at study termination, N-demethylase activity was increased by 30% over controls in males, and O-demethylase activity was increased by 65% in the females. Cytochrome P-450 activity was increased by 88% in the males. Albumin was decreased by 9-15% and bile acids increased by 50-100% throughout treatment in both sexes. Lactate dehydrogenase was increased in males by 179-259%. Gamma glutamyl transferase was increased by 43% in females at Day 363. Increased platelets were observed in both sexes (all time points, 18-38% in males and 9-27% in females). Reductions in % eosinophils in females throughout the study were seen but a clear dose-response was not observed. Mild abnormal neurological signs were observed in three females at 6 months (abnormal postural reactions) and one female at 12 months (abnormal postural reactions, Hami-standing deficit and lateral hopping deficit). The study authors concluded that these deficits were not due to direct neurotoxicity but were instead neuromuscular effects possibly secondary to thymic atrophy (see Discussion of this DER for details). Absolute and relative thymus weights were decreased by 35-40% in the 75 ppm and 1200 ppm males. These decreases corresponded to the minimal to slight thymic atrophy observed microscopically; however, they were not dose-related or significantly different from the controls. Thymic atrophy was observed in the 1200 ppm males (from control to high dose, 0/4, 2/4, 1/4, 0/4 and 4/4, $p \leq 0.05$ at 1200 ppm) and females (1/4 treated vs 0/4 controls, NS). The female with persistent neuromuscular effects had thymic atrophy and reduced B cells; findings appeared to be treatment-related. However, in males thymic atrophy was not dose-related in severity and did not show a strong dose-response for incidence. Based on findings in females thymic atrophy in males may be treatment related, but a clear dose-response was not observed. The only findings at 100 ppm were increased O-demethylase activity (incr. 42%) and triglycerides (incr. 43-74%) in males and increased cholesterol in females (incr. 21-29%) but were insufficient to be considered adverse. Slightly reduced hematocrit and hemoglobin in males at 181 days (decr. 5-8%, $p \leq 0.05$) T 100, 300 and 1200 ppm may have been related to treatment but were not considered adverse due to small change and lack of persistence. There were no effects of treatment on survival, quantitative electroencephalography, body weights/weight gains, food consumption, ophthalmoscope, immunoglobulin quantification, urinalysis or gross pathology.

The LOAEL is 300 ppm (equivalent to 8.9/8.7 mg/kg/day, M/F), based on effects on the liver, including: increased absolute and relative liver weights, and O-demethylase in males; increased globulin and cytochrome P450 in females; and increased triglycerides and cholesterol in both sexes. The NOAEL is 100 ppm (equivalent to 2.5/2.3 mg/kg/day, M/F).

This study is classified as acceptable/guideline and satisfies the guideline requirement (OPPTS 870.4100b, OECD 452) for a chronic oral toxicity study in dogs.

870.4300. Carcinogenicity in Mice. MRID 45121604.

EXECUTIVE SUMMARY - In a carcinogenicity study (MRID 45121604), 50 CD-1 (ICR)BR mice/sex/dose were exposed to MKH 3586 (amicarbazone; 98.1-98.6% a.i.; Lot/Batch #: 05362/0005) in the diet at concentrations of 0, 100, 1500, or 4000 ppm nominally (equivalent to 0/0, 15.7/17.9, 244.7/275.0, and 709.0/806.3 mg/kg/day in males/females) for up to 78 weeks.

No adverse treatment-related effects were observed on mortality, food consumption, or gross pathology. No treatment-related findings were noted at 100 ppm.

At 4000 ppm, body weights were generally decreased ($p<=0.05$) at Weeks 3-57 in the males (decr. 3-5%), and at Weeks 53-74 in the females (decr. 5-7%). This was reflected in decreased body weight gains in males at Weeks 1-13 (decr. 16), and in both sexes at Weeks 1-52 (decr. 14-22) and Weeks 1-78 (decr. 9-13).

A subclinical treatment-related anemia was observed in the 4000 ppm males. Decreases (not statistically significant [NS]) in erythrocytes (decr. 11%), hemoglobin (decr. 8%), and hematocrit (45% treated vs 49% controls) were noted at 12 months, and decreases (NS) in erythrocytes (decr. 13%) and hemoglobin (decr. 11%) were noted at 18 months. At 18 months, decreased ($p<=0.05$) hematocrit (41% treated vs 47% controls), and increased ($p<=0.05$) red cell distribution width (15.1% treated vs 13.4% controls) and reticulocytes (4.9% vs 2.6% controls) were observed. Increased ($p<=0.05$) hemoglobin distribution width was noted at 12 and 18 months (incr. 12-16%). Also, hemosiderin pigmentation of the spleen, thought to be hemoglobin-derived, was noted ($p<=0.05$) in the 1500 ppm males (34%; mild) and the 4000 ppm group (62-66%; moderate).

The LOAEL is 4000 ppm (equivalent to 709.0/806.3 mg/kg/day [M/F]), based on decreased body weight and body weight gains in males and females, and subclinical anemia and hemosiderin pigmentation of the spleen in males. The NOAEL is 1500 ppm (equivalent to 244.7/275.0 mg/kg/day [M/F]).

At the doses tested, there was not a treatment-related increase in tumor incidence when compared to controls. Dosing was considered adequate based on decreased body weight and body weight gains in males and females, and subclinical anemia and hemosiderin pigmentation of the spleen in males.

This study is classified as acceptable/guideline and satisfies the guideline requirements (OPPTS 870.4200b; OECD 451) for a carcinogenicity study in mice.

870.7485. Metabolism Study in Rats. MRID 45121701.

EXECUTIVE SUMMARY: In a rat metabolism study (MRID 45121701), [triazolinone-3-^{14}C] MKH 3586 (amicarbazone; Vial No.: C-710A; radiochemical purity >99%) in water, was administered to 4 male Fischer rats as a single gavage dose at 5.25-5.99 mg/kg. Samples were collected from these animals to determine elimination kinetics and to quantify metabolites.

Overall recovery of the radioactive dose was 95% by 72 hours following administration. The majority of the dose was recovered in the urine within 24 hours (64% dose), indicating substantial absorption. Fecal excretion accounted for 27% dose within 24 hours

post-dose. Only 2% dose was recovered in the excreta after 24 hours. At 72 hours following administration, ≤1% dose was found in each of the following fractions: carcass and selected tissues, CO_2 trap, volatile organic trap, urine trap wash, and cage and feces trap wash. Concentrations of radioactivity in the selected tissues at 72 hours post-dose were low (0.004-0.073 µg/g).

HPLC and LC-MS/MS analyses were used to identify the parent and 9 metabolites in excreta from male rats following oral dosing with [triazolinone-3-^{14}C] MKH 3586. Identified compounds in excreta accounted for 74% of the dose. Unidentified peaks in urine and fecal extracts each accounted for <1% dose, and unanalyzed fractions from urine and feces each accounted for <2% dose. Overall accountability of the administered dose was ~84%, with minor fractions and unknowns being reported at accounting for "<1% dose".

Minor amounts of parent were detected in both urine (~2% dose) and feces (<1% dose). The major metabolite in excreta was a hydroxylated, deanimated derivative of the parent, iPr-2-OH DA (34% dose), mainly found in the urine (32% dose). Other hydroxylated deanimated derivatives were also identified: tBu-OH DA (11% dose in urine and 4% dose in feces), and iPr-1,2-diOH DA (6% dose in urine and <1% dose in feces). The glucuronic acid conjugate of the parent, MKH 3586 GA, accounted for 11% dose, mainly in the feces (10% dose). Other minor metabolites (each ≤3% dose) identified in excreta included: tBu-1,2-di-iPr-tri-OH DA; tBu-iPr-di-OH DA; iPR-1,3-di-OH DA; tBu-OH-iPr-ene and DA. Based on the metabolite profile, the metabolism of MKH 3586 in males rats primarily involves deanimation followed by hydroxylation with elimination in the urine. Parent also undergoes glucuronic acid conjugation and elimination in the feces.

This metabolism study in the rat is classified acceptable/guideline and satisfies the guideline requirement for a Tier 1 metabolism study [OPPTS 870.7485, OPP 85-1] in rats.

870.7485. Metabolism Study in Rats. MRID 45121634.

EXECUTIVE SUMMARY: In a rat metabolism study (MRID 45121634), [triazolinone-3-^{14}C] 4-methyl MKH 3586 (soil metabolite of amicarbazone; Vial No.: C-803; radiochemical purity >99%) in water, was administered to 4 male Fischer rats as a single gavage dose at 4.86-5.22 mg/kg. Samples were collected from these animals to determine elimination kinetics and to identify and quantify metabolites.

Overall recovery of the radioactive dose was 91% by 96 hours following administration. The majority of the dose was recovered in the urine within 12 hours (70% dose), with total urinary excretion accounting for 80% dose, indicating substantial absorption. Fecal excretion (0-24 hours post-dose) accounted for 8% dose. Approximately 3% dose was recovered in the excreta after 24 hours. At 96 hours following administration, 2% dose was found in the urine trap wash and <1% dose was found in each of the following fractions: CO_2 trap, volatile organics trap, cage and feces trap wash, carcass and selected tissues. At 96 hours post-dose, the concentration of radioactivity in the tissues was low (0.002-0.028 µg/g), with <0.1% dose in each tissue.

HPLC, ^{1}H-NMR, and LC-MS/MS analyses identified 12 components in excreta from rats following oral dosing with [triazolinone-3-^{14}C] 4-methyl MKH 3586. Four of these compounds were present as chiral pairs. Identified compounds accounted for 75% of the dose. All unidentified peaks each accounted for <=1% of the dose, and individual

unanalyzed fractions each accounted for <2% dose. The overall accountability of the administered dose was ~82%. The proposed pathway for biotransformation of [triazolinone-3-^{14}C] 4-methyl MKH 3586 in rats is presented in the Appendix to this DER.

The test substance was not detected in the urine or feces. Hydroxylation of the soil metabolite at the isopropyl moiety formed the major metabolite, 4-Me-i-Pr-2-OH DA MKH 3586 (29% dose), which was found primarily in urine (28% dose). Further hydroxylation at the tertiary butyl group resulted in the formation of 4-Me-t-Bu-iPr-2-di-OH DA MKH 3586, which accounted for 12% dose. Alternatively, additional hydroxylation of the isopropyl moiety formed 4-Me-iPr-1,2-di-OH DA MKH 3586, which constituted 9% dose in urine. Desmethylation of the parent formed DA MKH 3586 (<1% dose), which was then hydroxylated at the isopropyl moiety to form iPr-2-OH DA MKH 3586 (8% dose). The parent was also be hydroxylated at the tertiary butyl moiety to form 4-Me-tBu-OH DA MKH 3586 (9% dose), which was only detected urine. The other minor identified metabolites occurred resulted from further oxidation, usually hydroxylation. The pathway for the metabolism of 4-methyl DA MKH 3586 in rats primarily involves a series of hydroxylation reaction. No conjugates were detected.

This metabolism study in the rat is classified acceptable/guideline and satisfies the guideline requirement for a Tier 1 metabolism study [OPPTS 870.7485, OPP 85-1] in rats.

870.5100. Bacterial Reverse Mutation Test. MRID 45121617.

EXECUTIVE SUMMARY - In independently conducted trials of a reverse gene mutation assay in bacteria (MRID 45121617), *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 were exposed to triazolinon (an intermediate of amicarbazone; 99.9% a.i., Lot/Batch #: LVK2304/11) in dimethyl sufoxide (DMSO) at concentrations of 16, 50, 158, 500, 1581, or 5,000 µg/plate in the presence and absence of S9-activation in both the plate incorporation and pre-incubation methods. Standard strain-specific mutagens served as positive controls in both trials. The S9 was derived from the livers of Sprague Dawley rats induced with Aroclor 1254.

Triazolinon was tested up to the limit dose (5000 µg/plate, +/-S9). No evidence of cytotoxicity was observed in either assay (+/-S9). No marked increase in the mean number of revertants compared to solvent controls was observed at any dose level in either trial in the presence or absence of S9-activation. The positive controls induced the appropriate responses. There was no evidence of induced mutant colonies over background.

The study is classified as acceptable/guideline and satisfies the guideline requirement for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

870.5100. Bacterial Reverse Mutation Test. MRID 45121616.

EXECUTIVE SUMMARY - In independently conducted trials of a reverse gene mutation assay in bacteria (MRID 45121616), *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 were exposed to oxadiazolinon (an intermediate of amicarbazone; 99.9% a.i., Lot/Batch #: LVK2302/3/1) in ethylene glycol dimethylether (EGDE) at concentrations of 16, 50, 158, 500, 1581, or 5,000 µg/plate in the presence and absence of S9-activation in both the plate incorporation and pre-incubation methods.

Standard strain-specific mutagens served as positive controls in both trials. The S9 was derived from the livers of adult male Sprague Dawley rats induced with Aroclor 1254.

Oxadiazolinon was tested up to the limit dose (5000 µg/plate, +/-S9). Cytotoxicity was observed at 5000 µg/plate in strains TA102 and TA1535 in Trial 1 (+S9). No marked increase in the mean number of revertants compared to solvent controls was observed at any dose level in either trial in the presence or absence of S9-activation. The positive controls induced the appropriate responses. There was no evidence of induced mutant colonies over background.

The study is classified as acceptable/guideline and satisfies the guideline requirement for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

870.5100. Bacterial Reverse Mutation Test. MRID 45121519.

EXECUTIVE SUMMARY - In independently conducted trials of a reverse gene mutation assay in bacteria (MRID 45121519), *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 were exposed to MKH 3586 (amicarbazone; 98.4% a.i., Lot/Batch #: 17004/93) in dimethyl sulfoxide (DMSO) at concentrations of 16, 50, 158, 500, 1581, or 5,000 µg/plate in the presence and absence of S9-activation in both the plate incorporation and pre-incubation methods. Standard strain-specific mutagens served as positive controls in both trials. The S9 was derived from the livers of adult male Sprague Dawley rats induced with Aroclor 1254.

MKH 3586 was tested up to the limit dose (5000 µg/plate, +/-S9). Cytotoxicity was observed at 5000 µg/plate in strain TA98, as indicated by a reduction in the cell titer but not the revertant colonies (Trial 1, +S9). No marked increase in the mean number of revertants compared to solvent controls was observed at any dose level in either trial in the presence or absence of S9-activation. The positive controls induced the appropriate responses. There was no evidence of induced mutant colonies over background.

The study is classified as acceptable/guideline and satisfies the guideline requirement for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

870.5300 *In vitro* Mammalian Cell Gene Mutation Test. MRID 45121514.

EXECUTIVE SUMMARY - In two independent trials of a mammalian cell gene mutation assay at the HGPRT locus (MRID 45121514), V79 (Chinese hamster lung fibroblast) cells cultured *in vitro* were exposed to MKH 3586 (amicarbazone; 98.1% a.i., Lot/Batch#: 05362/0005) in dimethylsulfoxide (DMSO) at concentrations of 250, 500, 1000, 2000, or 4000 µg/mL in the presence and absence of S9-activation for 5 hours. The S9 fraction was derived from the livers of male Wistar rats induced with Aroclor 1254.

MKH 3586 was tested up to the limit of solubility (4000 µg/mL, +/-S9). A reproducible increase in mutant frequency was not observed in either trial in the presence or absence of S9. The positive controls induced the appropriate response. There was no evidence that MKH 3586 induced mutant colonies over background in the presence or absence of S9-activation.

This study is classified as acceptable/guideline and satisfies the guideline requirement (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

870.5375. *In vitro* Mammalian Chromosome Aberration Test. MRID 45121513.

EXECUTIVE SUMMARY - In independently performed mammalian cell cytogenetic assays (chromosome aberration) (MRID 45121513), V79 Chinese hamster lung fibroblast cultures were exposed, both in the presence and absence of S9-activation (+/-S9) for 4 hours, to MKH 3586 (Amicarbazone; 98.1% a.i., Lot/Batch #: 05362/0005) in dimethylsulfoxide (DMSO) at concentrations of 1000, 2000, 3000, 4000, or 5000 µg/mL (Trial 1, cells harvested at 18 hours post-exposure) and 3000, 4000, or 5000 µg/mL (Trial 2, cells harvested at 30 hours post-exposure). The S9 was derived from the livers of male Sprague Dawley rats induced with Aroclor 1245.

MKH 3586 was tested up to the limit of solubility, a precipitate was observed at 5000 µg/mL (+/-S9), and evaluated for chromosome aberrations at up to 3000 µg/mL. In Trial 1, mitotic indices were significantly reduced compared to solvent controls at >=3000 µg/mL (+/-S9). In Trial 2, mitotic indices were significantly reduced at >=4000 µg/mL (+/-S9). No biologically relevant increases in aberration frequency were observed at up to 3000 µg/mL at 18 or 30 hours post-exposure in the presence or absence of S9-activation. The positive controls induced the appropriate response. There was no evidence of chromosome aberration induced over background in the presence or absence of S9-activation.

This study is classified as acceptable/guideline and satisfies the guideline requirement (OPPTS 870.5375, OECD 473) for *in vitro* mutagenicity (chromosome aberration) data.

870.5395. Mammalian Erythrocyte Micronucleus Test. MRID 45121515.

EXECUTIVE SUMMARY - In a bone marrow micronucleus assay (MRID 45121515), 5 Hsd/Win NMRI mice/sex/dose were treated once via intraperitoneal injection (i.p., 10 mL/kg) with MKH 3586 (amicarbazone; 98.1% a.i., Lot/Batch #: 05362/0005) in 0.5% aqueous Cremaphor at doses of 0 or 100 mg/kg bw. Bone marrow cells were harvested from the test material and vehicle control groups at 16, 24, and 48 hours post-dosing and from the positive control group at 24 hours post-dosing.

MKH 3586 was tested at 100 mg/kg. Clinical signs observed included apathy, spasm, twitching, hind legs spread wide apart, and labored breathing. Significantly ($p<0.01$) increased NCE: PCE (indicative of bone marrow toxicity) was observed at the 48 hour sampling time. The positive control induced the appropriate response. There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow at any treatment time.

The study is classified as acceptable/guideline and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for *in vivo* cytogenetic mutagenicity data.

Non-Guideline Studies

Subchronic Mechanistic Feeding Study in Rats. MRID 45121603.

EXECUTIVE SUMMARY: In a non-guideline mechanistic subchronic oral toxicity study (MRID 45121603), MKH 3586 (Amicarbazone; ≥98% a.i., Lot/batch # PT:05362/0005) was administered to 25 Fisher 344 rats/sex/group in the diet at dose levels of 0, 50, 1250 or 2500 ppm (equivalent to 0/0, 0.8/0.6, 19.4/13.5 or 40.0/28.8 mg/kg bw/day in males/females) for 10 weeks. Additionally, 5 rats/sex/group were fed test diets containing 0 and 2500 ppm for 10 weeks followed by a four-week recovery period. The purpose of this study was to determine whether thyroid hormone changes (observed in subchronic and chronic toxicity studies with MKH 3586 in the rat) are due to a direct effect of the test substance on the thyroid or to effects on liver enzymes that affect thyroid hormone metabolism. Thyroid function was assessed using the perchlorate discharge assay and by determining circulating levels of thyroid hormones T3 and T4 (total and free measured for both) and TSH. Liver function was assessed by determining liver weight and levels of hepatic UDP-glucuronosyltransferase and 5'deiodinase activities. Additionally, estrous cycle duration and frequency were examined.

In the 1250 ppm females, TSH, free T3, T3, free T4 and T4 were increased by 27-61%. In the 2500 ppm females, levels of these thyroid hormones were increased over controls by 5-24% (not significant [NS]) but to a lesser extent than at 1250 ppm. Increased T3 (incr. 52%) and free T3 (incr. 31%; NS) were observed in males at 2500 ppm. At the end of the recovery period, levels of TSH, T3 and T4 in 2500 ppm males and females were comparable to controls. In the perchlorate assay, thyroid to blood ratio of ^{125}I in MKH 3586 treated groups were comparable to negative controls in males and females, indicating that the increase in thyroid hormones is not due to increased synthesis. Thus, an organ or tissue other than the thyroid must be responsible for altered levels of thyroid hormones in rats treated with ≥1250 ppm test diets. There were no treatment-related effects on thyroid weight or microscopic changes in the thyroid at any dose.

In the liver, UDP-GT activity was increased by 109-241% in the ≥1250 ppm males and females and remained increased by 27% in the 2500 ppm females at the end of recovery. Absolute/relative (to body) liver weights were increased in the main study 1250 ppm males (incr. 9/14%) and females (incr. 5/12%) and in the 2500 ppm males (incr. 25/32%) and females (incr. 18/31%). Relative liver weights remained increased (incr. 7%) in the 2500 ppm males at the end of the recovery period. Absolute liver weights in males and absolute and relative liver weights in females were comparable to controls at the end of the recovery period. Because metabolism of the test substance is primarily via glucuronidation by UDP-GT, it is postulated that MKH 3586 competitively inhibited UDP-GT glucuronidation of T3 and T4 at 1250 ppm, resulting in increased levels of these thyroid hormones in the blood serum. At 2500 ppm, further induction of UDP-GT began to compensate for this competitive inhibition, allowing T3 and T4 levels to decline.

In the females, terminal body weights were decreased by 7-10% at ≥1250 ppm during the main study and remained decreased by 8% at 2500 ppm at the end of recovery. An increase in the number of animals spilling food was noted at 2500 ppm. Food consumption was intermittently decreased on a g/animal/day basis by 3-16% ($p \leq 0.05$) in the: (i) main study and recovery group males at 2500 ppm during treatment; (ii) main study and recovery group females at ≥1250 ppm during treatment; and (iii) 2500 ppm females at the end of the recovery period. On a g/kg bw/day basis, food consumption was

similar among groups. Absolute/relative uterine weights were decreased at 1250 ppm (decr. 15/9%) and 2500 ppm (decr. 24/15%). Decreases in absolute (decr. 20%) and relative (decr. 13%; NS) uterine weights continued in the 2500 ppm recovery group animals. There were no effects of treatment on the number of estrous cycles. However, a minor increase of 4% in the duration of the estrous cycle was observed at 2500 ppm.

In conclusion, thyroid hormones were increased in the >=1200 ppm females and 2500 ppm males. However, thyroid to blood ratios of ^{125}I in treated groups were comparable to negative controls, indicating there was no impairment thyroid hormone synthesis. Thus, the differences in thyroid hormones must be due to metabolism at an extra-thyroidal site. The liver was implicated as this site based on increased liver weights and UDP-glucuronosyltransferase activity.

The submitted special mechanistic subchronic oral toxicity study in the rat is classified as acceptable/non-guideline and satisfies the purpose for which it was intended.

***In vitro* Studies on Enzymes of Thyroid Hormone Regulation. MRID 45121618.**

EXECUTIVE SUMMARY: In a non-guideline study (MRID 45121618), possible interactions of MKH 3586 (Amicarbazone; 98.2% a.i., Lot/batch # 05362/0005, TOX 4490), MKH 3594 (N-desamino metabolite; 99.4% a.i., batch # 200898) and KOK 9422 (putative hydrolysis metabolite; 100% a.i., batch # 130898) with the enzymes involved in the synthesis of thyroid hormones and the regulation of the hypothalmus-pituitary-thyroid axis were investigated using three *in vitro* systems: (i) thyroid peroxidase (TPO), the key enzyme responsible for organification of iodide and the coupling of iodinated tyrosine residues to both T3 and T4; (ii) iodothyronine deiodinase type I (ID-I), catalyzing the phenolic ring deiodination of T4 to form T3 in peripheral organs like thyroid, liver and kidney; and (iii) iodothyronine deiodinase type II (ID-II), catalyzing the phenolic ring deiodination of T4 to T3 in the hypothalamus and pituitary gland.

Positive controls adequately demonstrated the sensitivity of each of these *in vitro* assays through strong, concentration-dependent inhibition of the enzyme of concern. Amitrole inhibited TPO-catalyzed guaiacol oxidation and iodine formation. Ethylenethiourea (ETU) temporarily inhibited iodine formation, not by inhibiting TPO itself but instead by reducing iodinating species (i.e., trapping). Propylthiouracil (PTU) inhibited ID-I, and iopanoic acid inhibited ID-II.

Neither MKH 3586, MKH 3594, nor KOK 9422 inhibited TPO, ID-I or ID-II at concentrations up to 1 mM, suggesting that MKH 3586 does not affect the iodide organification step of thyroid hormone synthesis (via either inhibition of TPO or trapping of iodine) or the peripheral metabolism of thyroid hormones via Type I or Type II deiodinases *in vivo*. These findings support the conclusion that MKH 3586 does not affect the major enzymes involved in the synthesis or regulation of thyroid hormones and are consistent with the findings of the non-guideline subchronic mechanistic study in rats (MRID 45121603) which indicate that upregulation of UDP-glucuronosyl transferase in the liver may account for alterations in the thyroid hormone profile.

The submitted special *in vitro* mechanistic study is classified as acceptable/non-guideline and satisfies the purpose for which it was intended, to investigate the possible inhibition of enzyme regulation of thyroid hormone synthesis and metabolism.

Non-Guideline Behavioral Study in Rats. MRID 45121511, 45121521, 45121629.

EXECUTIVE SUMMARY: In three special studies (MRIDs 45121511, 45121521 and 45121629), BAY 31-4666 (amicarbazone, 98.4% a.i., Batch #: 05362/0005) was administered in a single gavage dose in 0.5% aqueous tylose suspension (w/v) plus 0.4% TWEEN 80 (5 mL/kg) to 6-10 male HsdCbp WU rats/dose at 0, 1.0, 2.5, 5, 10, 20 or 100 mg/kg bw. The purpose of the studies was to assess central nervous system (CNS) effects in rats dosed with BAY 31-4666 and to determine a LOAEL. In the first two studies, rats were dosed with 0, 1,0, 20.0 or 100.0 mg/kg BAY 31-4666. In (1) MRID 45121529, 6 rats/dose were evaluated for behavioral or physiological changes by the modified Irwin test every 15 minutes for 3 hrs postdosing, then at 24 hrs postdosing. In (2) MRID 45121521, 5 rats/dose were evaluated for catalepsy (5 rats/dose) and 6 rats/dose for body temperature (both assessed every 30 minutes postdosing up to 240 minutes; predosing body temperature measurements also taken), and 10 rats/dose were evaluated for psychomotoric activity (traveling distance, resting time and rearing) at 30, 60 and 120 minutes postdosing. In the third study (MRID 45121511), 6 rats/dose were administered BAY 31-4666 at 0, 2.5, 5.0 or 10.0 mg/kg and evaluated by the modified Irwin test every 15 minutes for 3 hrs postdosing, then at 24 hrs postdosing.

No treatment-related effect was observed in the 1 mg/kg group, nor on clinical signs at 2.5 and 5 mg/kg. No treatment-related effect was observed during the catalepsy test. A treatment-related decrease in body temperature was observed in the 100 mg/kg males; however, the minor decreases (<=0.6°C) were considered not to be biologically adverse.

The following clinical signs (# rats) were observed: (i) sedation at 10 (1), 20 (3) and 100 (6) mg/kg; (ii) ptosis at 10 (1), 20 (3) and 100 (6) mg/kg; and (iii) salivation at 10 (2), 20 (2) and 100 (6). Additionally, piloerection (2), Straub phenomenon (4), and prone position (2) were observed in the 100 mg/kg group. The effects were first observed at 30 minutes post-dose, and no effect was observed in any animal after 150 minutes post-dose, with the higher dose groups showing greater persistence of effects.

A dose- and time-dependent effect was demonstrated on motor activity. In the 20 and 100 mg/kg groups, decreased (↓17-28%; p<=0.05) distance traveled and increased (↑19-36%; p<=0.05) resting time was observed at 30 minutes post-dose. Rearing was decreased (↓52-54%; p<=0.05) at 100 mg/kg at 30 and 60 minutes post-dose; a non-significant reduction of ↓34% was also observed at 20 mg/kg at 60 minutes. The CNS effects LOAEL is 10 mg/kg, based on transient sedation, ptosis and salivation. The NOAEL is 5 mg/kg.

This study is classified as acceptable/non-guideline and satisfies the purpose for which it was intended.

Non-Guideline Study of Central Nervous System Safety Pharmacology in Mice. MRID 45121522.

EXECUTIVE SUMMARY - In a non-guideline study (MRID 45121522), a single dose of MKH 3586 (Amicarbazone; Lot/batch # 05362/0005; 98.4% a.i.), in a 0.5% aqueous tylose suspension (w/v) plus 0.4% Tween-80, was administered orally, in a dosing volume of 10 mL/kg, to 40 male HsdWin: NMRI mice/group at dose levels of 0, 1, 20 or 100 mg/kg/day. Control animals received an appropriate volume of vehicle without test substance. The following tests were performed (10 animals/test): (1) hot plate test (analgesia), 56°C at pretreatment, 30, 45, 60 and 120 min postdosing; (2) traction, balance

rod and electroshock test (reduction of traction force, sensorimotor disturbances and anticonvulsive activity at 30, 40 and 50 minutes postdosing, respectively); (3) pentylenetetrazole test (pro- and anticonvulsive activity at 45 minutes postdosing; volume of pentylenetetrazole required to induce clonic seizure) and (4) hexobarbital test (potentiation or reduction of narcotic effects at 60 minutes postdosing, duration of anesthesia measured). The objective of this study was to assess the effects of a single oral administration of MKH 3586 on the central nervous system of mice.

At 100 mg/kg, mice showed increased (not significant unless noted) response times compared to controls to a nociceptive stimulus at 30 (incr. 24%), 45 (incr. 45%), 60 ($p<=0.05$; incr. 67%) and 120 (incr. 32%) minutes after dosing. 2/10 mice were observed to have reduced traction force and impaired motor coordination, compared to 0/10 in the controls and in the 1 and 20 mg/kg groups. An increased ($p<=0.05$) mean threshold dose of pentylenetetrazole was noted (incr. 16%) compared to controls, indicating an anticonvulsant effect by the test compound. Mice also were observed to exhibit sedation and partial ptosis approximately 20 minutes after treatment.

Following treatment with the test compound, all mice at all doses exhibited tonic convulsions in the electroshock test. Additionally, no treatment-related effects were observed on the duration of hexobarbital-induced anesthesia. There were no effects on the CNS of the 1 and 20 mg/kg mice in any test.

The data indicate that a single oral dose of MKH 3586 at 100 mg/kg causes minimal CNS functional impairment, characterized by increased reaction times to nociceptive stimuli, reduced traction force, impaired motor coordination, sedation, partial ptosis and a mild anticonvulsive effect.

The submitted study is classified as acceptable/non-guideline and satisfies the intended purpose of assessing the effects of a single oral administration of MKH 3586 on the central nervous system of mice.

3.0 METABOLISM CONSIDERATIONS

1.01 Introduction
- 1.1 Description of Issues
- 1.2 Team Proposal

2.0 Nature of the Residue Studies in Plants

2.1.1 Nature of the Residue in Corn.

A plant metabolism study was conducted on corn with soil application of [triazolinone-3-^{14}C]amicarbazone at 1.6X the maximum seasonal rate; application to the soil was made pre-emergence, 4 hours after planting corn seed into containers in a greenhouse. The total radioactive residues (TRRs) were 0.988, 2.369, and 0.054 ppm in corn forage, fodder, and grain, respectively, with preharvest intervals (PHIs) of 104-126 days. Total extractable residues were 91-100% TRR. Residues were characterized/identified primarily by HPLC analysis with confirmatory analysis by TLC and LC/MS.

Total identified residues were 74-79% TRR in corn commodities. Parent amicarbazone, was the major residue identified in forage and fodder, and was found in smaller amounts in grain. The major residue identified in grain was the metabolite iPr-2-OH DA MKH 3586, which was also found in forage and fodder. Other identified residues were DA MKH 3586, 4-N-glu-DA MKH 3586 and iPr-2-O-glu-DA MKH 3586 in grain, forage and fodder; and tBu-iPr-2-diOH DA MKH 3586 in forage only. Four additional glucose/glucoside conjugates were identified in small amounts (≤5% TRR) in fodder.

The nature of the residue in plants is adequately understood for the purpose 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 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; and 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 residues of concern in field corn commodities are amicarbazone and its metabolites DA amicarbazone and iPr-2-OH DA amicarbazone for both tolerance setting and risk assessment purposes. The corn metabolism study will also cover the proposed use on sugarcane, given the physiological similarities between corn and sugarcane. However, additional plant metabolism studies would be required to support use on other crops.

2.1.2 Tabular Summary of the Field Corn Metabolism Study

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Corn Matrices.

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.

3.0 Nature of the Residue in Livestock

3.1.1 Executive Summary of Ruminant Study

A study investigating the metabolism of [triazolinone-3-^{14}C]amicarbazone in goats was submitted in conjunction with the proposed use on corn. 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). 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.

Radioactive residues were highest in the meat by-products liver and kidney, followed by muscle, fat, and milk. The majority of the radioactivity (~85-99% TRR) was extracted from goat tissues using methanol/acetic acid and acetone (liver, kidney, and muscle) or acetonitrile (ACN; milk and fat), followed by methanol/water extraction and subsequent acid hydrolysis. Nonextractable residues accounted for <1-3% TRR.

Total residues amounting to 91-99% TRR were identified in goat matrices. Both parent amicarbazone and metabolite DA amicarbazone were major residues; amicarbazone was a major residue in all matrices with the exception of kidney. Metabolite iPr-2-OH DA amicarbazone was a major residue in kidney but was a minor residue in all other matrices. Metabolite tBu-OH MKH 3586 was also identified as a major residue in milk, muscle, and kidney, but was a minor residue in fat and liver. Metabolite iPr-Ene DA MKH 3586 was a major residue in liver 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.

The proposed metabolic pathway for amicarbazone in goats includes deamination followed by hydroxylation. Loss of the triazole amino group (deamination) yields 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.

Pending submission of data to address several deficiencies in the ruminant metabolism study, the nature of the residue in ruminants is adequately understood for the purpose of a conditional registration. The residues of concern in ruminants are amicarbazone and its metabolites DA amicarbazone and iPr-2-OH DA amicarbazone, for both tolerance setting and risk assessment purposes.

3.1.2 Tabular Summary of the Goat Metabolism Study

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Ruminant Matrices

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.

3.1.3 Executive Summary of the Poultry Metabolism Study

A study investigating the metabolism of [triazilinone-3-^{14}C]amicarbazone in hens was submitted in conjunction with the proposed use on corn. Radiolabeled amicarbazone was administered orally to laying hens at an average of 15.3 ppm in the diet (49x the maximum theoretical dietary burden to poultry). 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.

Total radioactive residues (TRRs) were highest in liver, followed by residues in muscle, fat and eggs; radioactive residues in eggs increased over the 3 days of dosing. A large portion of the TRR (~58-91% TRR) was extracted from hen commodities, except fat, using ACN/water or ACN; additional radioactivity was released from fat and eggs via acid

hydrolysis and enzyme hydrolysis. Nonextractable residues accounted for 3-14% of radioactivity in hen matrices.

Parent amicarbazone was a minor residue in the extracts of all matrices but was identified in the protease hydrolysate of liver. The major residue identified in eggs and tissues was iPr-2-OH DA amicarbazone; metabolite DA amicarbazone was a significant residue in eggs but was found in smaller amounts in tissues. Metabolite iPr-1-OH DA MKH 3586 was a significant residue in liver 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 but not in muscle and liver. Metabolite iPr-Acid DA MKH 3586 was identified in liver only. Other identified metabolites were triazolinone MKH 3586, triazolinone DA MKH 3586, tBu-OH MKH 3586, and iPr-1,2-diOH MKH 3586 (muscle and liver only, each at <7% of radioactivity). Remaining radioactivity consisted of unknowns, which were generally polar in nature.

The proposed metabolic pathway for amicarbazone in hens includes 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. 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.

Pending submission of data to address several deficiencies in the hen metabolism study, the nature of the residue in poultry is adequately understood. The residues of concern in poultry commodities are amicarbazone and its metabolites DA amicarbazone and iPr-2-OH DA amicarbazone for both tolerance setting and risk assessment purposes.

3.1.4 Tabular Summary of the Poultry Metabolism Study

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Hen Matrices.

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.

4.0 Confined Rotational Crop Studies

4.1 Executive Summary of Confined Rotational Crop Study

A confined rotational crop study with [triazolinone-3-^{14}C]amicarbazone was submitted to support the proposed use on corn, as well as proposed rotational crop tolerances. Radiolabeled amicarbazone was applied directly to silt loam soil at 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). Although a 30-day plantback interval (PBI) was originally proposed, crops planted 30 DAT failed due to 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. Total radioactive residues 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, residues were

highest in turnip tops and wheat hay and straw, and were significantly lower in other commodities. In rotational crop matrices from the 138-day and 364-day PBIs, radioactivity was highest in wheat hay and straw, while residues in other commodities were significantly lower.

Methanol extraction released 60->100% of the radioactivity from rotational crop matrices, with the exception of wheat grain, which required methanol extraction at reflux. Nonextractable residues in 138- and 364-DAT rotational crop commodities were <1-9% of total radioactivity. The extraction procedures extracted sufficient residues from rotational crop matrices from the 138- and 364-day PBIs; problems with extraction of bound residues of 47-DAT samples were due to the smaller amount of sample material available.

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 rotational 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, and metabolite iPr-2-OH DA amicarbazone was also a significant residue in all rotational commodities. Metabolite DA amicarbazone was identified in kale, turnip top, wheat forage, and wheat straw at. Glucose conjugates DA OH Glu I MKH 3586 and DA OH Glu II MKH 3586 were major residues in wheat hay and straw, but were found in smaller quantities in kale, turnip top, and wheat forage. Other identified residues 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), all at <3% of radioactivity.

In 138-DAT matrices, amicarbazone was a major residue in all commodities except turnip root and wheat grain; amicarbazone residues were very low in turnip root, and it was not found in wheat grain. Metabolite iPr-2-OH DA amicarbazone was found to be a significant residue in all rotational commodities. Metabolite DA amicarbazone was identified in all commodities except wheat grain. Glucose conjugates DA OH Glu I MKH 3586 and DA OH Glu II MKH 3586 were major residues in wheat hay, grain, and straw, but were found in smaller quantities in kale, turnip top, and wheat forage. Other identified residues 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), all at <5% of radioactivity.

In 364-DAT matrices, amicarbazone was a major residue in kale, turnip top, and wheat forage; amicarbazone residues were lower in wheat hay and straw, and it 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. Metabolite DA amicarbazone was identified in all commodities except wheat grain at. Two glucose conjugates (DA OH Glu I MKH 3586 and DA OH Glu II MKH 3586) were major residues in wheat hay, grain, and straw, but were found in smaller quantities in kale, turnip top, and wheat forage. Other identified residues 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), each at <10% of radioactivity.

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 tertiary carbon of the isopropyl moiety had undergone deamination and oxidation. Two minor metabolites identified in rotational crops, triazolinone MKH 3586 and N-Me DA amicarbazone, were not found in the corn metabolism study.

Although the characterization/identification of the radioactivity 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 exception of turnip roots and wheat grain, which had lower residues, 60-87% of the radioactivity was identified in rotational crop samples from the 47-day PBI, and the majority of these residues were accounted for by amicarbazone, DA amicarbazone, and iPr-2-OH DA amicarbazone. The only other major 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.

The data indicate uptake by rotational crops is likely to occur. Overall, metabolism of amicarbazone in rotational kale, turnips and wheat was similar to metabolism in the primary crop, corn, although the relative levels of the glucose conjugates were higher in rotated wheat commodities than in corn commodities. 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.

4.2 Tabular Summaries of Confined Rotational Crop Studies

TABLE C.2.3.1. Summary of Characterization and Identification of Radioactive Residues in Rotational Crop Matrices (47-day PBI).

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.

TABLE C.2.3.2. Summary of Characterization and Identification of Radioactive Residues in Rotational Crop (138-Day PBI).

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 (364-Day PBI)

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.

5.0 Tabular Summary of Analytical Methodology

Method Name	Applicable Commodities	Analytes	Extraction Solvent(s)	Clean-up Step(s)	Determinative Step	LOQ, ppm	LOD, ppm
Bayer Method 108340	Plants	Amicarbazone DA MKH 3586 iPr-2-OH DA MKH 3586	0.05% phosphoric acid	SPE	LC/MS/MS	0.01	0.001
Bayer Method 200258	Plants	Amicarbazone DA MKH 3586 iPr-2-OH DA MKH 3586	0.1% aqueous acetic acid	mixed mode C_8 and SCX SPE	LC/MS/MS	0.01	0.001-0.01
Bayer Method 108111	Livestock commodities	Same as plants but measured by oxidation to iPr-2-OH DA MKH 3586	0.05% phosphoric acid	SPE	LC/MS/MS	0.01	0.005

6.0 Summary of Magnitude of Residue (MOR) Studies

6.1 Plants

Adequate field trial data have been submitted to support the proposed use on corn. A total of 23 field corn field residue trials were conducted in accordance with required number and location of field trials [OPPTS Guideline 860.1500]. The 23 field corn field trials each consisted of 3 application scenarios - a single broadcast application made preemergence, a single preplant incorporated application (both at 0.45 lb ai/A) and an early postemergence application (0.25 lb ai/A). All applications were made using ground application equipment.

Even though the early postemergence application rate was the lowest of the 3 applications, it resulted in the highest combined residues of amicarbazone and its metabolites DA MKH 3586 and iPr-2-OH DA MKH 3586; residues following preplant incorporated application were slightly higher than those following preemergence application. Combined residues were at most 0.56 ppm in forage, 0.43 ppm in stover, and approximately 0.05 ppm in field corn grain.

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

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	0.44-0.46	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
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

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.
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.

6.2 Livestock

A ruminant feeding study was conducted in which encapsulated amicarbazone was administered orally to lactating cows for 30 days. Cows were dosed at 0.374, 1.238, or 4.538 ppm in the diet. Milk was sampled twice daily, and samples from study days 0, 4, 8, 12, 16, 18, 20, 22, 24, 26, and 28 were analyzed; samples of muscle, liver, kidney and fat were collected at sacrifice.

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 highest in liver and kidney, and lower in muscle and fat. In the high dose group, 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.

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.

6.3 Rotational Crops

A series of limited field rotational crop trials were conducted in which bare soil was treated once with amicarbazone at 0.42-0.46 lb ai/A (1x the maximum seasonal use rate on corn) using ground equipment. 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 (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, samples of mustard green leaves, turnip tops and roots, and wheat forage, hay, grain, and straw were harvested at intervals reflecting normal agricultural practices: 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 analyzed for residues of amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 using the proposed enforcement method. Quantifiable residues were observed in all rotated crops except turnip roots; residues of all analytes were <0.01 ppm in turnip roots at all PBIs. At the 1-month PBI, maximum combined residues of amicarbazone and its metabolites DA MKH 3586 and *i*Pr-2-OH DA MKH 3586 (expressed as parent equivalents), were <0.10 ppm in mustard greens, 0.12 ppm in turnip tops, <0.21 ppm in wheat forage, <0.40 ppm in hay, <0.07 ppm in grain, and 0.48 ppm in straw.

At the 4-month PBI, maximum combined residues were <0.04 ppm in mustard greens, <0.06 ppm in turnip tops, <0.10 ppm in wheat forage, <0.13 ppm in hay, <0.05 ppm in grain, and <0.19 ppm in straw.

At the 8-month PBI, maximum combined residues were 0.17 ppm in mustard greens, 0.23 ppm in turnip tops, <0.073 ppm in wheat forage, <0.13 ppm in hay, <0.04 ppm in grain, and <0.08 ppm in straw. At the 10-month PBI, maximum combined residues were <0.04 ppm in mustard greens, <0.04 ppm in turnip tops, <0.06 ppm in wheat forage, <0.12 ppm in hay, <0.03 ppm in grain, and <0.05 ppm in straw.

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 *i*Pr-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.

7.0 International Considerations

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

8.0 Environmental Degradation

See Sections 3.3 and 3.5 of the main document for information on environmental fate.

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