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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: July 12, 2005

Subject: Aminopyralid. Petition for the Establishment of Permanent Tolerances for Use of Aminopyralid on Grasses and Wheat. Summary of Analytical Chemistry and Residue Data. PP#4F6827.

DP Number:	D305665	Decision Number:	341121
PC Code:	005100/005209	MRID Numbers:	46235708-46235712, 46235714, 46235716-46235719, 46235721-46235725
40 CFR 180:	To be assigned	Chemical Class:	Herbicide

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JUL 18 2005

Executive Summary

Aminopyralid (4-amino-3,6-dichloropyridine-2-carboxylic acid) is the proposed common name of XDE-750, a new active ingredient developed by Dow AgroSciences. Aminopyralid is a systemic postemergence herbicide which belongs to the pyridine carboxylic acid class of compounds. The petitioner is currently proposing food/feed uses on grasses grown in rangelands and permanent pastures and on wheat for the selective control of invasive and noxious broadleaf weeds. It is also proposed for weed control in sites such as parks, electric utility rights-of way, forestry, woodlands, and wildlife openings, with smaller amounts used in railroads, utility substations, pipelines, and pumping stations.

The proposed end-use product (EP) is a soluble-concentrate liquid (SC/L) referred to by the trade name GF-871 (EPA Reg. No. 62719-LRI). The active ingredient in GF-871 is formulated as the triisopropanolammonium (TIPA) salt, with the product containing 40.6% of aminopyralid TIPA salt at an acid equivalent (ae) of 21.1% or 2 lb ae/gal. The 2 lb ae/gal SC/L formulation is proposed for broadcast foliar application at maximum rates of 0.11 lb ac/A (120 g ae/hectare) on rangeland and permanent pastures and 0.009 lb ae/A (10 g ae/ha) on wheat. The proposed PHIs are 0 days for wheat hay and 50 days for wheat grain and straw. No PHI is listed or proposed for grasses or wheat forage.

In support of the proposed uses, Dow AgroSciences has submitted a petition, PP#4F6827, for the establishment of permanent tolerances for residues of aminopyralid expressed as total parent, free and conjugated, in/on the following agricultural commodities:

Grass, forage	25 ppm
Grass, hay	65 ppm
Wheat, forage	2.0 ppm
Wheat, hay	4.0 ppm
Wheat, grain	0.05 ppm
Wheat, straw	0.5 ppm
Wheat bran	0.1 ppm
Wheat middlings	0.02 ppm
Wheat shorts	0.05 ppm
Wheat flour	0.01 ppm
Wheat germ	0.02 ppm
Wheat, aspirated grain fractions	0.5 ppm

Dow AgroSciences is also proposing the establishment of permanent tolerances for residues of aminopyralid *per se* in the following animal commodities:

Milk	0.02 ppm
Milk, cream	0.02 ppm
Meat of cattle, goats, hogs, horses, and sheep	0.05 ppm
Fat of cattle, goats, hogs, horses, and sheep	0.05 ppm
Liver of cattle, goats, hogs, horses, and sheep	0.05 ppm

Kidney of cattle, goats, hogs, horses, and sheep ..... 1.0 ppm

The subject petition is a joint review between the EPA, the Pest Management Regulatory Agency (PMRA) of Canada, and the Comisión Intersecretarial para el Control del Proceso y Uso de Plaguicidas y Sustancias Tóxicas (CICOPLAFEST) of Mexico.

The available data from metabolism studies with grass and wheat indicate that metabolism of aminopyralid is similar in these crops. In metabolism studies reflecting foliar applications to grass and wheat, aminopyralid was found to be metabolized to a multi-component mixture of water-soluble complexes which consist mostly of isomeric mixtures of acid- and base-labile N-glucosides and glucose ester conjugates of aminopyralid. Metabolism studies with lactating goats and laying hens show that most of the administered dose is rapidly excreted (~80% for hens and ~95% for goat). Residues in all poultry commodities, including eggs, were too low to allow identification of residues (TRR were less than 0.004 ppm aminopyralid-equivalents across all commodities). In the goat, residues were less than 0.008 ppm aminopyralid-equivalents in all commodities except kidney. In kidney, 80% of the TRR (0.07 ppm) was identified as parent aminopyralid. Although residues in other tissues were too low to permit identification, the weight-of-the-evidence is that the limited amount of aminopyralid that is not excreted remains as the parent compound; therefore, a new ruminant metabolism study is not required.

Adequate data are available to support a conditional registration for the use of the 2 lb ae/gal SC/L TIPA salt formulation on grasses. The final results for the ongoing storage stability study are needed to support the grass crop field trials. Adequate crop field trial data have been submitted for the emulsion-in-oil (EO) formulation of the TIPA salt. Based on side-by-side trials using the EO formulation and soluble concentrate liquid (SC/L) formulations (TIPA and K salts), the EO data are sufficient to support the requested use of the SC/L formulation. Adequate processing data have been submitted for wheat which indicate that a tolerance is needed for wheat bran. In addition, data for wheat aspirated grain fractions submitted in conjunction with the processing study indicate that a tolerance is needed for aspirated grain fractions. An adequate cattle feeding study has been submitted. It has been determined that a poultry feeding study is not needed to support this petition [40 CFR 180.6(a)(3)]. The proposed enforcement methods for plant and ruminant commodities have been sent to the EPA/OPP's Analytical Chemistry Laboratory for a tolerance method validation (TMV). The available confined rotational crop data indicate that field rotational crop studies are required to support the proposed rotational crop restrictions. There are currently no U.S. tolerances or international Codex maximum residue levels established for aminopyralid.

Regulatory Recommendations and Residue Chemistry Deficiencies

HED has examined the residue chemistry database for the new active ingredient aminopyralid. Pending resolution of the directions for use and reference standard deficiencies noted below, and successful method validation by the Agency, there are no residue chemistry issues that would preclude granting a conditional registration for this herbicide or establishment of tolerances for aminopyralid as follows:

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Tolerances for free and conjugated residues of aminopyralid:

Grass, forage	25	ppm
Grass, hay	50	ppm
Wheat, forage	2.0	ppm
Wheat, hay	4.0	ppm
Wheat, grain	0.04	ppm
Wheat, straw	0.25	ppm
Wheat, bran	0.1	ppm
Aspirated grain fractions	0.2	ppm

Tolerances for aminopyralid *per se*:

Milk	0.03	ppm
Cattle, meat	0.02	ppm
Goat, meat	0.02	ppm
Horse, meat	0.02	ppm
Sheep, meat	0.02	ppm
Cattle, fat	0.02	ppm
Goat, fat	0.02	ppm
Horse, fat	0.02	ppm
Sheep, fat	0.02	ppm
Cattle, meat byproducts, except kidney	0.02	ppm
Goat, meat byproducts, except kidney	0.02	ppm
Horse, meat byproducts, except kidney	0.02	ppm
Sheep, meat byproducts, except kidney	0.02	ppm
Cattle, kidney	0.3	ppm
Goat, kidney	0.3	ppm
Horse, kidney	0.3	ppm
Sheep, kidney	0.3	ppm

HED recommends that the interference study and storage stability data be made conditions of any registration for the requested uses.

860.1200 Directions for Use

- The petitioner should modify the proposed label to amend the recommendation regarding use of a surfactant; the label should be modified by removing the recommendations to use a surfactant in conjunction with application of aminopyralid to wheat. If the petitioner wishes to include the option to use a surfactant for application to wheat, then all the required wheat crop field trials should reflect the use of surfactant in the test substance application.

- The petitioner should modify the proposed label to specify a rotational crop plant-back interval of 4 months for barley, canola (rapeseed), flax, grasses, field corn, grain sorghum, oats, mustard, popcorn, and sweet corn.

860.1340 Residue Analytical Methods

- HED is concerned that the proposed enforcement method may not be able to differentiate between aminopyralid, picloram, and clopyralid. HED is requesting that the petitioner complete an interference study using these three compounds.

- The analytical methods for aminopyralid have not completed validation by the Agency. They are currently being evaluated.

860.1380 Storage Stability

- The available storage stability data for grass are not adequate to support the storage intervals and conditions for samples from the submitted grass crop field trials. To fully support the sample storage intervals and conditions, storage stability data for aminopyralid are needed for grass forage and hay reflecting up to approximately 15 months of frozen storage. The petitioner has stated that a final storage stability study, reflecting storage intervals of up to 18 months, will be submitted upon completion.

860.1650 Submittal of Analytical Reference Standards

- As of 09/07/2004, no reference standard for aminopyralid was available at the EPA National Pesticide Standards Repository. The petitioner should submit a reference standard for aminopyralid to the repository. In addition, because the proposed enforcement methods require the use of an internal standard for quantification, the petitioner should submit a quantity of the internal standard, $^{13}C_2{}^{15}N$-aminopyralid, to the repository.

Background

Aminopyralid [4-amino-3,6-dichloro-2-pyridinecarboxylic acid] is the proposed common name of XDE-750, a new active ingredient developed by Dow AgroSciences. Aminopyralid is a systemic postemergence herbicide which belongs to the pyridine carboxylic acid class of herbicides. HED notes that aminopyralid differs from the active ingredient picloram by the elimination of one chlorine atom from the 5 position on the pyridine ring (i.e., picloram is 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid) and from the active ingredient clopyralid by the addition of an amino group (clopyralid is 3,6-dichloro-2-pyridinecarboxylic acid). As with other chemicals in this class, aminopyralid's mode of action toward target weeds is not completely understood. The principle action of these compounds appears to affect cell wall plasticity and nucleic acid metabolism. The petitioner is currently proposing food/feed uses on grasses grown in rangelands and permanent pastures, and on wheat for the selective control of invasive and noxious broadleaf weeds. It is also proposed for weed control in sites such as parks, campgrounds, electric utility rights-of way, forestry, woodlands, and wildlife openings, with smaller amounts used in railroads, utility substations, pipelines, and pumping stations. The subject petition, PP#4F6827, represents the first food/feed uses of aminopyralid in the U.S. Concurrent with the registration application in the U.S., the petitioner is seeking a NAFTA

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review for registration of aminopyralid uses in Canada and Mexico. The proposed end-use product of aminopyralid is formulated as the triisopropanolammonium (TIPA) salt.

The PC Code and nomenclature of aminopyralid and aminopyralid TIPA salt, as well as the physicochemical properties, are presented in the tables below. The chemical names and structures of aminopyralid and its transformation products are presented in Appendix 1.

Table 1. Aminopyralid Nomenclature.

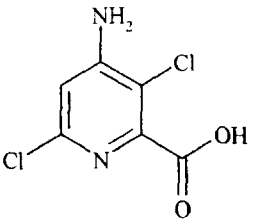
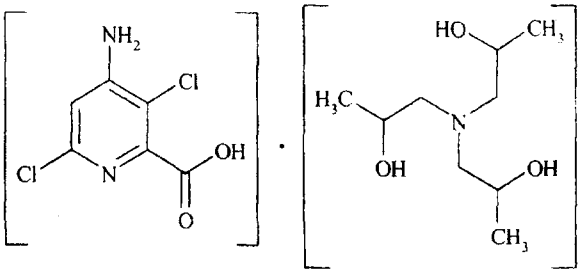
Chemical structure	
Common name	Aminopyralid
PC Code	005100
Company experimental name	XDE-750
IUPAC name	4-amino-3,6-dichloropyridine-2-carboxylic acid
CAS name	4-amino-3,6-dichloro-2-pyridinecarboxylic acid
CAS registry number	150114-71-9
End-use product (EP)	2 lb ae/gal TIPA salt SC/L formulation (GF-871 Herbicide; EPA Reg. No. 62719-LRI)
Chemical structure	
Common name	Aminopyralid, triisopropanolammonium (TIPA) salt
PC Code	005209
Company experimental name	XDE-750 TIPA salt
IUPAC name	Not provided
CAS name	Not provided
CAS registry number	Not provided
End-use product (EP)	2 lb ae/gal TIPA salt SC/L formulation (GF-871 Herbicide; EPA Reg. No. 62719-LRI)

Table 2. Physicochemical Properties of the Technical Grade Aminopyralid.			
Parameter	Value		Reference
Melting point	163.5 °C		MRID 46235703
pH	2.31 at 23.4 °C (1% solution in water)		MRID 46235703
Relative density	1.72 at 20 °C		MRID 46235703
Water solubility	2.48 g/L unbuffered water at 18 °C 212 g/L pH 5 buffer at 20 °C 205 g/L pH 7 buffer at 20 °C 203 g/L pH 9 Buffer at 20 °C		MRID 46235703
Solvent solubility at 20 °C	methanol acetone n-octanol ethyl acetate 1,2-dichloroethane xylene heptane	52.2 g/L 29.2 g/L 3.9 g/L 3.9 g/L 0.2 g/L 0.04 g/L <10 µg/mL	MRID 46235703
Vapor pressure	2.59 x 10^{-8} Pa at 25 °C; 9.52 x 10^{-9} Pa at 20 °C		MRID 46235703
Dissociation constant, pK_a	2.56		MRID 46235703
Octanol/water partition coefficient, $Log(K_{OW})$	0.201 unbuffered water at 19 °C; -1.76 at pH 5; -2.87 at pH 7; -2.96 at pH 9		MRID 46235703
UV/visible absorption spectrum	Solution Neutral Basic (pH 12.6) Acidic (pH 1.4)	Wavelength λ max, nm 217 220 245 217 270	Extinction coefficient ε, L/(mol*cm) 29100 26100 10150 22800 9140	MRID 46235703

860.1200 Directions for Use

The aminopyralid end-use product proposed for use on food/feed crops is presented in Table 3. The proposed directions for use on grasses and wheat are summarized in Table 4.

Table 3. Summary of Aminopyralid End-Use Products.

Trade Name	Reg. No.	ai (% of formulation)	Formulation Type	Target Crops	Target Pests	Label Date
GF-871	62719-LRI	40.6% triisopropanol-ammonium (TIPA) salt of aminopyralid 21.1% acid equivalent (ae) aminopyralid; 2 lb ae/gal	Soluble concentrate liquid (SC/L)	Rangeland, permanent grass pastures, Conservation Reserve Program (CRP) acres, noncropland areas (such as rights-of-way, roadsides and non-irrigation ditch banks), natural areas (such as wildlife trails), and grazed areas in and around these sites Wheat (including spring wheat, winter wheat, and durum)	Annual and perennial broadleaf weeds	3/4/04

Table 4. Summary of Directions for Use of Aminopyralid.

Trade Name	Applic. Timing, Type, and Equip.	Applic. Rate (lb ae/A) [g ae/ha]	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ae/A) [g ae/ha]	PHI (days)	Use Directions and Limitations
Rangeland and Permanent Grass Pastures						
GF-871	Broadcast foliar spray or spot treatment; Ground or aerial	0.11 [120]	Not specified (NS)	0.11 [120]	None proposed	Applications may be made alone or as a tank mix with other herbicides. Applications may be made in a minimum of 2 gal/A by air and 10 gal/A by ground. Application through any type of irrigation system is prohibited.
Wheat, Including Durum (not underseeded with a legume)						
GF-871	Broadcast foliar spray or spot treatment; Ground or aerial	0.009 [10]	NS	0.009 [10]	50 (grain and straw) 0 (hay)	Broadcast applications may be made to actively growing wheat from the 3-leaf crop growth stage up to early jointing stage (Zadoks scale 30). Application to a cereal crop underseeded with a legume is prohibited. Applications may be made alone or as a tank mix with other herbicides such as fluroxypyr 1-methylheptyl ester (Starane), 2,4-D ester or amine, MCPA ester or amine, thifensulfuron-methyl (Harmony GT), tribenuron-methyl (Express XP), and metsulfuron-methyl (Ally XP).

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The product label for GF-871 additionally specifies that an approved agricultural surfactant may be used and that any tank mixes should be pre-tested to determine physical compatibility between formulations and to confirm safety to the target crop. A re-entry interval of 12 hours is proposed.

The following plant-back intervals have been proposed: (i) 0 months for wheat (including durum); (ii) 3 months for barley, canola (rapeseed), flax, grasses, field corn, grain sorghum, oats, mustard, popcorn, sweet corn; (iii) 9 months for safflower; and (iv) 18 months for all other crops.

Conclusions. The proposed label is adequate to allow evaluation of the residue data submitted in support of this petition.

The petitioner should modify the proposed label to amend the recommendation regarding use of a surfactant. The submitted grass crop field trials reflected the use of a surfactant but no surfactant was used in the wheat trials. Therefore, the label should be modified by removing the recommendations to use a surfactant in conjunction with application of aminopyralid to wheat. If the petitioner wishes to include the option to use a surfactant for application to wheat, then wheat crop field trials will be required to demonstrate the effect of surfactants on residues in/on wheat commodities. Furthermore, the petitioner should modify the proposed label to specify a rotational crop plant-back interval of 4 months for barley, canola (rapeseed), flax, grasses, field corn, grain sorghum, oats, mustard, popcorn, and sweet corn.

HED notes that the petitioner has proposed that use on grass be restricted to rangeland and permanent pastures only. Because permanent pasture is defined as "pastureland composed of perennial or self-seeding annual plants kept for grazing indefinitely" (Crop Science Society of Agronomy; personal communication with B. Schneider, 09/29/04), HED concludes that the proposed label language is appropriate.

860.1300 Nature of the Residue - Plants

46235710.der.wpd (Grasses)
46235709.der.wpd (Wheat)

Grasses

Dow AgroSciences has submitted a grass metabolism study with aminopyralid. The aminopyralid test substance used in the study was labeled at the 2- and 6-positions of the pyridine ring and formulated as the potassium salt. The test substance was foliarly applied once to each of three types of pasture grasses (Big bluestem, Perennial rye grass, and *Panicum maximum*) approximately 8-10 weeks after planting at a rate of 0.321 lb ai/A (360 g ai/ha; 2.9x the maximum proposed label rate). Grasses were grown in individual plastic tubs that were maintained outdoors on a concrete patio that was adjacent to a greenhouse.

Samples were collected at 0, 14, 21, and 42 days after treatment (DAT). Portions of the 42-DAT samples for each grass species were allowed to air dry in order to produce hay samples.

Following sample preparation, the total radioactive residues (TRR) were determined by combustion/liquid scintillation counting (LSC). The TRR in grass matrices are tabulated below.

Table 5. Total Radioactive Residue in Grass Matrices.

Matrix	Sample Collection Timing	[^{14}C]Aminopyralid Equivalents, ppm		
		Ryegrass	Big Bluestem	*Panicum maximum*
Forage	0 DAT	48.84	25.84	17.96
	7 DAT	36.91	21.03	18.87
	14 DAT	22.90	8.29	10.08
	21 DAT	20.85	9.03	10.03
	42 DAT	6.57	5.61	4.81
Hay	42 DAT	23.34	12.64	19.13

The 0-DAT samples were sequentially rinsed with water and methanol, and residues in rinsed grasses were extracted by homogenizing in acetonitrile/water (70:30, v:v). All other samples were extracted by first homogenizing in acetonitrile/water (70:30, v:v) followed by refluxing in acetonitrile/2 N HCl (50:50, v:v). Potential conjugate fractions from the 21-DAT samples were further analyzed using a base hydrolysis step followed by organic solvent partitioning. All extracts and rinses were analyzed for aminopyralid and metabolites by reverse-phase HPLC. Selected fractions following isolation and cleanup were also analyzed by LC/MS.

Radioactive residues in/on grass forage and hay were readily extractable at all harvest intervals as evidenced by the fact that only 2.8-4.4% of the TRR remained as nonextractable residues in the 42-DAT samples after solvent extraction. Chromatographic analyses of the extractable residues identified the parent aminopyralid as the major residue component, and indicated that the residue level of the parent declined at subsequent intervals, suggesting rapid metabolism of the applied test substance. Aminopyralid accounted for approximately 92-97% of TRR in 0-DAT grass forage, 48-68% of TRR in 7-DAT forage, 33-45% of TRR in 14-DAT forage, 25-38% of TRR in 21-DAT forage, 22-31% TRR in 42-DAT forage, and 24-35% of TRR in 42-DAT hay.

In all three grasses, metabolism resulted in the formation of three metabolite complexes (Fractions C-1, C-2, and C-3). By 42 DAT, the least polar of these fractions represented 50-60% of TRR, while each of the other two fractions represented about 5-10% of the TRR. Subsequent characterization work confirmed that the two largest of these fractions were multi-component in nature and that most of all three fractions could be converted back to aminopyralid following base or acid hydrolysis. Based on these findings, the petitioner concluded that the metabolite fractions observed in the study consisted primarily of isomeric mixtures of acid- and base-labile N-glucosides and glucose conjugate esters of aminopyralid. Other than the formation of these conjugates, the only other metabolic alteration observed as part of this study involved the addition of a hydroxyl group to the pyridine ring to form a minor conjugated metabolite that was observed at levels estimated to be <1% of the TRR.

Wheat

Dow AgroSciences has submitted a wheat metabolism study with aminopyralid. The aminopyralid test substance used in the study was labeled at the 2- and 6-positions of the pyridine ring and formulated as the potassium salt. The test substance was foliarly applied once to spring wheat when plants were at the BBCH 26-28 stage (6 to 8 tillers) using two treatment rates: a low rate (0.036 lb ai/A or 40.1 g ai/ha; 4.0x the maximum proposed label rate) and a high rate (0.072 lb ai/A or 80.3 g ai/ha; 8.0x the maximum proposed label rate). The wheat plants were grown to maturity outdoors. Plant samples were collected 0 DAT, 14 DAT (forage), 35 DAT (hay) and 86 DAT (straw and grain).

Following sample preparation, the TRR in/on treated samples of wheat matrices were determined by combustion/LSC. The TRR in wheat matrices are tabulated below.

Table 6. Total Radioactive Residue in Wheat Matrices.

Wheat Matrix	Sample Collection Timing	TRR (ppm) (Expressed as [2,6-^{14}C]aminopyralid equivalents)	
		Low rate (0.036 lb ai/A)	High rate (0.072 lb ai/A)
Wheat, early forage	0 DAT	2.022	4.121
Wheat, forage	14 DAT	0.418	0.874
Wheat, hay	35 DAT	0.284	0.691
Wheat, grain	86 DAT	0.039	0.084
Wheat, straw	86 DAT	0.281	0.623

All wheat samples were homogenized and then extracted, and for some samples also refluxed with acetonitrile/water (70:30, v:v). The aqueous extracts and nonextractable residues from straw and grain were subjected to base and/or acid hydrolysis to release conjugated radioactivity. [For the purpose of brevity, the results for only the high-rate samples are included here; the distribution of radioactive residues in the chromatographic profiles for low and high rate samples were similar.] For all wheat matrices, the extractability of radioactive residues was high and ranged from 75.0% to 98.7% of TRR. The nonextractable residues following solvent extraction and acid/base hydrolysis were 0.7-9.4% of TRR.

Parent aminopyralid, either conjugated or free, was identified as the major residue component and comprised approximately 90% of TRR in 0-DAT forage, 38% of TRR in 14-DAT forage, 15% of TRR in 35-DAT hay, 79% of TRR in 86-DAT straw, and 60% of TRR in 96-DAT grain. In addition, the following metabolites were found in 35-DAT wheat hay: the glucose conjugate of aminopyralid (15.6% TRR) and the glucose conjugate of hydroxylated aminopyralid (4.8% TRR).

Conclusions. The submitted grass and wheat metabolism studies are adequate to satisfy plant metabolism data requirements for the purposes of this petition. The major residue identified from these studies is the parent aminopyralid (free and conjugated).

Based on the results of the grass metabolism study, it appears that there were no significant metabolic alterations to the basic structure of the parent compound with the exception of a minor conjugated metabolite formed by addition of a hydroxyl group to the parent molecule and found to be present at <1% TRR. Aminopyralid was rapidly conjugated to yield a multi-component mixture of water-soluble complexes which consisted mostly of isomeric mixtures of acid- and base-labile N-glucosides and glucose ester conjugates of aminopyralid.

Based on the results of the wheat metabolism study, the petitioner concluded that the major metabolic pathway of aminopyralid in wheat proceeds by conjugation of aminopyralid and hydroxylated aminopyralid with glucose. The petitioner further stated that, while only two metabolites were isolated as glucose conjugates, any other metabolites present in wheat which were not identified are believed to be conjugates of glucose or similar endogenous compounds, based on the fact that most of the radioactivity in the wheat samples that was not initially detected as the parent could be hydrolyzed to aminopyralid.

Based on these studies, HED has determined that the residues of concern in grass and small grain commodities are free and conjugated aminopyralid (Risk Assessment Review Committee meeting, 3/6/05). Additional metabolism data will be required to support uses on non-grass or non-grain commodities.

860.1300 Nature of the Residue - Livestock

46235708.der.wpd (Goat)
46235711.der.wpd (Poultry)

Goat

Dow AgroSciences has submitted a goat metabolism study with aminopyralid. The aminopyralid test substance used in the study was labeled at the 2- and 6-positions of the pyridine ring and was administered to one lactating goat for six consecutive days. The target dose level was 15 ppm in the total diet, while the actual achieved daily dose received was 13.96 ppm (0.23x the maximum dietary burden of 60 ppm for dairy cattle; see Table 7). Milk was collected twice daily throughout the study, and tissues (liver, kidney, muscle, and fat) were collected at sacrifice.

TRR in samples of milk and tissues, collected from the treated goat, were <0.01 ppm, except in kidney which bore a TRR of 0.071 ppm. Residues in milk, kidney, and liver were extracted and partitioned with organic solvents, and the nonextractable residues were subjected to enzyme hydrolysis. However, no residues were identified in milk and liver because the extracts from these matrices contained low levels (<0.01 ppm) of radioactivity. In kidney, the parent aminopyralid was the only residue identified, at 79.9% TRR (0.057 ppm) by HPLC. The identification of the parent was confirmed by LC/MS/MS.

The study reported that elimination via urine and feces was approximately the same, and each accounted for approximately 46% of the total dose administered. A steady state of daily excretion in feces was established after 48 hours, while output in urine was variable. The identity of unchanged aminopyralid was confirmed in urine and feces.

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No storage stability data were generated as part of this study, and none are required since milk and tissue samples were stored frozen for <6 months from sample collection to analysis.

Based on the results of the submitted study, the petitioner concluded that aminopyralid is rapidly absorbed and excreted by ruminants, that no significant bioaccumulation of aminopyralid residues is expected in the edible tissues of ruminants as a result of the proposed uses, and that any residues that are found in milk or tissue will consist almost exclusively of aminopyralid.

Poultry

Dow AgroSciences has submitted a hen metabolism study with aminopyralid. The aminopyralid test substance used in the study was labeled at the 2- and 6-positions of the pyridine ring and was administered to ten laying hens at a dose level of 1.024 mg/kg bw/day. Based on feed consumption data during the dosing phase of the study, this dose level was equivalent to ~12 ppm of feed consumed (160x the maximum dietary burden of 0.075 ppm for poultry; see Table 7). Hens were dosed for seven consecutive days by oral administration each day of a single gelatin capsule containing the test material. During the dosing phase, excreta was collected at 24-hour intervals, while eggs were collected twice a day (morning and evening) and pooled to give a single sample for each day. Within approximately 25 hours of the final dose, the hens were sacrificed, and samples of muscle, fat, liver and skin with subcutaneous fat were collected for analysis.

Following preparation, all samples were assayed for TRR either by solubilization (fat only) or by combustion analysis. TRR in/on all collected samples of eggs and tissues were <0.01 ppm. Due to the low TRR levels in all egg and tissue samples, none of these samples was subjected to residue characterization/identification.

The study reported that a large portion (~79%) of the administered dose was excreted. To provide information concerning the nature of the residue in hen excreta, samples of Day-7 excreta were subjected to residue characterization. The parent aminopyralid was identified as the major residue in excreta, at 92.9% TRR. Two poorly resolved fractions (3.3% TRR) were characterized as conjugates of aminopyralid following acid and base hydrolysis.

Based on the results of this study, the petitioner concluded that aminopyralid is rapidly excreted in laying hens with minimal transference of residues to eggs and tissues. Furthermore, any metabolism which might take place would result in the formation of conjugated residues of aminopyralid.

Conclusions. The submitted goat metabolism study is insufficient, on its own, to support the subject petition because the dosing level represented ~0.23x the maximum theoretical dietary burden and insufficient radioactivity was found in milk and tissues to allow characterization and/or identification of residues in all fractions.

Radioactivity levels in eggs and tissues were also low in the poultry metabolism study. At this time an additional study will not be required to support the proposed uses because the

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dosing rate reflected 160x the maximum theoretical dietary burden to poultry. The petitioner should note that if additional uses of aminopyralid are proposed in the future which significantly increase the poultry dietary burden, a new poultry metabolism study may be required.

Although the residues in the goat and hen studies were too low to allow adequate characterization/identification of residues, new studies are not being requested. The available metabolism data from the goat, hen, and rat (MRIDs 46235807 and 46235833) indicate that the majority of the administered aminopyralid is excreted as unchanged parent in all three species, and the small amount which is absorbed remains unchanged. Therefore, the residue of concern in livestock is aminopyralid, *per se*. This finding is supported by the residues of concern for the related compounds picloram and clopyralid which, in each case, show parent compound to be the major residue.

860.1340 Residue Analytical Methods

46235712 (Plant method; also includes review of MRID 46235717)
46235714 (Livestock method; also includes review of MRID 46235716)

Plant commodity method

Enforcement method: Dow AgroSciences has proposed the LC/MS/MS Method GRM 02.31 for the enforcement of tolerances for residues of aminopyralid in plant commodities. The method is titled "Determination of Residues of Aminopyralid in Agricultural Commodities by Liquid Chromatography with Tandem Mass Spectrometry Detection."

Briefly, ground samples are extracted with 0.1 N sodium hydroxide, releasing bound residues and hydrolyzing base-labile conjugates to free aminopyralid. The extract is then acidified with hydrochloric acid and heated to release acid-labile conjugates. Following hydrolysis, the extract is cleaned up through an anion-exchange solid-phase extraction column. The internal standard, $^{13}C_2{}^{15}N$-aminopyralid, is added to the eluate, and residues are derivatized with butyl chloroformate to form the 1-butyl esters of aminopyralid for LC/MS/MS analysis. The validated limit of quantitation (LOQ) is 0.01 ppm for all matrices, and the calculated limit of detection (LOD) is 0.002 ppm.

Method validation data for LC/MS/MS Method GRM 02.31 demonstrated adequate method recoveries of aminopyralid from barley grain, forage, and straw; grass forage and hay; sorghum grain, forage, and stover; and wheat grain, forage, and straw fortified at the LOQ (0.01 ppm) and at up to 0.50 ppm for cereal grain, 5.00 ppm for cereal forage and straw, and 20.0 ppm for grasses. The fortification levels and samples used in method validation are not adequate to bracket expected residue levels, and processed commodities for which tolerances have been proposed were not included in the validation study. However, acceptable concurrent method recovery data, bracketing the reported residue levels, were included with the crop field trial and processing studies submitted in conjunction with this petition.

Adequate radiovalidation data have been submitted for the extraction procedures of Method GRM 02.31 using samples of grass and wheat commodities bearing incurred residues,

from the respective metabolism studies, in which crops were treated with [2,6-^{14}C]aminopyralid. Adequate independent laboratory validation data have been submitted using grass forage and wheat grain.

Data-collection methods: The proposed LC/MS/MS enforcement method was used to determine residues of free and conjugated aminopyralid in/on grass and wheat samples from the storage stability, field trial, and processing studies associated with this petition.

Conclusions. The plant commodity analytical method residue data are adequate to satisfy data requirements. The proposed enforcement method has been forwarded to ACB for tolerance method validation. No confirmatory method was included with the submission. The petitioner concluded that confirmatory analysis procedures are not required for the proposed enforcement method due to the high specificity of the LC/MS/MS method. Because the method only monitors one transition ion, HED is concerned that it will not be able to differentiate between aminopyralid, picloram, and clopyralid, and is requesting an interference study using these three compounds. With respect to the more general issue of specificity and a single transition ion, HED defers to ACB to determine whether confirmatory analysis procedures are needed for the method.

Livestock commodity method

Enforcement method: Dow AgroSciences has proposed the LC/MS/MS Method GRM 03.18 for the enforcement of tolerances for residues of aminopyralid in ruminant milk and tissues. The method is titled "Determination of Residues of Aminopyralid in Bovine Tissues by Liquid Chromatography with Tandem Mass Spectrometry."

Briefly, milk or ground tissue samples are extracted with methanol/sodium bicarbonate. The extract is cleaned up through an anion-exchange solid-phase extraction plate. The internal standard, $^{13}C_2{}^{15}N$-aminopyralid, is added to the eluate, and residues are derivatized with butyl chloroformate to form the 1-butyl esters of aminopyralid for LC/MS/MS analysis. The validated LOQ is 0.01 ppm for all matrices, and the calculated LOD is 0.003 ppm.

Method validation data for LC/MS/MS Method GRM 03.18 demonstrated adequate method recoveries of aminopyralid from bovine whole milk, cream, skimmed milk, fat, kidney, liver, and muscle fortified at the LOQ (0.01 ppm) and up to 100x LOQ (1.0 ppm) for milk, fat, liver, and muscle, or 250x LOQ (2.5 ppm) for kidney. The fortification levels and samples used in method validation adequately bracket expected residue levels in ruminant milk and tissues. Two recoveries were below the acceptable 70-120% range: 67% in one kidney sample fortified at the LOQ and 64% in one milk cream sample fortified at the higher level (1.0 ppm). The petitioner stated that the low recoveries were due to random error and not systemic error. Acceptable concurrent method recovery data were included with the feeding study submitted in conjunction with this petition.

Adequate independent laboratory validation data have been submitted using bovine milk and kidney. A radiovalidation study was not conducted for the LC/MS/MS enforcement method

because residues in samples from a goat metabolism study were very low; only kidney samples had TRR >0.01 ppm. Since the extraction solvent used in the proposed enforcement method is similar to that used in the goat metabolism study and was able to extract most of the radioactivity, specific radiovalidation of the method is not being requested.

Data-collection method: The proposed LC/MS/MS enforcement method was used to determine residues of aminopyralid in/on samples from the cattle feeding study associated with DP Barcode D305665.

Conclusions. The livestock analytical method residue data are classified as adequate to satisfy data requirements. HED concludes that the radiovalidation requirements for the method have been satisfied based on the rationale provided by the petitioner.

The proposed enforcement method has been forwarded to ACB for tolerance method validation. The petitioner concluded that confirmatory analysis procedures are not required for the proposed enforcement method due to the high specificity of the LC/MS/MS method. Because the method only monitors one transition ion, HED defers to ACB to determine whether confirmatory analysis procedures are needed for the method. As with the plant method, HED is concerned about the method's ability to distinguish between aminopyralid, picloram, and clopyralid, and is requesting an interference study using these three compounds.

860.1360 Multiresidue Methods

46235718.der.wpd

Dow AgroSciences has submitted multiresidue method data for aminopyralid. Aminopyralid was analyzed according to the FDA Multi-Residue Method Test guidelines in PAM Vol. I (dated 1/94). Aminopyralid was tested through Protocols A and C, and as a result of Protocol C testing, was also tested through Protocols D and E. Based on the results of Protocol E testing, testing under Protocol F was not required. Since methylated aminopyralid provided good response on the column/detector combinations outlined in Protocol C, additional testing was performed with aminopyralid methyl ester under Protocol B.

Aminopyralid is not an N-methylcarbamate and was not found to be naturally fluorescent; therefore, further testing under Protocol A was not required. Methylation efficiency was low for aminopyralid using Protocol B. Aminopyralid was not recovered using Protocol D (with no cleanup), or using Florisil cleanup under Protocols E and F.

We note that the testing laboratory (Pyxant Labs Inc.) did not address the testing of aminopyralid using Protocol G; however, testing of this compound using Protocol G is not required because the compound is not a substituted urea. The petitioner and the testing laboratory should note that the most recent version of PAM Vol. I is dated 10/99.

Conclusions. The submitted multiresidue method residue data are adequate to satisfy data requirements. The results of the study indicate that the FDA MRM guidelines in PAM Vol. I are not suitable for the analysis of aminopyralid. These data will be forwarded to the U.S. FDA for

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further evaluation.

860.1380 Storage Stability

46235719.der.wpd

Dow AgroSciences has submitted interim data from a study investigating the stability of aminopyralid in/on grass hay and forage, and wheat grain and straw under frozen storage conditions. Samples of untreated, homogenized grass hay, grass forage, wheat grain, and wheat straw were fortified with aminopyralid at 0.1 ppm and stored frozen (~-20 C) for up to 187 days for grass hay and forage, 168 days for wheat grain, and 175 days for wheat straw. Samples of grass hay and forage were analyzed, along with fresh fortification samples, after 0, 28, 130, and 187 days of storage. Samples of wheat grain and straw were analyzed, along with fresh fortification samples, after 0, 113, and 168/175 days of storage. The final analysis data, targeted for 18 months of storage, will be submitted when the study has been completed.

The results indicate that under these conditions residues of aminopyralid are stable for up to 187 days in/on grass hay and forage, 168 days in/on wheat grain, and 175 days in/on wheat straw.

Samples from the storage stability study were analyzed for residues of aminopyralid using an adequate method, LC/MS/MS Method GRM 02.31.

The maximum storage interval of residue trial crop samples from harvest to analysis was 454 days (14.9 months) for grass forage, 441 days (14.5 months) for grass hay, and 167 days (5.5 months) for wheat forage, hay, grain, and straw. The maximum storage interval of wheat processing study samples from harvest/processing to analysis was 22 days (0.7 months); samples of wheat grain were processed within 48 days of harvest.

Conclusions. The available storage stability data are adequate to support the storage intervals and conditions of samples from the submitted wheat crop field trials but are not adequate to support the storage intervals and conditions of samples from the grass crop field trials. The petitioner has stated that the final storage stability study will reflect storage intervals of up to 18 months. To fully support the sample storage intervals and conditions, storage stability data for aminopyralid are needed for grass forage and hay reflecting up to ~15 months of frozen storage. The information available to date indicate that residues of concern for aminopyralid are stable in frozen grass forage and hay and that the lack of full storage stability support will not result in an underestimation of residue levels. Even so, HED is recommending that registration of aminopyralid, if granted, be made conditional pending receipt and review of the final storage stability study report.

No storage stability data are required for livestock commodities or wheat processed commodities because all samples from the cattle feeding study and the wheat processing study were stored frozen from collection to analysis and were analyzed within 30 days of collection.

860.1400 Water, Fish, and Irrigated Crops

There are no proposed uses that are relevant to this guideline topic.

860.1460 Food Handling

There are no proposed uses that are relevant to this guideline topic.

860.1480 Meat, Milk, Poultry, and Eggs

46235723.wpd

The petitioner submitted a cattle feeding study with the subject petition. The theoretical dietary burden of aminopyralid to livestock is presented in Table 7.

Table 7. Calculation of Theoretical Dietary Burdens of Aminopyralid to Livestock.

Feedstuff	% Dry Matter [1]	% Diet [1]	Estimated Tolerance (ppm)	Dietary Contribution (ppm) [2]
Beef and Dairy Cattle				
Grass, forage	25	60	25	60
Wheat, aspirated grain fractions	85	20	0.20	0.050
TOTAL BURDEN	--	80 [3]	--	**60.05**
Poultry				
Wheat, grain	89	50	0.05	0.025
Wheat, milled byproducts (bran)	88	50	0.10	0.05
TOTAL BURDEN	--	100	--	**0.075**
Swine				
Wheat, grain	89	50	0.05	0.025
Wheat, milled byproducts (bran)	88	50	0.10	0.05
TOTAL BURDEN	--	100	--	**0.075**

[1] Table 1 (OPPTS Guideline 860.1000).
[2] Contribution = ([tolerance /% DM] x % diet) for beef and dairy cattle; contribution = (tolerance x % diet/100) for poultry and swine.
[3] The remainder of the diet will be composed of feedstuff derived from crops that do not have aminopyralid uses/tolerances proposed (e.g., peanut or cotton seed meal).

Cattle: Dow AgroSciences has submitted a dairy cattle feeding study with aminopyralid. Holstein dairy cattle were orally dosed with aminopyralid at levels equivalent to 32.8, 64.5, 181.5, or 644.7 ppm in the diet. The dosing levels are equivalent to 0.54x, 1.1x, 3.0x, and 10.8x the maximum theoretical dietary burden to beef and dairy cattle, and 440x, 860x, 2420x, and 8600x the maximum theoretical dietary burden to swine. Cattle were dosed once per day after the morning milking for 28 consecutive days. The 0.54x, 1.1x, and 3.0 dosing groups each consisted of three cows. The 10.8x dosing group consisted of a total of nine cows; the six additional cows were used to evaluate depuration of residues following completion of dosing. Cows were milked twice daily, and samples were composited daily for each cow. Milk samples were collected for analysis the first 7 days of dosing and every third day thereafter until the end of the dosing period; subsamples of milk collected on study days 13 and 28 were separated into

cream and skim milk. All cows in the 0.54x, 1.1x, and 3.0x dosing groups and three cows in the 10.8x dosing group were sacrificed within 24 hours of completion of the dosing period, and samples of muscle, liver, kidney and fat were collected. For the depuration study, samples of milk were collected following 0, 1, 2, 3, 6, and 13 days of withdrawal. At each of three withdrawal intervals of 3, 7, and 14 days, two cows were sacrificed, and tissue samples were collected.

Samples of milk and tissues were analyzed for residues of aminopyralid using an adequate method, LC/MS/MS Method GRM 03.18, with a validated LOQ of 0.01 ppm. No storage stability data are required because all samples from the ruminant feeding study were stored frozen from collection to analysis and were analyzed within 30 days of collection.

The maximum residues of aminopyralid found in milk and cattle tissues are listed in the table below. Residues of aminopyralid were generally found to have a linear relationship with the dosing level in milk, kidney, and liver. Residues appeared to plateau in milk by the second dosing day and did not appear to preferentially partition into cream.

Table 8. Summary of Residues from the Aminopyralid Cattle Feeding Study.

Matrix	Maximum Residue Levels (ppm) of Aminopyralid by Feeding Level (ppm in diet)			
	32.8 [0.54x]	64.5 [1.1x]	181.5 [3.0x]	644.7 [10.8x]
Whole milk	<0.01	0.024	0.030	0.152
Cream	--	0.012	--	0.065
Skim milk	--	0.015	--	0.074
Fat	0.011	0.013	0.095	0.042
Kidney	0.102	0.202	1.580	2.549
Liver	<0.01	0.014	0.054	0.117
Muscle	<0.01	<0.01	0.046	0.029

The study results indicate that there is the potential for transfer of aminopyralid residues to livestock, most notably to the kidneys.

The results of the depuration study indicate that aminopyralid residues in cow milk and tissues decline rapidly following withdrawal from dosing. Residues in milk declined to below the method LOQ (<0.01 ppm) following one day of withdrawal; residues were nondetectable in all milk samples from subsequent withdrawal intervals. Residues in tissues declined to below the method LOQ (<0.01 ppm) following 3 days withdrawal; one fat sample bore residues at the LOQ (0.01 ppm) at the 3-day withdrawal period. Residues were nondetectable in all fat, kidney, liver, and muscle samples from subsequent withdrawal intervals.

Conclusions. The submitted cattle study data are adequate to satisfy livestock feeding study data requirements for ruminants.

The feeding study data indicate that the proposed uses would necessitate the establishment of livestock tolerances for residues of aminopyralid. The available data support

tolerances, as aminopyralid *per se*, at: 0.03 ppm for milk; 0.02 ppm for the meat and meat byproducts, excluding kidney, of cattle, goats, horses, and sheep; 0.02 ppm for the fat of cattle, goats, horses, and sheep; and 0.30 ppm for the kidney of cattle, goats, horses, and sheep[1]. A separate tolerance is not needed for cream.

Based on residue levels in milk and tissues from the lowest dosing level in the cattle feeding study, and the fact that the lowest dosing level represents 440x the maximum theoretical dietary burden to swine, HED concludes that tolerances for hog commodities are not needed to support the proposed uses of aminopyralid.

Poultry: The petitioner did not submit a poultry feeding study with the subject petition.

The dosing level of 12 ppm used in the poultry metabolism study corresponds to 160x the maximum theoretical dietary burden to poultry. TRR were <0.01 ppm in eggs and all poultry tissues at this dosing level. Therefore, HED concludes that the proposed uses of aminopyralid in this petition result in a 40 CFR §180.6(a)(3) situation for poultry commodities; i.e., there is no reasonable expectation of finite residues in poultry commodities. No poultry feeding study need be submitted to support the subject petition. HED notes that if additional uses of aminopyralid with significant poultry feed items are proposed in the future, then a poultry feeding study may be required.

860.1500 Crop Field Trials

46235721.de1.wpd (Wheat)
46235722.der.wpd (Grass)

Table 9. Summary of Residues from the Crop Field Trials with Aminopyralid.

Commodity	Formulation [1]	Total Applic. Rate (lb ae/A) [g ae/ha]	PHI (days)	Residue Levels (ppm)						
				n	Min.	Max.	HAFT[2]	Median (STMdR[3])	Mean (STMR[4])	Std. Dev.
Wheat (proposed use = 0.009 lb ai/A total application rate; 0-day PHI for hay; 50-day PHI for grain & straw)										
Canadian Trials										
Wheat forage	0.08 lb ae/gal EO TIPA salt	0.009 [9.7-9.9]	0	4	0.105	0.494	0.492	0.301	0.300	0.221
			7	4	0.052	0.093	0.088	0.069	0.071	0.021
	2 lb ae/gal SC/L TIPA salt	0.009 [10-10.1]	0	4	0.523	0.777	0.719	0.595	0.623	0.121
			7	4	0.034	0.189	0.112	0.048	0.080	0.073
	2.1 lb ae/gal SC/L K salt	0.008-0.009 [9.4-9.9]	0	4	0.409	0.883	0.850	0.625	0.635	0.249
			7	4	0.042	0.221	0.216	0.129	0.130	0.099
Wheat hay	0.08 lb ae/gal EO TIPA salt	0.009 [9.7-9.9]	0	4	0.371	1.375	1.306	0.808	0.840	0.540
			7	4	0.121	0.217	0.194	0.151	0.160	0.044

[1]These recommended tolerance levels are based on the residue observed in each commodity at the 64.5 ppm (1.1x) feeding level.

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Table 9. Summary of Residues from the Crop Field Trials with Aminopyralid.

Commodity	Formulation [1]	Total Applic. Rate (lb ae/A) [g ae/ha]	PHI (days)	Residue Levels (ppm)						
				n	Min.	Max.	HAFT[2]	Median (STMdR[3])	Mean (STMR[4])	Std. Dev.
	2 lb ae/gal SC/L TIPA salt	0.009 [10-10.1]	0	4	1.318	2.377	2.318	1.954	1.901	0.503
			7	4	0.047	0.620	0.619	0.367	0.350	0.311
	2.1 lb ae/gal SC/L K salt	0.008-0.009 [9.4-9.9]	0	4	1.390	2.608	2.358	1.817	1.908	0.561
			7	4	0.117	0.648	0.637	0.374	0.378	0.299
Wheat grain	0.08 lb ae/gal EO TIPA salt	0.009 [9.7-9.9]	49-55	4	<0.01	0.012	0.012	0.008	0.008	0.004
	2 lb ae/gal SC/L TIPA salt	0.009 [10-10.1]	49-54	4	<0.01	0.013	0.013	0.009	0.009	0.005
	2.1 lb ae/gal SC/L K salt	0.008-0.009 [9.4-9.9]	49-54	4	0.011	0.013	0.013	0.012	0.012	0.001
Wheat straw	0.08 lb ae/gal EO TIPA salt	0.009 [9.7-9.9]	49-55	4	0.067	0.080	0.074	0.073	0.073	0.005
	2 lb ae/gal SC/L TIPA salt	0.009 [10-10.1]	49-54	4	0.046	0.069	0.069	0.062	0.060	0.011
	2.1 lb ae/gal SC/L K salt	0.008-0.009 [9.4-9.9]	49-54	4	0.066	0.145	0.138	0.101	0.103	0.041
U.S. Trials										
Wheat forage	0.08 lb ae/gal EO TIPA salt	0.009 [9.9-10.4]	0	40	0.112	0.858	0.803	0.397	0.426	0.176
			6/7	40	0.022	0.269	0.261	0.125	0.121	0.057
	2 lb ae/gal SC/L TIPA salt	0.009 [9.7-10.5]	0	10	0.158	0.440	0.428	0.371	0.324	0.108
			7	10	0.044	0.171	0.157	0.055	0.073	0.045
	2.1 lb ae/gal SC/L K salt	0.009 [10.1-10.5]	0	10	0.165	0.666	0.631	0.385	0.368	0.165
			7	10	<0.01	0.182	0.126	0.068	0.081	0.056
Wheat hay	0.08 lb ae/gal EO TIPA salt	0.009 [9.9-10.4]	0	40	0.358	2.121	2.005	0.925	1.002	0.469
			6/7	39	0.058	1.031	0.995	0.314	0.336	0.203
	2 lb ae/gal SC/L TIPA salt	0.009 [9.7-10.5]	0	10	0.259	1.441	1.376	0.710	0.824	0.477
			7	10	0.151	0.368	0.357	0.207	0.230	0.074
	2.1 lb ae/gal SC/L K salt	0.009 [10.1-10.5]	0	10	0.329	1.393	1.328	0.840	0.868	0.457
			7	10	<0.01	0.355	0.267	0.167	0.163	0.105
Wheat grain	0.08 lb ae/gal EO TIPA salt	0.009 [9.9-10.4]	50-80	40	<0.01	0.026	0.025	0.005	0.011	0.008
	2 lb ae/gal SC/L TIPA salt	0.009 [9.7-10.5]	50-72	10	<0.01	0.018	0.014	0.010	0.010	0.005
	2.1 lb ae/gal SC/L K salt	0.009 [10.1-10.5]	50-65	10	<0.01	0.026	0.023	0.013	0.014	0.006
Wheat straw	0.08 lb ae/gal EO TIPA salt	0.009 [9.9-10.4]	50-80	40	<0.01	0.170	0.136	0.038	0.051	0.044

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Table 9. Summary of Residues from the Crop Field Trials with Aminopyralid.

Commodity	Formulation [1]	Total Applic. Rate (lb ae/A) [g ae/ha]	PHI (days)	Residue Levels (ppm)						
				n	Min.	Max.	HAFT[2]	Median (STMdR[3])	Mean (STMR[4])	Std. Dev.
	2 lb ae/gal SC/L TIPA salt	0.009 [9.7-10.5]	50-72	10	0.020	0.076	0.072	0.060	0.050	0.024
	2.1 lb ae/gal SC/L K salt	0.009 [10.1-10.5]	50-65	10	0.021	0.092	0.078	0.058	0.059	0.024
Grass (proposed use = 0.11 lb ai/A total application rate with no proposed PHI)										
Canadian Trials										
Grass forage	2 lb ae/gal SC/L TIPA salt	0.103-0.112 [116-125]	0	18	7.452	14.033	12.832	10.783	10.801	1.838
			7-8	18	1.276	6.108	5.901	3.746	3.716	1.214
			13-14	18	1.009	3.326	3.201	2.110	2.124	0.608
Grass hay	2 lb ae/gal SC/L TIPA salt	0.103-0.112 [116-125]	0	18	12.909	51.496	49.167	21.857	24.472	10.619
			13-14	18	1.837	9.531	9.215	3.547	4.173	2.325
			20-22	18	2.119	8.708	7.957	3.466	3.976	1.825
U.S. Trials										
Grass forage	2 lb ae/gal SC/L TIPA salt	0.103-0.112 [115-125]	0	30	3.516	15.551	13.801	7.389	7.264	2.869
			6-7	30	0.232	6.514	5.793	2.361	2.652	1.556
			13-15	30	0.535	8.009	5.464	1 785	1.998	1.315
Grass hay	2 lb ae/gal SC/L TIPA salt	0.103-0.112 [115-125]	0	28	9.407	29.962	29.443	17.416	18.269	6.046
			13-15	30	1.762	13.152	10.573	5.148	5.830	3.317
			20-22	30	0.784	17.150	15.623	3.662	4.630	3.593

[1] EO = Water emulsion in oil formulation.
[2] HAFT = Highest Average Field Trial.
[3] STMdR = Supervised Trial Median Residue.
[4] STMR = Supervised Trial Mean Residue.

Wheat

Dow AgroSciences has submitted field trial data depicting the magnitude of the residue of aminopyralid in/on wheat commodities. A total of 22 wheat field trials were conducted in Canada and the U.S. during the 2003 growing season. For the 0.08 lb ae/gal EO formulation, two Canadian wheat field trials were conducted in Region 7 (SK; 2 trials), and twenty U.S. wheat field trials were conducted in Regions 2 (VA; 1 trial), 4 (AR; 1 trial), 5 (IN, MN, NE, ND, and SD; 5 trials), 6 (OK; 1 trial), 7 (NE, ND, and SD; 5 trials), 8 (KS and TX; 6 trials), and 11 (WA; 1 trial). For the 2 lb ae/gal SC/L TIPA salt formulation and 2.1 lb ae/gal SC/L K salt formulation, a total of seven trials were conducted with each formulation in Region 7 (two trials in Canada and five trials in the U.S.). The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 for the EO formulation but are not in accordance for the SC/L formulations.

At each test location, wheat plants at the 3-leaf to heading stage were treated with a single broadcast foliar application of a 0.08 lb ae/gal water emulsion in oil (EO) formulation of

aminopyralid TIPA salt containing fluroxypyr 1-methylheptyl (Starane). Application was made at ~0.009 lb ae/A (~10 g ae/ha). Wheat forage and hay were sampled 0 and 6-7 days post application, and wheat grain and straw were sampled at maturity, 49-80 days post application. For data bridging purposes, at seven test locations (Region 7; 2 trials in Canada and 5 trials in the U.S.) two soluble concentrate liquid (SC/L) formulations, a 2 lb ae/gal SC/L aminopyralid TIPA salt formulation and a 2.1 lb ae/gal SC/L aminopyralid potassium (K) salt formulation, were applied to separate plots at the same rate and timing as stated above. Applications at all test sites were made using ground equipment in ~11-20 gal/A of water. No spray adjuvant was used. Additional forage and hay samples were collected from three U.S. test locations at 14, 21, and 28 days after application to evaluate residue decline.

Samples of wheat forage, hay, grain, and straw were analyzed for residues of aminopyralid using an adequate method, LC/MS/MS Method GRM 02.31, with an LOQ of 0.01 ppm.

The results of the wheat crop field trials are reported in Table 9. Residue decline data showed that aminopyralid residues generally decreased in wheat forage and hay with increasing sampling intervals, with maximum residues occurring at the 0-day sampling interval.

In the side-by-side trials, the SC/L formulations generally yield slightly higher residues in forage and hay than the EO formulation in the Canadian trials, and the EO formulation generally yields slightly higher residues in forage and hay than the SC/L formulations in the U.S. trials. However, the differences in residue levels do not appear to be significant. Residue levels in wheat grain and straw samples from the side-by-side trials are similar across formulations.

Grass

Dow AgroSciences has submitted field trial data depicting the magnitude of the residue of aminopyralid in/on pasture and rangeland grass forage and hay. A total of 20 grass field trials were conducted in Canada and the U.S. during the 2002 growing season. Seven Canadian grass field trials were conducted in Regions 7 (AB and SK; 2 trials) and 14 (AB, MB, and SK; 5 trials). Thirteen U.S. grass field trials were conducted in Regions 1 (NY and PA; 2 trials), 2 (GA and VA; 2 trials), 4 (LA; 1 trial), 5 (ND and OH; 2 trials), 5A (WI; 1 trial), 7 (MT; 1 trial), 8 (TX; 1 trial), 9 (MT; 1 trial), and 11 (ID and WA; 2 trials). The number and locations of field trials are in accordance with OPPTS Guideline 860.1500.

At each trial location, pasture and rangeland grass were treated with a single broadcast foliar application of the 2 lb ae/gal soluble concentrate/liquid (SC/L) formulation of aminopyralid as the triisopropanolammonium (TIPA) salt at ~0.11 lb ae/A (120 g ae/ha). Grass forage was harvested 0, 6-8, and 13-15 days after application, and grass hay was harvested 0, 13-15, and 20-22 days after application and allowed to dry for 2 to 5 days prior to collection. Applications at all test sites were made using ground equipment in ~10-20 gal/A of water with a non-ionic spray adjuvant added. In addition, a separate plot at four test locations (2 trials in Canada and 2 trials in U.S.) received a single application of the 2 lb ae/gal SC/L aminopyralid formulation tank mixed with a 5.7 lb ae/gal SC/L formulation of 2,4-D. Data for the 2,4-D acid

equivalent are not reviewed herein. Additional forage samples were collected from these four test locations (both treatment plots) 3 and 21/22 days after application, and additional grass hay samples were collected 28 days after application to evaluate residue decline.

Samples of grass hay and forage were analyzed for residues of aminopyralid using an adequate method, LC/MS/MS Method GRM 02.31, with an LOQ of 0.01 ppm.

The results of the grass field trials are presented in Table 9. Residue decline data showed that aminopyralid residues generally decreased in grass forage and hay with increasing sampling intervals, with maximum residues occurring at the 0-day sampling interval.

Conclusions. The submitted field trial residue data are adequate to satisfy data requirements for wheat even though geographic representation of the SC/L formulations are inadequate (only seven field trials were conducted with the SC/L formulation the petitioner wishes to register). The petitioner conducted side-by-side trials which indicated no significant differences between the EO and SC/L formulation types. Generally, HED prefers that side-by-side trials be conducted in diverse geographic regions. For aminopyralid, all side-by-side trials were conducted in Region 7. Since the data for aminopyralid show that there is no discernable effect of geographic location on residue levels, HED considers the data sufficient to support registration of the SC/L formulations in this case. The petitioner has included language on the proposed label recommending that a surfactant be used for wheat. The petitioner should modify the proposed label to remove the recommendation that a surfactant be used with application to wheat. If the petitioner wishes to include the option to use a surfactant for application to wheat, then all the required wheat crop field trials should reflect the use of surfactant in the test substance application.

The submitted field trial residue data are adequate to satisfy data requirements for grass. For U.S. trials, OPPTS 860.1500 (Tables 2 and 5) specifies that four trials should be conducted for each of three grass cultivars (Bermuda grass, bluegrass, and bromegrass or fescue) for a total of 12 trials, in all areas across the country. The U.S. crop field trials were conducted using Bermuda grass (2 trials), bromegrass or fescue (6 trials), crested wheatgrass (1 trial), orchard grass (2 trials), reed canarygrass (1 trial), and a grass mixture (1 trial). Although the grass cultivars used by the petitioner did not exactly match the requirements of OPPTS 860.1500 (more trials were conducted with cool season grasses than warm season grasses), HED concludes that the grass cultivars used are sufficient to represent grasses grown for pasture and rangeland in the U.S. and Canada.

The submitted crop field trial data support the following tolerances for residues of aminopyralid, free and conjugated: grass forage at 25.0 ppm; grass hay at 50.0 ppm; wheat forage at 2.0 ppm; wheat hay at 4.0 ppm; wheat grain at 0.04 ppm; and wheat straw at 0.25 ppm.

Residue data for wheat aspirated grain fractions were included with the processing study (see 860.1520). Based on the observed processing factor of 6.1x for aspirated grain fractions and a HAFT residue of 0.025 ppm for wheat grain, the expected residues in aspirated grain fractions following treatment at 1x would be 0.15 ppm. Because the residues are greater than the

supported tolerance of 0.05 ppm for wheat grain, a tolerance for aspirated grain fractions is needed. Because the petitioner is not proposing use of aminopyralid on corn, sorghum, or soybeans, the data for wheat may be used to set the aspirated grain fractions tolerance; a tolerance of 0.20 ppm is appropriate.

860.1520 Processed Food and Feed

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Table 10. Residue Data from Wheat Processing Study with Aminopyralid.

RAC	Processed Commodity	Total Rate (lb ae/A) [g ae/ha]	PHI (days)	Aminopyralid Residues (ppm) [1]	Processing Factor [2]
Wheat grain (bulk)	RAC	0.045 [50.0]	69	0.054, 0.055 (0.055)	–
	Bran			0.143	2.6x
	Flour			*0.008*	<0.1x
	Shorts			0.067	1.2x
	Middlings			0.032	0.6x
	Germ			0.019, 0.021	0.3x, 0.4x
	Aspirated Grain Fractions			0.338	6.1x

[1] The petitioner presented both uncorrected residue values and residue values corrected for concurrent method recovery. The uncorrected values are reported here. Residues reported between the method LOQ (<0.01 ppm) and LOD (0.003 ppm) are *italicized*. Average residues are reported in parentheses for the grain RAC.

[2] Calculated by the study reviewer using the average residues in the grain RAC samples from the processor.

Dow AgroSciences has submitted a processing study with wheat. In one trial conducted in Colony, OK, wheat grain (RAC) was harvested 69 days following a single broadcast foliar spray application of the 0.08 lb ae/gal water emulsion in oil (EO) formulation of aminopyralid triisopropylamine (TIPA) salt at 0.045 lb ae/A (~50 g ae/ha; 5x the maximum proposed seasonal rate); we note that the test substance also contained fluroxypyr 1-methylheptyl ester (Starane). Wheat grain was processed into germ, bran, middlings, shorts, and flour using simulated commercial processing procedures. In addition, a sample of wheat aspirated grain fractions was generated.

Residues of aminopyralid were 0.054-0.055 ppm in/on wheat grain RAC. The processing data indicate that residues of aminopyralid may concentrate in wheat bran (2.6x processing factor), wheat shorts (1.2x processing factor), and aspirated grain fractions (6.1x processing factor), but do not appear to concentrate in wheat flour, middlings, and germ (processing factors of <0.1x, 0.6x, and 0.4x, respectively).

The maximum theoretical concentration factors for wheat bran, flour, and shorts are 7.7x, 1.4x, and 8.3x, respectively (based on separation into components, Table 3 of OPPTS 860.1520). The reported concentration factors do not exceed the theoretical concentration factors.

Samples of wheat grain, aspirated grain fractions, and wheat processed commodities were

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analyzed for residues of aminopyralid using an acceptable method, LC/MS/MS Method GRM 02.31, with a validated LOQ of 0.010 ppm for each wheat commodity.

Conclusions. The submitted wheat processing data are adequate to satisfy data requirements. The processing data indicate that aminopyralid residues may concentrate in wheat bran and, to a lesser degree, shorts.

Based on a processing factor of 2.6x for bran and a HAFT residue of 0.025 ppm for wheat grain, the maximum expected residues in wheat bran following treatment at 1x would be 0.065 ppm. Because the residues are greater than the proposed tolerance of 0.05 ppm for wheat grain, a tolerance for wheat bran is needed; a tolerance of 0.1 ppm is appropriate.

Based on a processing factor of 1.2x for wheat shorts and a HAFT residue of 0.025 ppm for wheat grain, the maximum expected residues in wheat shorts following treatment at 1x would be 0.03 ppm. Because the expected residues do not exceed the proposed tolerance of 0.05 ppm for wheat grain, a tolerance for wheat shorts is not needed.

860.1650 Submittal of Analytical Reference Standards

As of 09/07/2004, no reference standard for aminopyralid was available at the EPA National Pesticide Standards Repository. The petitioner should submit a reference standard for aminopyralid. In addition, because the proposed enforcement methods require the use of an internal standard for quantification, the petitioner should submit a quantity of the internal standard, $^{13}C_2{}^{15}N$-aminopyralid, to the repository. The standards should be directed to Fred Siegelman, Chief of the Analytical Chemistry Branch, at:

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

860.1850 Confined Accumulation in Rotational Crops

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Table 11. Total Radioactive Residues (TRR) in Lettuce, Sorghum, and Turnip Matrices.

Matrix	[2,6-^{14}C]Aminopyralid, ppm [1]	
	90-Day Plant-back Interval	120-Day Plant-back Interval
Lettuce, leaf, immature	(0.002)	(0.001)
Lettuce, leaf, mature	(<0.002)	(<0.001)
Sorghum, early forage	0.027	0.017
Sorghum, late forage	0.003	0.003
Sorghum, stover	0.027	0.003
Sorghum, grain	0.006	0.003
Turnip tops, immature	0.007	0.007

Table 11. Total Radioactive Residues (TRR) in Lettuce, Sorghum, and Turnip Matrices.		
Matrix	[2,6-^{14}C]Aminopyralid, ppm [1]	
	90-Day Plant-back Interval	120-Day Plant-back Interval
Turnip, tops, mature	0.004	0.010
Turnip, roots, mature	(<0.001)	(<0.001)

[1] Expressed as aminopyralid equivalents. For values reported in parentheses one or more combustion values may have been less than the LOD of 0.0005 ppm, and all combustion values were below the LOQ (0.002-0.003 ppm).

Table 12. Summary of Characterization and Identification of Radioactive Residues in Rotational Crop Matrices Following Application of Radiolabeled Aminopyralid to Bare Soil at 0.009 lb ai/A (10 g ai/ha).								
Compound	90-Day Sorghum Early Forage		120-Day Sorghum Early Forage		90-Day Sorghum Stover		120-Day Turnip Tops	
	TRR = 0.027 ppm		TRR = 0.017 ppm		TRR = 0.027 ppm		TRR = 0.010 ppm	
	%TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Aminopyralid	44.2	0.012	26.9	0.005	18.1	0.005	17.2	0.002
C-1 Fraction [1]	20.5	0.006	17.0	0.003	17.3	0.005	5.4	0.001
C-2 Fraction [1]	23.1	0.006	38.2	0.006	37.9	0.010	67.9	0.007
Reflux, aqueous	--	--	--	--	16.7 [2]	0.004	--	--
ACN/water (2nd extract)	--	--	--	--	--	--	7.2	0.001
Total identified	44.2	0.012	26.9	0.005	18.1	0.005	17.2	0.002
Total characterized	43.6	0.012	55.2	0.009	71.9	0.019	80.5	0.009
Total extractable	88.9	0.024	84.2	0.014	89.9	0.024	97.7	0.010
Unextractable (PES) [3]	11.1	0.003	15.8	0.003	10.1	0.003	2.3	<0.001
Accountability [4]	100		100		100		100	

[1] The petitioner stated that these fractions consist primarily of glucose conjugates of aminopyralid based on base hydrolysis of the aqueous fraction and characterization work performed in conjunction with primary plant metabolism studies.
[2] Following base hydrolysis, additional aminopyralid 7.7% TRR, 0.002 ppm) and C-2 Fraction (3.0% TRR, 0.001 ppm) residues were released.
[3] Residues remaining after exhaustive extractions.
[4] Values were normalized by the petitioner; therefore, accountabilities were 100% for all matrices.

Dow AgroSciences has submitted a confined rotational crop study with [2,6-^{14}C]aminopyralid (specific activity 40 µCi/mg; 88,800 dpm/µg). The radiolabeled test substance was applied directly to sandy loam soil in lined wooden boxes at 0.009 lb ai/A (10 g ai/ha; 1x the maximum seasonal rate for wheat). Rotational lettuce, sorghum, and turnips were planted 90 and 120 days after treatment (DAT).

Total radioactive residues (TRR) accumulated at ≥0.01 ppm in several rotated crop matrices planted 90 and 120 days following a single soil application of [2,6-^{14}C]aminopyralid at 0.009 lb ai/A. TRR accumulated at ≥0.01 ppm in 90- and 120-DAT early sorghum forage (0.027 ppm and 0.017 ppm, respectively), 90-day sorghum stover (0.027 ppm), and 120-DAT mature turnip tops (0.010 ppm); residues in all other rotational crop commodities ranged <0.001-0.007 ppm. TRR were generally found to decrease from the 90-day plant-back interval (PBI) to the 120-day PBI.

Analysis of soil samples at planting of the rotational crops indicated that ≤30% of the

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applied aminopyralid was present in the soil following the fallow periods. Between planting and harvest, aminopyralid residues in soil declined slowly.

Only 90-DAT sorghum early forage and stover, and 120-DAT sorghum early forage and turnip tops contained radioactivity ≥0.01 ppm and were extracted for metabolite characterization. Extraction with acetonitrile (ACN)/water released the majority of the TRR (66-98% TRR). Additional radioactivity was released by ACN/acid reflux of sorghum stover (24% TRR). Nonextractable residues in 90-DAT sorghum early forage and stover, and 120-DAT sorghum early forage and turnip tops were 2.3-15.8% TRR (≤0.003 ppm). The extraction procedures extracted sufficient residues from rotational crop matrices from the 90- and 120-day PBIs. Because the petitioner normalized extraction results, reported accountabilities were 100%; extraction/hydrolysis recoveries prior to normalization were 80.2-114.5%.

Total identified residues ranged 17-44% TRR in sorghum early forage and stover and in turnip tops and consisted entirely of free aminopyralid; residue profiles were similar between the matrices. Free aminopyralid was the major residue identified in sorghum early forage (44.2% TRR, 0.012 ppm at 90 DAT; 26.9% TRR, 0.005 ppm at 120 DAT), sorghum stover (18.1% TRR, 0.005 ppm at 90 DAT), and turnip tops (17.2% TRR, 0.002 ppm at 120 DAT). Two metabolite fractions were also characterized in each matrix. Fraction C-1, which accounted for 5.4-20.5% TRR (0.001-0.006 ppm) was more polar than aminopyralid, and Fraction C-2, which accounted for 23.1-67.9% TRR (0.006-0.010 ppm) was slightly less polar than aminopyralid. These metabolites were further characterized as base-labile conjugates of aminopyralid following base hydrolysis of aqueous-soluble residues, which demonstrated significant conversion of the metabolite fractions to aminopyralid. In conjunction with the results of the primary plant metabolism studies, the petitioner indicated that these conjugates are believed to consist primarily of N-glucoside and glucose ester conjugates of aminopyralid.

Conclusions. The submitted confined rotational crop study is adequate to satisfy data requirements. Based on the study results, the petitioner concluded that residues of aminopyralid are metabolized in the same manner in rotated crops as in primary crops and noted that initial uptake of residues resulted in aminopyralid-related residues >0.01 ppm in commodities used exclusively as animal feedstock items. Uptake of residues appeared to be more limited (≤0.01 ppm) in rotated crop commodities used for direct human consumption.

The submitted confined rotational crop studies indicate the potential for quantifiable residues of aminopyralid in rotated cereal grain forage at a 3-month plant-back interval. The residues of concern in rotational crop commodities are the same as for primary crop commodities, aminopyralid, free and conjugated.

We note that because the petitioner has specified that application of aminopyralid to grass is to be restricted to permanent pastures and rangelands, rotational crop data are not needed to support the requested use on grass.

860.1900 Field Accumulation in Rotational Crops

The petitioner did not submit any field rotational crop studies. The following plant-back intervals have been proposed for aminopyralid use: (i) 0 months for wheat (including durum); (ii) 3 months for barley, canola (rapeseed), flax, grasses, field corn, grain sorghum, oats, mustard, popcorn, sweet corn; (iii) 9 months for safflower; and (iv) 18 months for all other crops.

Conclusions. Because wheat is a primary crop on the label, the 0-day plant-back interval for wheat is appropriate. In the confined rotational crop study, residues of free aminopyralid were observed at >0.01 ppm in sorghum forage at the 3-month plant-back interval. Residues of total aminopyralid (free and conjugated) only slightly exceed the 0.01-ppm threshold at the 4-month plant-back interval, with free parent present at only 0.005 ppm. Therefore, HED is requesting that the label for aminopyralid be modified to specify a 4-month plant-back interval for barley, canola, flax, grasses, field corn, grain sorghum, oats, mustard, popcorn, and sweet corn. Alternatively, the petitioner may submit field rotational crop data designed to support a tolerance for residues in rotational crops at a shorter plant-back interval. The petitioner has presumably proposed 9-month and 18-month plant-back intervals for certain crops due to phytotoxicity concerns.

860.1550 Proposed Tolerances

Dow AgroSciences has proposed the establishment of permanent tolerances for residues of aminopyralid expressed as total parent, free and conjugated, in/on grass and wheat commodities, and the establishment of permanent tolerances for residues of aminopyralid, *per se*, in animal commodities. HED agrees with this tolerance definition.

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

Pending receipt of the required storage stability data for grass forage and hay, the available crop field trial data will support tolerances for residues of aminopyralid, free and conjugated, in/on grass and wheat commodities. The available crop field trial data indicate that the proposed tolerance of 65 ppm for grass hay is not appropriate. A revised tolerance of 50 ppm for grass hay should be proposed. Similarly, the proposed tolerance for wheat straw should be revised to be 0.25 ppm. The available data indicate that the proposed tolerance of 0.5 ppm for wheat aspirated grain fractions is too high; a revised tolerance of 0.2 ppm should be proposed. The available processing data indicate that the proposed tolerances for wheat middlings, shorts, flour, and germ are not needed.

The available animal feeding study data indicate that the proposed tolerance for muscle is too high and that the proposed tolerance for milk is adequate. The feeding study data indicate that a separate tolerance for kidney is appropriate, although the proposed tolerance is too high. The proposed tolerance for liver should be amended to "meat byproducts, excluding kidney" to account for meat byproducts in addition to liver, and the proposed level should be reduced. The appropriate levels for these tolerances are listed in Table 6. The cattle feeding study data indicate that tolerances for cream and for hog commodities are not needed.

The proposed tolerances should be revised to reflect the correct commodity definitions as specified in Table 13.

Table 13. Tolerance Summary for Aminopyralid.

Commodity	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments/ *Correct commodity definition*
Aminopyralid expressed as total parent, free and conjugated			
Grass, forage	25	25	
Grass, hay	60	50	
Wheat, forage	2.0	2.0	
Wheat, hay	4.0	4.0	
Wheat, grain	0.05	0.04	
Wheat, straw	0.5	0.25	
Wheat bran	0.1	0.10	*Wheat, bran*
Wheat middlings	0.02	Remove	No tolerance needed.
Wheat shorts	0.05	Remove	No tolerance needed.
Wheat flour	0.01	Remove	No tolerance needed.
Wheat germ	0.02	Remove	No tolerance needed.
Wheat, aspirated grain fractions	0.5	0.20	*Aspirated grain fractions*
Aminopyralid *per se*			
Milk	0.02	0.03	
Milk, cream	0.02	Remove	No tolerance needed.
Meat of cattle, goats, hogs, horses, and sheep	0.05	0.02	*Cattle, meat*
		0.02	*Goat, meat*
		--	No tolerance needed for hog meat.
		0.02	*Horse, meat*
		0.02	*Sheep, meat*
Fat of cattle, goats, hogs, horses, and sheep	0.05	0.02	*Cattle, fat*
		0.02	*Goat, fat*
		--	No tolerance needed for hog fat.
		0.02	*Horse, fat*
		0.02	*Sheep, fat*
Liver of cattle, goats, hogs, horses, and sheep	0.05	0.02	*Cattle, meat byproducts, except kidney*
		0.02	*Goat, meat byproducts, except kidney*
		--	No tolerance needed for hog meat byproducts.
		0.02	*Horse, meat byproducts, except kidney*
		0.02	*Sheep, meat byproducts, except kidney*

Table 13. Tolerance Summary for Aminopyralid.			
Commodity	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments/ *Correct commodity definition*
Kidney of cattle, goats, hogs, horses, and sheep	1.0	0.30	*Cattle, kidney*
		0.30	*Goat, kidney*
		--	No tolerance needed for hog kidney.
		0.30	*Horse, kidney*
		0.30	*Sheep, kidney*

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

Template Version November 2003

INTERNATIONAL RESIDUE LIMIT STATUS

Chemical Name: 4-amino-3,6-dichloropyridine-2-carboxylic acid	Common Name: Aminopyralid	X Proposed tolerance □ Reevaluated tolerance □ Other	Date: 09/02/04

Codex Status (Maximum Residue Limits)	U. S. Tolerances
X No Codex proposal step 6 or above □ No Codex proposal step 6 or above for the crops requested	Petition Number: PP#4F6827 DP Barcode: D305665 Other Identifier:
Residue definition (step 8/CXL): N/A	Reviewer/Branch: C. Swartz and M. Doherty /RAB2
	Residue definition: Aminopyralid, expressed as total parent, free and conjugated; and aminopyralid *per se*

Crop (s)	MRL (mg/kg)	Crop(s)	Tolerance (ppm)
		Aminopyralid, expressed as total parent, free and conjugated	
		Grass, forage	25
		Grass, hay	65
		Wheat, forage	2.0
		Wheat, hay	4.0
		Wheat, grain	0.05
		Wheat, straw	0.5
		Wheat bran	0.1
		Wheat middlings	0.02
		Wheat shorts	0.05
		Wheat flour	0.01
		Wheat germ	0.02
		Wheat, aspirated grain fractions	0.5
		Aminopyralid *per se*	
		Milk	0.02
		Milk, cream	0.02
		Meat of cattle, goats, hogs, horses, and sheep	0.05
		Fat of cattle, goats, hogs, horses, and sheep	0.05
		Liver of cattle, goats, hogs, horses, and sheep	0.05
		Kidney of cattle, goats, hogs, horses, and sheep	1.0

Limits for Canada	Limits for Mexico

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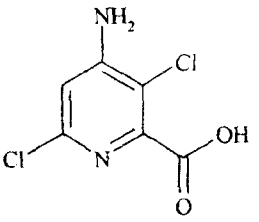
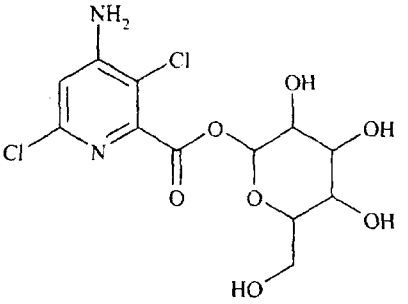
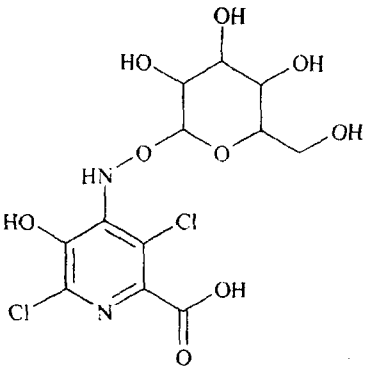
X No Limits ☐ No Limits for the crops requested		X No Limits ☐ No Limits for the crops requested	
Residue definition: N/A		Residue definition: N/A	
Crop(s)	MRL (mg/kg)	Crop(s)	MRL (mg/kg)
Notes/Special Instructions: S.Funk, 09/02/04.			

Appendix 1. Chemical Name and Structure of Aminopyralid and its Transformation Products.		
Company Name	Chemical Name	Structure
Aminopyralid; XDE-750	4-amino-3,6-dichloro-2-pyridinecarboxylic acid	
Aminopyralid - glucose conjugate	glucose conjugate of 4-amino-3,6-dichloro-2-pyridinecarboxylic acid	
Hydroxylated aminopyralid - glucose conjugate	glucose conjugate of 4-amino-3,6-dichloro-5-hydroxypyridine-2-carboxylic acid	

Primary Evaluator _____ Date: 6/23/05
Michael A. Doherty, Ph.D., Chemist, RAB2

Peer Reviewer _____ Date: June 6/05
Tamara Sheremata, Ph.D., Evaluation Officer,
FREAS, HED, PMRA

Approved by _____ Date: 7/5/05
Richard A. Loranger, Ph.D., Branch Senior Scientist, RAB2

Approved by _____ Date: Jun 13/05
Henri Bietlot, Ph.D., A/Section Head,
FREAS, HED, PMRA

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 11/08/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

END-USE PRODUCTS:

TABLE 1. Summary of Aminopyralid End-Use Products.

Trade Name	Reg. No.	ai (% of formulation)	Formulation Type	Target Crops	Target Pests	Label Date
GF-871	62719-LRI	40.6% triisopropanol-ammonium (TIPA) salt of aminopyralid 21.1% acid equivalent (ae) aminopyralid; 2 lb ae/gal	Soluble concentrate liquid (SC/L)	Rangeland, permanent grass pastures, Conservation Reserve Program (CRP) acres, noncropland areas (such as rights-of-way, roadsides and non-irrigation ditch banks), natural areas (such as wildlife trails), and grazed areas in and around these sites; Wheat (including spring wheat, winter wheat, and durum)	Annual and perennial broadleaf weeds	3/4/04
Canadian Label: Aminopyralid Liquid Concentrate		Canadian Label: Aminopyralid 240 g L, present as triisopropan-olamine salt		Canadian Label: Rangeland, pasture (without legumes), industrial and other nn-crop areas; wheat (spring and durum) in brown soil region of Western Canada.	Canadian Label: Broadleaf weeds and woody plants in rangeland; broadleaf weeds in wheat	

TABLE 2. Summary of Directions for Use of Aminopyralid.						
Trade Name	Applic. Timing, Type, and Equip.	Applic. Rate (lb ae/A) [g ae/ha]	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ae/A) [g ae/ha]	PHI (days)	Use Directions and Limitations
Rangeland and Permanent Grass Pastures						
GF-871 Canadian Label: Aminopyralid Liquid Concentrate	Broadcast foliar spray or spot treatment; Ground or aerial	0.11 [120]	Not specified (NS)	0.11 [120]	None proposed	Applications may be made alone or as a tank mix with other herbicides. Applications may be made in a minimum of 2 gal/A [19 L/ha] by air and 10 gal/A [100 L/ha] by ground. Application through any type of irrigation system is prohibited in the U.S., but there is no such restriction in Canada.
Wheat, Including Durum (not underseeded with a legume)						
GF-871 Canadian Label: Aminopyralid Liquid Concentrate	Broadcast foliar spray or spot treatment; Ground or aerial	0.009 [10]	NS	0.009 [10]	50 (grain and straw) 0 (hay)	Broadcast applications may be made to actively growing wheat from the 3-leaf crop growth stage up to early jointing stage (Zadoks scale 30). The Canadian Label indicates application at the 3-6 leaf stage for spring wheatand 2-6 leaf stage for durum wheat. Application to a cereal crop underseeded with a legume is prohibited. Applications may be made alone or as a tank mix with other herbicides such as fluroxypyr 1-methylheptyl ester (Starane), 2,4-D ester or amine, MCPA ester or amine, thifensulfuron-methyl (Harmony GT), tribenuron-methyl (Express XP), and metsulfuron-methyl (Ally XP). The Canadian Label only supports tank-mixing with Starane herbicide.

The product label for GF-871 additionally specifies that an approved agricultural surfactant may be used and that any tank mixes should be pre-tested to determine physical compatibility between formulations and to confirm safety to the target crop. The Canadian Label does not specify the need to use a surfactant under any conditions. A re-entry interval of 12 hours is proposed.

The following rotational crop restrictions are proposed: (i) 0 months for wheat (including durum); (ii) 3 months for barley, canola (rapeseed), flax, grasses, field corn, grain sorghum, oats,

mustard, popcorn, sweet corn; (iii) 9 months for safflower; and (iv) 18 months for all other crops. The following rotational crop restrictions have been approved for the Canadian Label: ~10 months for spring wheat and canola and ~ 2 years for all other crops.

CONCLUSION

The submitted label is adequate to allow evaluation of the residue data relative to the proposed uses on grasses and wheat. However, additional label additions/revisions/clarifications are recommended for regulatory purposes. These recommendations are addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D305665 and in Canada's Regulatory Decision Document.

REFERENCES

None.

DOCUMENT TRACKING

RDI: MADoherty, 06/28/05; TSheramata, 06/13/05; RLoranger, 7/5/05; HBietlot, 6/13/05
Petition Number(s): PP#4F6827
DP Barcode(s): D305665
PC Code: 005100/005209

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Aminopyralid (including TIPA salt)/XDE-750/PC Codes 005100 & 005209/Dow AgroSciences/62719
DACO 7.4.5/OPPTS 860.1520/OECD IIA 6.5.4 and IIIA 8.5
Processed Food and Feed - Wheat

Primary Evaluator _____ Date: 6/25/05
Michael A. Doherty, Ph.D.; Chemist, RAB2

Peer Reviewer _____ Date: June 7/05
Tamara Sheremata, Ph.D.
Evaluation Officer, FREAS, HED, PMRA

Approved by _____ Date: 6/13/05
Henri Bietlot, Ph.D.
A/Section Head, FREAS, HED, PMRA

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 11/08/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46235721 Roberts, D; Schelle, G.; Knuteson, J. (2004) Magnitude of Residue of XDE-750 in Wheat Agricultural Commodities: Amended Report. Project Number: 030042. Unpublished study prepared by Dow AgroSciences LLC. 232 p.

EXECUTIVE SUMMARY:

Dow AgroSciences has submitted a processing study with wheat. In one trial conducted in Colony, OK, wheat grain (RAC) was harvested 69 days following a single broadcast foliar spray application of the 0.08 lb ae/gal (9.6 g ae/L) water emulsion in oil (EO) formulation of aminopyralid triisopropanolammonium (TIPA) salt at 0.045 lb ae/A (~50 g ae/ha); we note that the test substance also contained fluroxypyr 1-methylheptyl ester (Starane). Wheat grain was processed into germ, bran, middlings, shorts, and flour using simulated commercial processing procedures. In addition, a sample of wheat aspirated grain fractions was generated.

Residues of aminopyralid were 0.054-0.055 ppm in/on wheat grain RAC. The processing data indicate that residues of aminopyralid may concentrate in wheat bran (2.6x processing factor), wheat shorts (1.2x processing factor), and aspirated grain fractions (6.1x processing factor), but do not appear to concentrate in wheat flour, middlings, and germ (processing factors of <0.1x, 0.6x, and 0.4x, respectively).

The maximum theoretical concentration factors for wheat bran, flour, and shorts are 7.7x, 1.4x, and 8.3x, respectively (based on separation into components, Table 3 of OPPTS 860.1520; Table 3 in Section 10 of Dir 98-02). The reported concentration factors do not exceed the theoretical concentration factors.

Samples of wheat grain, aspirated grain fractions, and wheat processed commodities germ, bran, middlings, shorts, and flour were analyzed for residues of aminopyralid using LC/MS/MS Method GRM 02.31. The validated limit of quantitation (LOQ) was 0.010 ppm for each wheat commodity. This method is adequate for data collection based on acceptable concurrent method recovery data.

The maximum storage interval of processing study samples from harvest/processing to analysis was 22 days (0.7 months). Samples of wheat grain were processed within 48 days of harvest. In support of the study, the petitioner cited storage stability data (refer to the DER for MRID 46235719) submitted in conjunction with the current petition; these interim data indicate that residues of aminopyralid are stable under frozen storage conditions in wheat grain for up to 168 days (5.5 months). The available storage stability data support the storage conditions and intervals of RAC samples from the submitted wheat processing study. Because wheat processing samples were stored frozen and analyzed within 30 days of collection, storage stability data are not required for the processed commodities.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the wheat processing residue data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D305665 and Canadian Regulatory Decision Document.

COMPLIANCE:

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

A. BACKGROUND INFORMATION

Aminopyralid (4-amino-3,6-dichloropyridine-2-carboxylic acid) is the proposed common name of XDE-750, a new active ingredient developed by Dow AgroSciences. Aminopyralid is a systemic postemergence herbicide which belongs to the pyridine carboxylic acid class of herbicides. The petitioner is currently proposing food/feed uses on grasses grown in rangelands and permanent pastures and on wheat for the selective control of invasive and noxious broadleaf weeds. It is also proposed for weed control in sites such as parks, electric utility rights-of way, forestry, woodlands, and wildlife openings, with smaller amounts used in railroads, utility substations, pipelines, and pumping stations.

The proposed end-use product (EP) is a soluble concentrate liquid (SC/L) referred to by the trade name GF-871 (EPA Reg. No. 62719-LRI). The active ingredient in GF-871 is formulated as the triisopropanolammonium (TIPA) salt, with the product containing 40.6% of aminopyralid TIPA salt at an acid equivalent (ae) of 21.1% or 2 lb ae/gal (240 g ae/L). The petitioner stated that the

aminopyralid TIPA salt dissociates rapidly in water to the acid (aminopyralid) at environmental pH values greater than 2.56 (the pKa).

Currently, the 2 lb ae/gal (240 g ae/L) SC/L formulation is proposed for broadcast foliar application at maximum rates of 0.11 lb ae/A (120 g ae/hectare) on rangeland and permanent pastures and 0.009 lb ae/A (10 g ae/ha) on wheat. The proposed PHIs are 0 days for wheat hay and 50 days for wheat grain and straw. No PHI is listed or proposed for grasses or wheat forage.

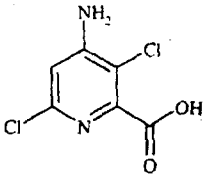
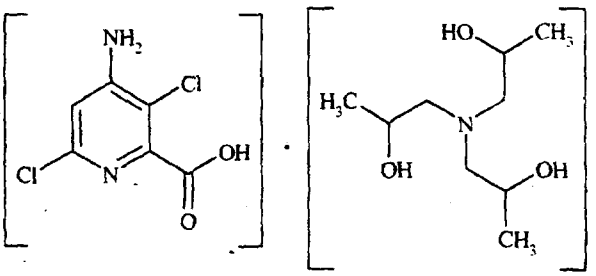
TABLE A.1. Test Compound Nomenclature.	
Chemical structure	
Common name	Aminopyralid
Company experimental name	XDE-750
IUPAC name	4-amino-3,6-dichloropyridine-2-carboxylic acid
CAS name	4-amino-3,6-dichloro-2-pyridinecarboxylic acid
CAS registry number	150114-71-9
End-use product (EP)	2 lb ae/gal TIPA salt SC/L formulation (GF-871 Herbicide: EPA Reg. No. 62719-LRI)
Chemical structure	
Common name	Aminopyralid, triisopropanolammonium (TIPA) salt
Company experimental name	XDE-750 TIPA salt
IUPAC name	Not provided
CAS name	Not provided
CAS registry number	Not provided
End-use product (EP)	0.08 lb ae/gal TIPA salt water emulsion in oil (EO) formulation (GF-982) 2 lb ae/gal (240 g ae/L) TIPA salt SC/L formulation (GF-871 Herbicide; EPA Reg. No. 62719-LRI; Aminopyralid Liquid Concentrate Herbicide in Canada)

TABLE A.2. Physicochemical Properties of the Aminopyralid Technical Grade Test Compound.			
Parameter	Value		Reference
Melting point	163.5 °C		MRID 46235703. PMRA LS
pH	2.31 at 23.4 °C (1% solution in water)		MRID 46235703. PMRA LS
Relative density	1.72 at 20 °C		MRID 46235703. PMRA LS
Water solubility	2.48 g/L unbuffered water at 18 °C 212 g/L pH 5 buffer at 20 °C 205 g/L pH 7 buffer at 20 °C 203 g/L pH 9 Buffer at 20 °C		MRID 46235703. PMRA LS
Solvent solubility at 20 °C	methanol acetone n-octanol ethyl acetate 1,2-dichloroethane xylene heptane	52.2 g/L 29.2 g/L 3.9 g/L 3.9 g/L 0.2 g/L 0.04 g/L <10 µg/mL	MRID 46235703. PMRA LS
Vapor pressure	2.59 x 10^{-8} Pa at 25 °C; 9.52 x 10^{-9} Pa at 20 °C		MRID 46235703. PMRA LS
Dissociation constant, pK_a	2.56		MRID 46235703. PMRA LS
Octanol/water partition coefficient, $Log(K_{ow})$	0.201 unbuffered water at 19 °C; -1.76 at pH 5; -2.87 at pH 7; -2.96 at pH 9		MRID 46235703. PMRA LS
UV/visible absorption spectrum	Solution Neutral Basic (pH 12.6) Acidic (pH 1.4)	Wavelength λ max, nm 217 220 245 217 270	Extinction coefficient ε, L/(mol*cm) 29100 26100 10150 22800 9140	MRID 46235703. PMRA LS

TABLE A.3. Physicochemical Properties of the Aminopyralid TIPA Salt 2 lb/gal (240 g ae/L) SC/L Formulation.		
Parameter	Value	Reference
Melting point	Not provided	
pH	7.33 at 19.8 °C	MRID 46235704. PMRA LS
Density	1.1401 g/mL at 20.0 °C	MRID 46235704. PMRA LS
Water solubility	Not provided	
Solvent solubility at 20 °C	Not provided	
Vapor pressure	Not provided	
Dissociation constant, pK_a	Not provided	
Octanol/water partition coefficient, $Log(K_{ow})$	Not provided	
UV/visible absorption spectrum	Not provided	

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B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

TABLE B.1.2. Study Use Pattern

Location (County, State: Year)	EP[1]	Application					Tank Mix Adjuvants
		Method; Timing	Vol (GPA[2]) [L/ha]	Rate (lb ae/A) [g ae/ha]	RTI[3] (days)	Total Rate (lb ae/A) [g ae/ha]	
Colony, OK; 2003	0.08 lb (9.6 g ae/L) ae/gal EO	Single broadcast foliar application; crop height 31-36 cm, 33 BBCH	12.6 [119]	0.045 [50.0]	N/A	0.045 [50.0]	None

[1] EP = End-use Product; 0.08 lb ae/gal (9.6 G AE/l) EO formulation of aminopyralid TIPA salt, containing fluroxypyr 1-methylheptyl.
[2] GPA = Gallons per acre [Liters per hectare]
[3] RTI = Retreatment Interval; Not applicable (N/A) because only one application was made.

B.2. Sample Handling and Processing Procedures

Samples of wheat grain used for processing were obtained from a field trial conducted during the 2003 growing season in OK (Region 6). Bulk samples of wheat grain (RAC) were collected at maturity, 69 days following a single broadcast foliar application of the 0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt formulation at 0.045 lb ae/A (50 g ae/ha) in 12.6 gal of water/A (118 L/ha) using ground equipment. The petitioner did not specify the sampling procedures used for collection of wheat grain.

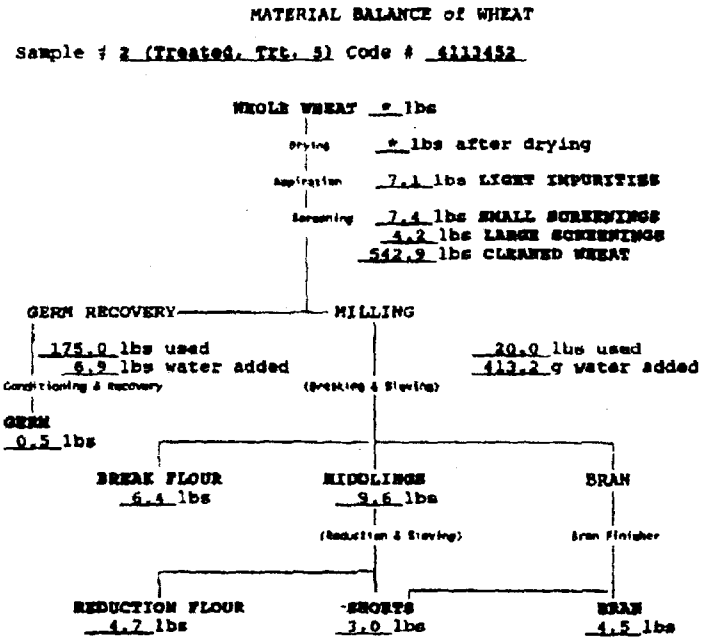
Bulk samples of treated and control wheat grain for processing were collected and stored frozen at the field facility for 4 days prior to shipment via ACDS freezer truck to the Food Protein Research and Development Center at Texas A&M University (Bryan, TX) where samples were stored frozen (-23 °C) prior to processing. Wheat grain was processed using simulated commercial procedures into germ, bran, middlings, shorts, and flour within 40-48 days of harvest. In addition, a sample of wheat aspirated grain fractions was generated. A brief description of the processing procedure follows.

Wheat grain samples were circulated through a dust-generating apparatus consisting of a bucket conveyor, drag conveyors, and holding bins. Dust was collected by aspiration and was separated by sieving into six aspirated grain fractions based on particle size; after classification, all six fractions were combined to generate an aspirated grain fractions sample for analysis. After the aspirated grain fractions were collected, the wheat grain was cleaned by aspiration; light impurities collected during this step were not combined with the aspirated grain fractions. A portion of the cleaned grain was moisture adjusted to 16%, broken into small pieces in a corrugated roller mill, and sieved to separate bran from germ (including endosperm), which was subsequently reduced and sifted to separate the germ from the endosperm. A second portion of cleaned wheat grain was moisture adjusted to 16%, broken four times in corrugated roller mills, and sieved to produce bran, middlings, and break flour. Middlings were reduced in a smooth roller mill, sieved to separate shorts and reduction flour, and break and reduction flour were

combined. Bran was further finished by screening to separate shorts and bran. The material passing through the screen was classified as shorts and was added to the reduction mill shorts. The material over the screen is classified as bran. Material balance and process flow charts were provided. After processing, samples were shipped frozen to Dow AgroSciences (Indianapolis, IN) for extraction and analysis.

The petitioner provided a flowchart and material balances for wheat processing, which is presented below (copied without alteration from MRID 46235721).

FIGURE 1. Processing Flowchart for Wheat.



B.3. Analytical Methodology

Samples of wheat grain, aspirated grain fractions, and processed commodities germ, bran, middlings, shorts, and flour were analyzed for residues of aminopyralid by Dow AgroSciences (Indianapolis, IN) using LC/MS/MS Method GRM 02.31. A description of the method was included in the submission. For a complete description of the method, refer to the Residue Analytical Method for Plants DER (MRID 46235712).

Briefly, ground samples of wheat commodities were extracted with 0.1 N sodium hydroxide, releasing bound residues and hydrolyzing base-labile conjugates to free aminopyralid. Acid-labile conjugates were hydrolyzed by the acidification of the extract with hydrochloric acid and heating. Following hydrolysis, the extract was cleaned up through a polymeric solid-phase extraction column. The eluate was evaporated to dryness after the addition of the internal standard solution, $^{13}C_2{}^{15}N$-aminopyralid, and the residues reconstituted with the derivitization

coupling reagent. The solution was derivatized with butyl chloroformate and diluted with methanol:water:acetic acid (50:49.9:0.1, v:v:v) for LC/MS/MS analysis. The validated limit of quantitation (LOQ) was 0.01 ppm, and the calculated limit of detection (LOD) was 0.003 ppm for all wheat matrices.

C. RESULTS AND DISCUSSION

Acceptable concurrent method validation data for residues of aminopyralid in/on wheat grain, aspirated grain fractions, and processed germ, bran, middlings, shorts, and flour are presented in Table C.1. The method is adequate for data collection based on acceptable concurrent method recovery data. Apparent residues of aminopyralid were less than the method LOQ (<0.01 ppm) in/on one sample each of untreated wheat grain, and germ, middlings, shorts, and flour processed from untreated wheat grain. Detectable residues of aminopyralid were observed in/on one untreated wheat bran sample at 0.017 ppm and one untreated aspirated grain fraction sample at 0.030 ppm; however, these residues were insignificant in comparison with the residues in treated samples.

Sample storage conditions and intervals are summarized in Table C.2. The maximum storage interval of processing study samples from harvest/processing to analysis was 22 days (0.7 months). Samples of wheat grain were processed within 48 days of harvest. In support of the study, the petitioner cited storage stability data (refer to the DER for MRID 46235719) submitted in conjunction with the current petition; these interim data indicate that residues of aminopyralid are stable under frozen storage conditions in wheat grain for up to 168 days (5.5 months). The available storage stability data support the storage conditions and intervals of RAC samples from the submitted wheat processing study. Because wheat processing samples were stored frozen and analyzed within 30 days of collection, storage stability data are not required for the processed commodities.

Residue data from the wheat processing study are reported in Table C.3. Residues of aminopyralid were 0.054-0.055 ppm (average = 0.055 ppm) in/on wheat grain treated with the 0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt formulation at 0.045 lb ae/A (50.0 g ae/ha). The processing data indicate that residues of aminopyralid reduce in wheat flour (0.008 ppm; <0.1x processing factor), wheat germ (0.019-0.021 ppm; 0.4x average processing factor), and middlings (0.032 ppm; 0.6x processing factor), and concentrate in wheat bran (0.143 ppm; 2.6x processing factor), wheat shorts (0.067 ppm; 1.2x processing factor), and aspirated grain fractions (0.338 ppm; 6.1x processing factor). The observed processing factors of <0.1x for wheat flour, 2.6x for wheat bran, and 1.2x for wheat shorts are less than the theoretical concentration factors of 1.4x, 7.7x, and 8.3x for wheat flour, bran, and shorts, respectively (OPPTS 860.1520, Table 3; Dir 98-02, Table 3, Section 10).

TABLE C.1. Summary of Concurrent Recoveries of Aminopyralid from Wheat Commodities.				
Matrix	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean
Wheat bran	0.01	1	109	106
	0.50	1	102	
Wheat middlings	0.01	1	115	110
	0.50	1	104	
Wheat shorts	0.01	1	127	118
	0.50	1	109	
Wheat flour	0.01	1	112	108
	0.50	1	104	
Wheat germ	0.01	1	97	97
Wheat, aspirated grain fractions	0.01	1	104	103
	0.50	1	102	

TABLE C.2. Summary of Storage Conditions			
Matrix (RAC) [1]	Storage Temp. (°C)	Actual Storage Duration (days)	Interval of Demonstrated Storage Stability (days)
Wheat grain	-20	22	Interim storage stability data indicate that residues of aminopyralid are stable under frozen storage conditions in/on fortified samples of wheat grain for up to 168 days. [2]
-Bran		12	None required; samples were analyzed within 30 days of sampling (processing).
-Flour		12	
-Shorts		12	
-Middlings		12	
-Germ		20	
Aspirated Grain Fractions		20	

[1] Wheat grain samples were processed within 48 days of harvest.
[2] Refer to the DER for MRID 46235719.

TABLE C.3. Residue Data from Wheat Processing Study with Aminopyralid.

RAC	Processed Commodity	Total Rate (lb ae/A) [g ae/ha]	PHI (days)	Aminopyralid Residues (ppm) [1]	Processing Factor [2]
Wheat grain (bulk)	RAC	0.045 [50.0]	69	0.054, 0.055 (0.055)	–
	Bran			0.143	2.6x
	Flour			*0.008*	<0.1x
	Shorts			0.067	1.2x
	Middlings			0.032	0.6x
	Germ			0.019, 0.021	0.3x, 0.4x
	Aspirated Grain Fractions			0.338	6.1x

[1] The petitioner presented both uncorrected residue values and residue values corrected for concurrent method recovery. The uncorrected values are reported here. Residues reported between the method LOQ (<0.01 ppm) and LOD (0.003 ppm) are *italicized*. Average residues are reported in parentheses for the grain RAC.
[2] Calculated by the study reviewer using the average residues in the grain RAC samples from the processor.

D. CONCLUSION

The processing data indicate that residues of aminopyralid may concentrate in wheat bran (2.6x processing factor), wheat shorts (1.2x processing factor), and aspirated grain fractions (6.1x processing factor), but do not appear to concentrate in wheat flour, middlings, and germ (processing factors of <0.1x, 0.6x, and 0.4x, respectively).

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: MADoherty, 06/28/05; TSheramata, 06/13/05; HBietlot, 6/13/05
Petition Number(s): PP#4F6827
DP Barcode(s): D305665
PC Code: 005100/005209

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Aminopyralid (including TIPA salt)/XDE-750/PC Codes 005100 & 005209/Dow AgroSciences/62719
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Rangeland and Pasture Grasses

Primary Evaluator _____ Date: 6/28/05
Michael A. Doherty, Ph.D., Chemist, RAB2

Peer Reviewer _____ Date: June 7/05
Tamara Sheremata, Ph.D.
Evaluation Officer, FREAS, HED, PMRA

Approved by _____ Date: 13/05
Henri Bietlot, Ph.D.
A/Section Head, FREAS, HED, PMRA

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 11/08/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46235722 McCormick, R.; Schelle, G.; Dolder, S. (2004) Magnitude of Residue of XDE-750 and 2,4-D in Rangeland and Pasture Grasses. Project Number: 020018. Unpublished study prepared by Dow AgroSciences LLC. 202 p.

EXECUTIVE SUMMARY:

Dow AgroSciences has submitted field trial data depicting the magnitude of the residue of aminopyralid in/on pasture and rangeland grass forage and hay. A total of 20 grass field trials were conducted in Canada and the U.S. during the 2002 growing season. Seven Canadian grass field trials were conducted in Regions 7 (AB and SK; 2 trials) and 14 (AB, MB, and SK; 5 trials). Thirteen U.S. grass field trials were conducted in Regions 1 (NY and PA; 2 trials), 2 (GA and VA; 2 trials), 4 (LA; 1 trial), 5 (ND and OH; 2 trials), 5A (WI; 1 trial), 7 (MT; 1 trial), 8 (TX; 1 trial), 9 (MT; 1 trial), and 11 (ID and WA; 2 trials). The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 and PMRA Directive 98-02, Section 9.

At each test location, pasture and rangeland grass were treated with a single broadcast foliar application of the 2 lb ae/gal (240 g ae/L) soluble concentrate/liquid (SC/L) formulation of aminopyralid as the triisopropanolammonium (TIPA) salt at ~0.11 lb ae/A (120 g ae/ha). Grass forage was harvested 0, 6-8, and 13-15 days after application, and grass hay was harvested 0, 13-15, and 20-22 days after application and allowed to dry for 2 to 5 days prior to collection. Applications at all test sites were made using ground equipment in ~10-20 gal/A (93.5-187 L/ha) of water with a non-ionic spray adjuvant added. In addition, a separate plot at four test locations (2 trials in Canada and 2 trials in the U.S.) received a single application of the 2 lb ae/gal (240 g ae/L) SC/L aminopyralid formulation tank mixed with a 5.7 lb ae/gal (570 g ae/L) SC/L

formulation of 2,4-D. Data for the 2,4-D acid equivalent are not reviewed herein. Additional forage samples were collected from these four test locations (both treatment plots) 3 and 21/22 days after application, and additional grass hay samples were collected 28 days after application to evaluate residue decline.

Samples of grass hay and forage were analyzed for residues of aminopyralid using LC/MS/MS, Method GRM 02.31. The validated limit of quantitation (LOQ) was 0.01 ppm. This method is adequate for data collection based on acceptable concurrent method recovery and radiovalidation data.

The maximum storage interval of crop samples from harvest to analysis was 454 days (14.9 months) for grass forage and 441 days (14.5 months) for grass hay. In support of the crop field trial study, the petitioner cited storage stability data (refer to the DER for MRID 46235719) submitted in conjunction with the current petition; these interim data indicate that residues of aminopyralid are stable under frozen storage conditions in grass forage and hay for up to 187 days (6.2 months). The available storage stability data do not support the storage intervals of the samples from the grass field trials; however, the petitioner stated that the full study will include storage intervals of up to 18 months for grass forage and hay.

The maximum aminopyralid residues in/on pasture and rangeland grass forage and hay from the submitted grass field trials are reported below.

Commodity	PHI (days)	Maximum Residue Levels (ppm)
Canadian Trials		
Grass forage	0	14.033
	7-8	6.108
	13-14	3.326
Grass hay	0	51.496
	13-14	9.531
	20-22	8.708
U.S. Trials		
Grass forage	0	15.551
	6-7	6.514
	13-15	8.009
Grass hay	0	29.962
	13-15	13.152
	20-22	17.150

Residue decline data showed that aminopyralid residues generally decreased in grass forage and hay with increasing sampling intervals, with maximum residues occurring at the 0-day sampling interval.

For U.S. trials, OPPTS 860.1500 (Tables 2 and 5) specifies that four trials should be conducted for each of three grass cultivars (Bermuda grass, bluegrass, and bromegrass or fescue) for a total of 12 trials, in all areas across the country. The U.S. crop field trials were conducted using Bermuda grass (2 trials), bromegrass or fescue (6 trials), crested wheatgrass (1 trial), orchard grass (2 trials), reed canarygrass (1 trial), and a grass mixture (1 trial). Although the grass cultivars used by the petitioner did not exactly match the requirements of OPPTS 860.1500 (more trials were conducted with cool season grasses than warm season grasses), HED concludes that the grass cultivars used are sufficient to represent grasses grown for pasture and rangeland in the U.S. and Canada.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the grass field trial residue data are classified as scientifically acceptable, provided the final storage stability report confirms the stability of aminopyralid in grasses during frozen storage for at least 15 months. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D305665, and in Canada's Regulatory Decision Document.

COMPLIANCE:

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

A. BACKGROUND INFORMATION

Aminopyralid (4-amino-3,6-dichloropyridine-2-carboxylic acid) is the proposed common name of XDE-750, a new active ingredient developed by Dow AgroSciences. Aminopyralid is a systemic postemergence herbicide which belongs to the pyridine carboxylic acid class of herbicides. The petitioner is currently proposing food/feed uses on grasses grown in rangelands and permanent pastures and on wheat for the selective control of invasive and noxious broadleaf weeds. It is also proposed for weed control in sites such as parks, electric utility rights-of way, forestry, woodlands, and wildlife openings, with smaller amounts used in railroads, utility substations, pipelines, and pumping stations.

The proposed end-use product (EP) is a soluble concentrate liquid (SC/L) referred to by the trade name GF-871 (EPA Reg. No. 62719-LRI). The active ingredient in GF-871 is formulated as the triisopropanolammonium (TIPA) salt, with the product containing 40.6% of aminopyralid TIPA salt at an acid equivalent (ae) of 21.1% or 2 lb ae/gal (240 g ae/L). The petitioner stated that the aminopyralid TIPA salt dissociates rapidly in water to the acid (aminopyralid) at environmental pH values greater than 2.56 (the pKa).

Currently, the 2 lb ae/gal (240 g ae/L) SC/L formulation is proposed for broadcast foliar application at maximum rates of 0.11 lb ae/A (120 g ae/hectare) on rangeland and permanent pastures and 0.009 lb ae/A (10 g ae/ha) on wheat. The proposed PHIs are 0 days for wheat hay and 50 days for wheat grain and straw. No PHI is listed or proposed for grasses or wheat forage.

TABLE A.1. Test Compound Nomenclature.

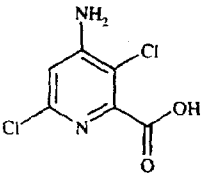
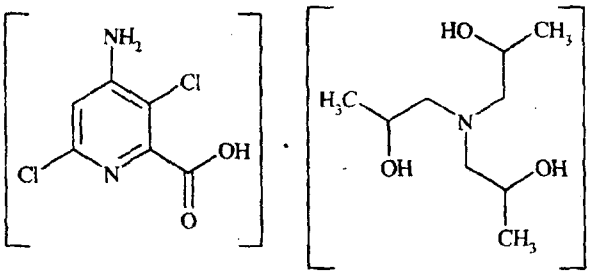
Chemical structure	
Common name	Aminopyralid
Company experimental name	XDE-750
IUPAC name	4-amino-3,6-dichloropyridine-2-carboxylic acid
CAS name	4-amino-3,6-dichloro-2-pyridinecarboxylic acid
CAS registry number	150114-71-9
End-use product (EP)	2 lb ae/gal TIPA salt SC/L formulation (GF-871 Herbicide; EPA Reg. No. 62719-LRI).
Chemical structure	
Common name	Aminopyralid, triisopropanolammonium (TIPA) salt
Company experimental name	XDE-750 TIPA salt
IUPAC name	Not provided
CAS name	Not provided
CAS registry number	Not provided
End-use product (EP)	2 lb ae/gal (240 g ae/L) TIPA salt SC/L formulation (GF-871 Herbicide; EPA Reg. No. 62719-LRI; Aminopyralid Liquid Concentrate Herbicide in Canada)

TABLE A.2. Physicochemical Properties of the Aminopyralid Technical Grade Test Compound.

Parameter	Value	Reference
Melting point	163.5 °C	MRID 46235703, PMRA LS
pH	2.31 at 23.4 °C (1% solution in water)	MRID 46235703, PMRA LS
Relative density	1.72 at 20 °C	MRID 46235703, PMRA LS
Water solubility	2.48 g/L unbuffered water at 18 °C 212 g/L pH 5 buffer at 20 °C 205 g/L pH 7 buffer at 20 °C 203 g/L pH 9 Buffer at 20 °C	MRID 46235703, PMRA LS
Solvent solubility at 20 °C	methanol 52.2 g/L acetone 29.2 g/L n-octanol 3.9 g/L ethyl acetate 3.9 g/L 1,2-dichloroethane 0.2 g/L xylene 0.04 g/L heptane <10 µg/mL	MRID 46235703, PMRA LS
Vapor pressure	2.59×10^{-8} Pa at 25 °C; 9.52×10^{-9} Pa at 20 °C	MRID 46235703, PMRA LS
Dissociation constant, pK_a	2.56	MRID 46235703, PMRA LS
Octanol/water partition coefficient, $Log(K_{OW})$	0.201 unbuffered water at 19 °C; -1.76 at pH 5; -2.87 at pH 7; -2.96 at pH 9	MRID 46235703, PMRA LS
UV/visible absorption spectrum	Solution: Neutral; Wavelength λ max, nm: 217; Extinction coefficient ε, L/(mol*cm): 29100 Basic: 220; 26100 (pH 12.6): 245; 10150 Acidic: 217; 22800 (pH 1.4): 270; 9140	MRID 46235703, PMRA LS

TABLE A.3. Physicochemical Properties of the Aminopyralid TIPA Salt 2 lb/gal (240 g ae/L) SC/L Formulation.

Parameter	Value	Reference
Melting point	Not provided	
pH	7.33 at 19.8 °C	MRID 46235704, PMRA LS
Density	1.1401 g/mL at 20.0 °C	MRID 46235704, PMRA LS
Water solubility	Not provided	
Solvent solubility at 20 °C	Not provided	
Vapor pressure	Not provided	
Dissociation constant, pK_a	Not provided	
Octanol/water partition coefficient, $Log(K_{OW})$	Not provided	
UV/visible absorption spectrum	Not provided	

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B. EXPERIMENTAL DESIGN

B.1. Study Site Information

TABLE B.1.1. Trial Site Conditions.

Trial Identification: City, State/Province; Year (Study ID)	Soil characteristics				Meteorological data	
	Type	%OM	pH	CEC	Overall monthly rainfall range (inches) [1]	Overall temperature range (°C)
Canadian Trials						
Vanscoy, SK; 2002 (AB-2)	Sandy loam	Optional			2.0-3.0 (I)	9.3-27.8
Gull Lake, AB; 2002 (AB-3)	Sandy loam	Optional			0.8-1.7 (I)	9.2-23.4
Monarch, AB; 2002 (AB-4)	Silty clay	Optional			1.9-11.0	8.7-28.9
Lacombe, AB; 2002 (AB-D)	Loam	Optional			0.8-1.7 (I)	9.2-23.4
Boissevain, MB; 2002 (MB-1)	Clay loam	Optional			1.0	11.1-26.2
Rosthern, SK; 2002 (SK-2)	Silt loam	Optional			1.1-2.4 (I)	9.0-27.0
Taber, AB; 2002 (SK-D)	Sandy loam	Optional			1.1-10.8	9.4-29.4
U.S. Trials						
Athens, GA; 2002 (GA)	Sandy loam	Optional			4.5	20.7-31.6
American Falls, ID; 2002 (ID)	Silt loam	Optional			0.3-0.7	4.5-24.6
Washington, LA; 2002 (MS)	Silt loam	Optional			4.0	20.4-31.2
Fort Benton, MT; 2002 (MT-1)	Sandy loam	Optional			2.2-5.4	2.4-24.2
Ennis, MT; 2002 (MT-2)	Sandy loam	Optional			0.0 (I)	2.3-23.7
Northwood, ND; 2002 (ND)	Loam	Optional			1.3-4.6	1.8-25.3
North Rose, NY; 2002 (NY)	Loamy fine sand	Optional			3.2-4.2	5.8-24.0
New Holland, OH; 2002 (OH)	Silt loam	Optional			4.8	8.5-21.2
Germansville, PA; 2002 (PA)	Loam	Optional			1.1-7.4	15.0-29.8
Claude, TX; 2002 (TX)	Loam	Optional			3.1-3.5	8.0-32.2
Suffolk, VA; 2002 (VA-D)	Fine sandy loam	Optional			3.2	12.2-27.4
Moses Lake, WA; 2002 (WA-D)	Loamy fine sand	Optional			5.3-7.4 (I)	5.8-26.6
Arkansaw, WI; 2002 (WI)	Sandy loam	Optional			3.5-12.1	5.3-26.1

[1] (I) indicates that supplemental irrigation was received.

The petitioner provided average maximum and minimum air temperatures and rainfall data for all of the grass field trials and historical values for temperatures and rainfall. The actual temperature recordings were within the average historical values for the residue study period. The actual rainfall average was within the normal rainfall encountered in rangeland and pasture grass production areas. Irrigation was used to supplement rainfall as needed. In general, variations in weather conditions were not considered by the petitioner to have an impact on the results of the study.

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TABLE B.1.2. Study Use Pattern.

Location: City, State/Province; Year (Study ID)	Treatment regime [1]	EP	Application					Tank Mix Adjuvants
			Method; Timing	Vol. (GPA[2]) [L/ha]	Rate (lb ae/A) [g ae/ha]	RTI[3] (days)	Total Rate (lb ae/A) [g ae/ha]	
Canadian Trials								
Vanscoy, SK; 2002 (AB-2)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 30 cm	15.9 [150]	0.106 [119]	N/A	0.106 [119]	non-ionic adjuvant (0.25% v/v)
Gull Lake, AB; 2002 (AB-3)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 10-15 cm	15.8 [149]	0.106 [119]	N/A	0.106 [119]	non-ionic adjuvant (0.25% v/v)
Monarch, AB; 2002 (AB-4)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 15 cm	10.7 [101]	0.108 [121]	N/A	0.108 [121]	non-ionic adjuvant (0.25% v/v)
Lacombe, AB; 2002 (AB-D)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 10-15 cm	15.7 [148]	0.105 [118]	N/A	0.105 [118]	non-ionic adjuvant (0.25% v/v)
	B	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 10-15 cm	15.8 [149]	0.106 [119]	N/A	0.106 [119]	non-ionic adjuvant (0.25% v/v)
		5.7 lb ae/gal (683 g ae/L) SC/L			1.269 [1423]		1.269 [1423]	
Boissevain, MB; 2002 (MB-1)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 15 cm	10.7 [101]	0.108 [121]	N/A	0.108 [121]	non-ionic adjuvant (0.25% v/v)
Rosthern, SK; 2002 (SK-2)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 10-15 cm	15.9 [150]	0.107 [120]	N/A	0.107 [120]	non-ionic adjuvant (0.25% v/v)
Taber, AB; 2002 (SK-D)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 15 cm	10.3 [97]	0.103 [116]	N/A	0.103 [116]	non-ionic adjuvant (0.25% v/v)
	B	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 15 cm	11.0 [104]	0.112 [125]	N/A	0.112 [125]	non-ionic adjuvant (0.25% v/v)
		5.7 lb ae/gal (683 g ae/L) SC/L			1.336 [1498]		1.336 [1498]	
U.S. Trials								
Athens, GA; 2002 (GA)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 10-20 cm	17.4 [164]	0.107 [120]	N/A	0.107 [120]	non-ionic adjuvant (0.25% v/v)
American Falls, ID; 2002 (ID)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 10-30 cm	17.3 [163]	0.107 [120]	N/A	0.107 [120]	non-ionic adjuvant (0.25% v/v)
Washington, LA; 2002 (MS)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 15 cm	15.7 [148]	0.112 [125]	N/A	0.112 [125]	non-ionic adjuvant (0.25% v/v)

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TABLE B.1.2. Study Use Pattern.

Location: City, State/Province; Year (Study ID)	Treatment regime [1]	EP	Application					Tank Mix Adjuvants
			Method; Timing	Vol. (GPA[2]) [L/ha]	Rate (lb ae/A) [g ae/ha]	RTI[3] (days)	Total Rate (lb ae/A) [g ae/ha]	
Fort Benton, MT; 2002 (MT-1)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 10-15 cm	20.1 [190]	0.109 [122]	N/A	0.109 [122]	non-ionic adjuvant (0.25% v/v)
Ennis, MT; 2002 (MT-2)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 13-15 cm	19.9 [188]	0.108 [121]	N/A	0.108 [121]	non-ionic adjuvant (0.25% v/v)
Northwood, ND; 2002 (ND)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 13 cm	16.0 [151]	0.107 [120]	N/A	0.107 [120]	non-ionic adjuvant (0.25% v/v)
North Rose, NY; 2002 (NY)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 61-76 cm	19.2 [181]	0.103 [115]	N/A	0.103 [115]	non-ionic adjuvant (0.25% v/v)
New Holland, OH; 2002 (OH)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 30-40 cm	18.6 [175]	0.110 [123]	N/A	0.110 [123]	non-ionic adjuvant (0.25% v/v)
Germansville, PA; 2002 (PA)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 30-60 cm	16.6 [157]	0.112 [125]	N/A	0.112 [125]	non-ionic adjuvant (0.25% v/v)
Claude, TX; 2002 (TX)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 15 cm	18.1 [171]	0.108 [121]	N/A	0.108 [121]	non-ionic adjuvant (0.25% v/v)
Suffolk, VA; 2002 (VA-D)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 25-36 cm	15.4 [145]	0.107 [120]	N/A	0.107 [120]	non-ionic adjuvant (0.25% v/v)
	B	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 25-36 cm	15.4 [145]	0.108 [121]	N/A	0.108 [121]	non-ionic adjuvant (0.25% v/v)
		5.7 lb ae/gal (683 g ae/L) SC/L			1.291 [1447]		1.291 [1447]	
Moses Lake, WA; 2002 (WA-D)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 25-30 cm	14.8 [140]	0.107 [120]	N/A	0.107 [120]	non-ionic adjuvant (0.25% v/v)
	B	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 25-30 cm	14.8 [140]	0.107 [120]	N/A	0.107 [120]	non-ionic adjuvant (0.25% v/v)
		5.7 lb ae/gal (683 g ae/L) SC/L			1.295 [1452]	N/A	1.295 [1452]	

TABLE B.1.2. Study Use Pattern.

Location: City, State/Province; Year (Study ID)	Treatment regime [1]	EP	Application					Tank Mix Adjuvants
			Method; Timing	Vol. (GPA[2]) [L/ha]	Rate (lb ae/A) [g ae/ha]	RTI[3] (days)	Total Rate (lb ae/A) [g ae/ha]	
Arkansaw, WI; 2002 (WI)	A	2 lb ae/gal (240 g ae/L) SC/L	Single broadcast foliar application; crop height 15 cm	18.7 [176]	0.108 [121]	N/A	0.108 [121]	non-ionic adjuvant (0.25% v/v)

[1] Treatment Regimes:

A = single broadcast foliar application of the 2 lb ae/gal SC/L formulation of aminopyralid TIPA salt.

B = single broadcast foliar application of a tank mix of the 2 lb ae/gal (240 g ae/L) SC/L formulation of aminopyralid TIPA salt and the 5.7 lb ae/gal (683 g ae/L) SC/L formulation of 2,4-D.

[2] GPA = Gallons per acre [Liters per hectare]

[3] RTI = Retreatment Interval; Not applicable (N/A) because only one application was made.

TABLE B.1.3. Trial Numbers and Geographical Locations.

NAFTA Growing Region	Grass			
	Submitted		Requested	
	Canada	US	Canada [1]	US [2]
1		2	1	
1A				
2		2		
3				
4		1		
5		2	2	
5A		1	1	
5B			1	
6				
7	2	1	1	
7A				
8		1		
9		1		
10				
11		2		
12				
13				
14	5		6	
15				
16				
17				
18				
19				
20				
21				
Total	7	13	12	12 [2]

[1] Directive 98-02, Section 9, Table 2 (requirements for tame hay).
[2] OPPTS 860.1500, Tables 2 and 5 require 4 trials for each of three grass cultivars (Bermuda grass, bluegrass, and bromegrass or fescue) for a total of 12 trials, conducted in all areas across the country.

Although one less trial was conducted in Region 14 than required, both of the trials conducted in Canada in Region 7 were conducted in areas near the border of Region 7 and Region 14. Therefore, one of the Canadian Region 7 trials may be used to satisfy the Region 14 requirements.

The U.S. crop field trials were conducted using Bermuda grass (2 trials), bromegrass or fescue (6 trials), crested wheatgrass (1 trial), orchard grass (2 trials), reed canarygrass (1 trial), and a grass mixture (1 trial).

B.2. Sample Handling and Preparation

One untreated and two treated samples of grass forage and hay were collected from each trial site. Grass forage was harvested 0, 6-8, and 13-15 days after application and grass hay was harvested 0, 13-15, and 20-22 days after application and allowed to dry for 2 to 5 days (to the proper moisture content for hay) prior to collection. Additional forage samples were collected from four trials 3 and 21/22 days after application and additional grass hay samples were collected 28 days after application to evaluate residue decline. Grass forage samples were frozen within 5 hours of sampling, and grass hay samples were allowed to dry prior to freezing. Samples were shipped to Dow AgroSciences (Indianapolis, IN), where samples were stored frozen (<-20 °C). Prior to analysis, samples were frozen using liquid nitrogen and then ground using a hammer mill.

B.3. Analytical Methodology

Samples of pasture and rangeland grass (forage and hay) were analyzed for residues of aminopyralid by Dow AgroSciences (Indianapolis, IN) using LC/MS/MS Method GRM 02.31. A description of the method was included in the submission. For a complete description of the method, refer to the DER for MRID 46235712.

Briefly, ground samples of grass forage and hay were extracted with 0.1 N sodium hydroxide, releasing bound residues and hydrolyzing base-labile conjugates to free aminopyralid. Acid-labile conjugates were hydrolyzed by the acidification of the extract with hydrochloric acid and heating. Following hydrolysis, the extract was cleaned up through a polymeric solid-phase extraction column. The eluate was evaporated to dryness after the addition of the internal standard, $^{13}C_2{}^{15}N$-aminopyralid, and the residues reconstituted with the derivitization coupling reagent. The solution was derivatized with butyl chloroformate and diluted with methanol:water:acetic acid (50:49.9:0.1, v:v:v) for LC/MS/MS analysis. The validated limit of quantitation (LOQ) was 0.01 ppm, and the calculated limit of detection (LOD) was 0.003 ppm.

C. RESULTS AND DISCUSSION

Sample storage conditions and intervals are summarized in Table C.2. The maximum storage interval of crop samples from harvest to analysis was 454 days (14.9 months) for grass forage and 441 days (14.5 months) for grass hay. In support of the crop field trial study, the petitioner cited storage stability data (refer to the DER for MRID 46235719) submitted in conjunction with the current petition; these interim data indicate that residues of aminopyralid are stable under frozen storage conditions in grass forage and hay for up to 187 days (6.2 months). The available storage stability data do not support the storage intervals of the samples from the grass field trials; however, the petitioner stated that the full study will include storage intervals of up to 18 months for grass forage and hay.

Concurrent method recovery data are presented in Table C.1. Samples of grass hay and forage were analyzed for residues of aminopyralid using LC/MS/MS Method GRM 02.31. The method

LOQ was 0.01 ppm. This method is adequate for data collection based on acceptable concurrent method recovery data. Apparent residues of aminopyralid were below the method LOQ (<0.01 ppm) in/on all untreated samples of grass forage and hay, except in/on five untreated grass hay samples (0.013-0.029 ppm) where detectable residues of aminopyralid were observed. Detectable residues (14.548 and 16.326 ppm) were also observed in two additional untreated grass hay samples; however, the petitioner concluded, based on the observed residues in the associated treated samples, that control and treated samples were apparently switched.

Residue data from the grass field trials are reported in Table C.3. A summary of residue data for grass forage and hay is presented in Table C.4. In the Canadian field trials, residues of aminopyralid were 7.452-14.033 ppm in/on grass forage and 12.909-51.496 ppm in/on grass hay harvested on the day (0-day PHI) of a single broadcast foliar application of the SC/L formulation of aminopyralid TIPA salt either alone or as a tank mix with 2,4-D at total seasonal rates of 0.103-0.112 lb ae/A (116-125 kg ae/ha). In the U.S. field trials, residues of aminopyralid were 3.516-15.551 ppm in/on grass forage and 9.407-29.962 ppm in/on grass hay harvested on the day (0-day PHI) of a single broadcast foliar application of the SC/L formulation of aminopyralid either alone or as a tank mix with 2,4-D at total seasonal rates of 0.103-0.112 lb ae/A (115-125 kg ae/ha).

In the Canadian field trials, residues of aminopyralid were 1.276-6.108 ppm and 1.009-3.326 ppm in/on grass forage harvested 7-8 and 13-14 days, respectively, following treatment. Residues of aminopyralid were 1.837-9.531 ppm and 2.119-8.708 ppm in/on grass hay harvested 13-14 and 20-22 days, respectively, following treatment.

In the U.S. field trials, residues of aminopyralid were 0.232-6.514 ppm and 0.535-8.009 ppm in/on grass forage harvested 6-7 and 13-15 days, respectively, following treatment. Residues of aminopyralid were 1.762-13.152 ppm and 0.784-17.150 ppm in/on grass hay harvested 13-15 and 20-22 days, respectively, following treatment.

Residue decline data show that aminopyralid residues generally decrease in/on grass forage and hay with increasing sampling intervals, with the maximum residues occurring at the 0-day sampling interval. In the two Canadian decline trials, aminopyralid residues declined in/on forage from 10.155-14.033 ppm at the 0-day PHI to 1.668-2.115 ppm at the 28-day PHI. Residues declined in/on hay from 13.113-29.220 ppm at the 0-day PHI to 2.472-4.322 ppm at the 28-day PHI. In the two U.S. decline trials, aminopyralid residues declined in/on forage from 3.516-8.425 ppm at the 0-day PHI to 0.936-3.734 ppm at the 28-day PHI. Residues declined in/on hay from 9.407-28.622 ppm at the 0-day PHI to 1.662-6.328 ppm at the 28-day PHI.

A total of 20 grass field trials were conducted in Canada and the U.S. during the 2002 growing season. Seven Canadian grass field trials were conducted in Regions 7 (AB and SK; 2 trials) and 14 (AB, MB, and SK; 5 trials). Thirteen U.S. grass field trials were conducted in Regions 1 (NY and PA; 2 trials), 2 (GA and VA; 2 trials), 4 (LA; 1 trial), 5 (ND and OH; 2 trials), 5A (WI; 1 trial), 7 (MT; 1 trial), 8 (TX; 1 trial), 9 (MT; 1 trial), and 11 (ID and WA; 2 trials). The

number and locations of field trials are in accordance with OPPTS Guideline 860.1500 and PMRA Directive 98-02, Section 9.

For U.S. trials, OPPTS 860.1500 (Tables 2 and 5) specifies that four trials should be conducted for each of three grass cultivars (Bermuda grass, bluegrass, and bromegrass or fescue) for a total of 12 trials, in all areas across the country. The U.S. crop field trials were conducted using Bermuda grass (2 trials), bromegrass or fescue (6 trials), crested wheatgrass (1 trial), orchard grass (2 trials), reed canarygrass (1 trial), and a grass mixture (1 trial). Although the grass cultivars used by the petitioner did not exactly match the requirements of OPPTS 860.1500 (more trials were conducted with cool season grasses than warm season grasses), HED concludes that the grass cultivars used are sufficient to represent grasses grown for pasture and rangeland in the U.S. and Canada.

TABLE C.1. Summary of Concurrent Recoveries of Aminopyralid from Grass Forage and Hay.

Matrix	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Grass forage	0.01	27	71, 73, 75, 78, 80, 83, 85, 86, 87, 87, 88, 88, 89, 89, 90, 90, 91, 92, 92, 93, 95, 97, 99, 100, 103, 104, 109	89 ± 9
	0.10	7	84, 85, 90, 92, 94, 95, 102	
	20.0	4	71, 95, 97, 104	
	30.0	2	76, 82	
	60.0	2	81, 89	
Grass hay	0.01	21	62, 70, 71, 75, 74, 75, 80, 82, 88, 88, 90, 92, 92, 93, 94, 95, 98, 101, 103, 106, 115	89 ± 13
	0.02	1	88	
	0.10	8	65, 82, 89, 93, 99, 102, 102, 103	
	1.0	1	97	
	20.0	1	100	
	30.0	3	82, 82, 102	
	60.0	2	71, 97	

TABLE C.2. Summary of Storage Conditions.

Matrix	Storage Temp. (°C)	Actual Storage Duration	Interval of Demonstrated Storage Stability [1]
Grass forage	~-20	≤454 days (14.9 months)	Interim storage stability data indicate that residues of aminopyralid are stable under frozen conditions in/on fortified samples of grass forage and hay for up to 187 days (6.2 months).
Grass hay	"	≤441 days (14.5 months)	

[1] Refer to the DER for MRID 46235719.

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TABLE C.3. Residue Data from Crop Field Trials with Aminopyralid.

Trial Location: City, State/Province; Year (Study ID)	Region	Crop; Variety	Commodity or Matrix	Treat. Regime [1]	Total Rate (lb ae/A) [g ae/ha]	PHI (days)	Aminopyralid Residues (ppm) [2]
Canadian Field Trials							
Vanscoy, SK; 2002 (AB-2)	7	Grass; Brome grass mix	forage	A	0.106 [119]	0	8.888, 9.029
						7	1.276, 1.317
						14	1.009, 1.324
			hay	A	0.106 [119]	0	24.753, 29.699
						14	2.362, 2.706
						22	2.252, 2.657
Gull Lake, AB; 2002 (AB-3)	14	Grass; Fescue brome mix	forage	A	0.106 [119]	0	10.183, 10.559 [3]
						7	3.707, 3.757
						14	1.730, 2.645
			hay	A	0.106 [119]	0	19.455, 20.707 [3]
						14	3.763, 5.408
						21	4.381, 4.706
Monarch, AB; 2002 (AB-4)	14	Grass; Orchard Fescue Timothy Mix	forage	A	0.108 [121]	0	7.452, 7.869
						7	3.888, 4.148 [3]
						13	2.330, 2.729
			hay	A	0.108 [121]	0	12.909, 14.044
						13	3.849, 3.957 [3]
						21	4.371, 6.243
Lacombe, AB; 2002 (AB-D) (decline study)	14	Grass; Brome Fescue mix	forage	A	0.105 [118]	0	10.155, 14.033
						3	4.366 [3], 6.120
						7	3.806, 4.301
						14	1.742, 2.140
						21	1.631, 2.110
						28	2.055, 2.115
			hay	A	0.105 [118]	0	13.113, 28.555 [3]
						14	1.970, 3.330
						21	2.650, 3.253
						28	3.511 [3], 3.722

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TABLE C.3. Residue Data from Crop Field Trials with Aminopyralid.

Trial Location: City, State/Province; Year (Study ID)	Region	Crop; Variety	Commodity or Matrix	Treat. Regime [1]	Total Rate (lb ae/A) [g ae/ha]	PHI (days)	Aminopyralid Residues (ppm) [2]
Lacombe, AB; 2002, (AB-D) (decline study)	14	Grass; Brome Fescue mix	forage	B	0.106 [119] + 1.269 [1423]	0	11.006, 12.295
						3	6.063, 7.689
						7	5.694, 6.108
						14	2.080, 2.488
						21	1.214, 1.910
						28	1.962, 2.046
			hay	B	0.106 [119] + 1.269 [1423]	0	20.304, 29.220
						14	2.616, 3.965
						21	2.119, 2.502
						28	4.171, 4.322
Boissevain, MB; 2002 (MB-1)	14	Grass; Brome grass	forage	A	0.108 [121]	0	10.232, 11.160
						7	2.575, 3.513
						14	1.756, 2.293
			hay	A	0.108 [121]	0	46.838, 51.496
						14	5.983, 7.161
						20	3.784, 4.705
Rosthern, SK; 2002 (SK-2)	14	Grass; Native grasses	forage	A	0.107 [120]	0	9.828, 11.675
						8	3.284, 3.294
						14	3.076, 3.326
			hay	A	0.107 [120]	0	23.006, 30.641
						14	8.898, 9.531
						21	7.205, 8.708
Taber, AB; 2002 (SK-D) (decline study)	7	Grass; Native grasses	forage	A	0.103 [116]	0	11.935, 13.729
						3	6.544, 6.836
						7	3.413, 3.735 [3]
						14	1.831, 2.468
						22	2.331, 2.517
						28	1.668, 1.994
			hay	A	0.103 [116]	0	15.998, 17.160
						14	1.837, 3.100
						22	3.371, 3.561
						28	2.472, 3.203

TABLE C.3. Residue Data from Crop Field Trials with Aminopyralid.

Trial Location: City, State/Province; Year (Study ID)	Region	Crop; Variety	Commodity or Matrix	Treat. Regime [1]	Total Rate (lb ae/A) [g ae/ha]	PHI (days)	Aminopyralid Residues (ppm) [2]
Taber, AB; 2002 (SK-D) (decline study)	7	Grass; Native grasses	forage	B	0.112 [125] + 1.336 [1498]	0	11.371, 13.018
						3	5.909, 6.720
						7	4.410, 4.654
						14	1.436, 1.837
						22	2.001, 2.045
						28	1.718, 1.840
			hay	B	0.112 [125] + 1.336 [1498]	0	19.583, 23.014
						14	2.289, 2.395
						22	2.526, 2.576
						28	2.297, 2.920
U.S. Field Trials							
Athens, GA; 2002 (GA)	2	Grass; Bermuda	forage	A	0.107 [120]	0	10.266, 10.810
						7	4.833 [3], 5.135
						14	0.535, 2.852
			hay	A	0.107 [120]	0	25.551, 28.185
						14	8.609 [3], 12.071 [3]
						21	3.427, 6.556
American Falls, ID; 2002 (ID)	11	Grass; Mixture	forage	A	0.107 [120]	0	4.641, 7.025
						7	0.663, 0.704
						14	0.752, 0.873
			hay	A	0.107 [120]	0	17.902, 19.950
						14	2.162, 2.501
						20	2.042, 2.378
Washington, LA; 2002 (MS)	4	Grass; Bermuda	forage	A	0.112 [125]	0	7.753, 9.065
						7	0.232, 3.499
						14	2.344, 2.717
			hay	A	0.112 [125]	0	18.098, 20.584
						14	5.219, 7.368
						21	3.153, 3.311
Fort Benton, MT; 2002 (MT-1)	7	Grass; Crested wheat	forage	A	0.109 [122]	0	9.156, 9.474
						7	5.072, 6.514
						14	1.818, 2.142
			hay	A	0.109 [122]	0	14.548 [4]
						14	4.158, 4.230
						21	3.651, 3.672

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TABLE C.3. Residue Data from Crop Field Trials with Aminopyralid.

Trial Location: City, State/Province; Year (Study ID)	Region	Crop; Variety	Commodity or Matrix	Treat. Regime [1]	Total Rate (lb ae/A) [g ae/ha]	PHI (days)	Aminopyralid Residues (ppm) [2]
Ennis, MT; 2002 (MT-2)	9	Grass; Orchard grass	forage	A	0.108 [121]	0	7.798, 7.940
						6	1.433, 1.441
						13	1.176, 1.208
			hay	A	0.108 [121]	0	16.326 [4]
						13	1.978, 2.230
						20	1.152, 1.296
Northwood, ND; 2002 (ND)	5	Grass; Brome grass	forage	A	0.107 [120]	0	9.334, 9.576
						7	3.345, 3.890 [3]
						14	0.822, 1.541
			hay	A	0.107 [120]	0	15.494, 17.647
						14	2.781, 3.123 [3]
						22	0.784, 0.826
North Rose, NY; 2002 (NY)	1	Grass; Orchard grass	forage	A	0.103 [115]	0	3.793, 3.983 [3]
						7	2.255, 2.810 [3]
						14	2.246, 2.295
			hay	A	0.103 [115]	0	9.782 [3], 10.175
						14	8.377, 11.262 [3]
						21	4.473, 8.747
New Holland, OH; 2002 (OH)	5	Grass; Fescue/ Orchard grass	forage	A	0.110 [123]	0	4.093, 4.328
						7	2.079, 2.108
						15	1.652, 1.736
			hay	A	0.110 [123]	0	12.490, 16.883
						15	7.789, 9.782
						21	4.389, 5.157
Germansville, PA; 2002 (PA)	1	Grass; Tall Fescue	forage	A	0.112 [125]	0	8.418, 8.590
						7	0.801, 2.466
						14	2.040, 2.378
			hay	A	0.112 [125]	0	28.924, 29.962
						14	5.029, 5.086
						21	4.923, 5.217
Claude, TX; 2002 (TX)	8	Grass; Tall Fescue	forage	A	0.108 [121]	0	12.051, 15.551 [3]
						7	1.367, 1.452 [3]
						14	1.674, 2.052
			hay	A	0.108 [121]	0	17.184, 21.668 [3]
						14	1.762 [3], 2.243
						21	3.563, 3.954

TABLE C.3. Residue Data from Crop Field Trials with Aminopyralid.

Trial Location: City, State/Province; Year (Study ID)	Region	Crop; Variety	Commodity or Matrix	Treat. Regime [1]	Total Rate (lb ae/A) [g ae/ha]	PHI (days)	Aminopyralid Residues (ppm) [2]
Suffolk, VA; 2002 (VA-D) (decline study)	2	Grass; Fescue/ Orchard grass	forage	A	0.107 [120]	0	3.516, 5.025 [3]
						3	4.115, 5.074 [3]
						7	3.153, 3.597
						14	2.240, 3.069
						21	1.433, 2.029
						28	1.851, 2.920 [3]
			hay	A	0.107 [120]	0	16.070, 19.664 [3]
						14	7.654, 8.589 [3]
						21	6.415, 8.299
						28	5.682, 6.328
Suffolk, VA; 2002 (VA-D) (decline study)	2	Grass; Fescue/ Orchard grass	forage	B	0.108 [121] + 1.291 [1447]	0	7.000, 8.425
						3	5.145, 6.946
						7	3.885, 4.564
						14	2.918, 8.009
						21	2.720, 3.774
						28	3.423, 3.734
			hay	B	0.108 [121] + 1.291 [1447]	0	23.312, 28.622
						14	7.994, 13.152 [3]
						21	14.096, 17.150
						28	3.026, 6.109
Moses Lake, WA; 2002 (WA-D) (decline study)	11	Grass; Fescue	forage	A	0.107 [120]	0	4.064, 4.494
						3	1.090, 1.263
						7	1.241, 1.258
						14	1.065, 1.244
						21	0.920, 1.376 [3]
						28	0.936, 0.992
			hay	A	0.107 [120]	0	10.132, 10.815
						14	2.638, 3.141
						21	2.189, 2.462 [3]
						28	1.662, 2.571

TABLE C.3. Residue Data from Crop Field Trials with Aminopyralid.

Trial Location: City, State/Province; Year (Study ID)	Region	Crop; Variety	Commodity or Matrix	Treat. Regime [1]	Total Rate (lb ae/A) [g ae/ha]	PHI (days)	Aminopyralid Residues (ppm) [2]
Moses Lake, WA; 2002 (WA-D) (decline study)	11	Grass; Fescue	forage	B	0.107 [120] + 1.295 [1452]	0	5.264, 5.500
						3	1.320, 1.382
						7	1.756, 1.974
						14	1.528, 1.755
						21	1.058, 1.304
						28	0.842, 1.002
			hay	B	0.107 [120] + 1.295 [1452]	0	9.407, 15.026
						14	3.235, 3.953
						21	2.834, 2.937
						28	1.949, 2.293
Arkansaw, WI; 2002 (WI)	5A	Grass; Reed Canary grass	forage	A	0.108 [121]	0	5.451, 5.549
						7	2.787, 3.240
						14	1.429, 1.815
			hay	A	0.108 [121]	0	15.790, 21.343
						14	7.904, 8.710
						21	4.870, 4.964

[1] Treatment Regimes:
A = single broadcast foliar application of the SC/L formulation of aminopyralid TIPA salt.
B = single broadcast foliar application of a tank mix of the SC/L formulation of aminopyralid TIPA salt and the SC/L formulation of 2,4-D.
[2] The petitioner presented both uncorrected residue values and residue values corrected for concurrent method recovery. The uncorrected values are reported here.
[3] Multiple (duplicate or quadruple) analyses of a single sample; highest residue is reported.
[4] The petitioner indicated that the treated and control samples were apparently switched; residues for the control sample are reported.

TABLE C.4. Summary of Residue Data from Crop Field Trials with Aminopyralid.

Commodity	Total Applic. Rate (lb ae/A) [g ae/ha]	PHI (days)	Residue Levels (ppm)						
			n	Min.	Max.	HAFT[1]	Median (STMdR[2])	Mean (STMR[3])	Std. Dev.
Canadian Trials									
Grass forage	0.103-0.112 [116-125]	0	18	7.452	14.033	12.832	10.783	10.801	1.838
		7-8	18	1.276	6.108	5.901	3.746	3.716	1.214
		13-14	18	1.009	3.326	3.201	2.110	2.124	0.608
Grass hay	0.103-0.112 [116-125]	0	18	12.909	51.496	49.167	21.857	24.472	10.619
		13-14	18	1.837	9.531	9.215	3.547	4.173	2.325
		20-22	18	2.119	8.708	7.957	3.466	3.976	1.825

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TABLE C.4. Summary of Residue Data from Crop Field Trials with Aminopyralid.

Commodity	Total Applic. Rate (lb ae/A) [g ae/ha]	PHI (days)	Residue Levels (ppm)						
			n	Min.	Max.	HAFT[1]	Median (STMdR[2])	Mean (STMR[3])	Std. Dev.
U.S. Trials									
Grass forage	0.103-0.112 [115-125]	0	30	3.516	15.551	13.801	7.389	7.264	2.869
		6-7	30	0.232	6.514	5.793	2.361	2.652	1.556
		13-15	30	0.535	8.009	5.464	1.785	1.998	1.315
Grass hay	0.103-0.112 [115-125]	0	28	9.407	29.962	29.443	17.416	18.269	6.046
		13-15	30	1.762	13.152	10.573	5.148	5.830	3.317
		20-22	30	0.784	17.150	15.623	3.662	4.630	3.593

[1] HAFT = Highest Average Field Trial.
[2] STMdR = Supervised Trial Median Residue.
[3] STMR = Supervised Trial Mean Residue.

D. CONCLUSION

Aminopyralid residue trials conducted on a variety of grasses in the U.S. and Canada indicate that residues of aminopyralid are fairly consistent across geographic region and grass variety. Generally, residues decline with increasing PHI, with a rapid decline noted in samples immediately following the 0-Day samples (i.e., the 7- or 14-Day samples, depending on the study location). In the few cases where residues increase at a longer PHI, there is no trend for this and the apparent increase is probably insignificant, since it falls within the variability of the PHI-specific residue data.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: MADoherty, 06/28/05; TSheramata, 06/13/05; HBietlot, 6/13/05
Petition Number(s): PP#4F6827
DP Barcode(s): D305665
PC Code: 005100/005209

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DACO 7.5.1/OPPTS 860.1480/OECD IIA 6.4.1, 6.4.2 and IIIA 8.2, 8.4.1, 8.4.2
Livestock Feeding Study - Ruminant

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In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 11/08/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46235723 Rosser, S.; Rutherford, L.; McFarlane, J. (2004) Magnitude of XDE-750 Residues in Bovine Tissues and Milk from a 28-Day Feeding Study. Project Number: 030061, 208/001/10. Unpublished study prepared by Dow AgroSciences LLC and Genesis Midwest Laboratories. 259 p.

EXECUTIVE SUMMARY:

Dow AgroSciences has submitted a dairy cattle feeding study with aminopyralid. Holstein dairy cattle were orally dosed once per day with aminopyralid via gelatin capsules at levels equivalent to concentrations of 32.8, 64.5 181.5, or 644.7 ppm in the cow's diet. Cattle were dosed once per day after the morning milking for 28 consecutive days. The 32.8-, 64.5-, and 181.5-ppm dosing groups each consisted of three cows. The highest dosing group consisted of a total of nine cows; the six additional cows were used to evaluate depuration of residues following completion of dosing. Cows were milked twice daily, and samples were composited daily for each cow. Milk samples were collected for analysis the first 7 days of dosing and every third day thereafter until the end of the dosing period; subsamples of milk collected on study days 13 and 28 were separated into cream and skim milk. All cows in the 32.8-, 64.5-, and 181.5-ppm dosing groups and three cows in the 644.7-ppm dosing group were sacrificed within 24 hours of completion of the dosing period, and samples of muscle, liver, kidney and fat were collected. For the depuration study, samples of milk were collected following 0, 1, 2, 3, 6, and 13 days of withdrawal. At each of three withdrawal intervals of 3, 7, and 14 days, two cows were sacrificed, and tissue samples were collected.

Samples of milk and tissues were analyzed for residues of aminopyralid using LC/MS/MS, Method GRM 03.18. The validated method limit of quantitation (LOQ) was 0.01 ppm and the calculated limit of detection (LOD) was 0.003 ppm for all matrices. This method is adequate for data collection based on acceptable concurrent method recovery data. No storage stability data are required because all samples from the ruminant feeding study were stored frozen from collection to analysis and were analyzed within 30 days of collection. The maximum residues of aminopyralid found in milk and cow tissues are listed in the table below. Residues of aminopyralid were generally found to have a linear relationship with the dosing level in milk, kidney, and liver. Residues appeared to plateau in milk by the second dosing day and do not appear to preferentially partition into cream.

Matrix	Maximum Residue Levels (ppm) of Aminopyralid by Feeding Level			
	32.8 ppm	64.5 ppm	181.5 ppm	644.7 ppm
Whole milk	<0.01	0.024	0.030	0.152
Cream	--	0.012	--	0.065
Skim milk	--	0.015	--	0.074
Fat	0.011	0.013	0.095	0.042
Kidney	0.102	0.202	1.580	2.549
Liver	<0.01	0.014	0.054	0.117
Muscle	<0.01	<0.01	0.046	0.029

The study results indicate that there is the potential for transfer of aminopyralid residues to livestock, most notably to the kidneys.

The results of the depuration study indicate that aminopyralid residues in cow milk and tissues decline rapidly following withdrawal from dosing. Residues in milk declined to below the method LOQ (<0.01 ppm) following one day of withdrawal; residues were nondetectable in all milk samples from subsequent withdrawal intervals. Residues in tissues declined to below the method LOQ (<0.01 ppm) following 3 days withdrawal; one fat sample bore residues at the LOQ (0.01 ppm) at the 3-day withdrawal period. Residues were nondetectable in all fat, kidney, liver, and muscle samples from subsequent withdrawal intervals.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the livestock feeding study residue data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D305665, and in Canada's Regulatory Decision Document.

COMPLIANCE:

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

A. BACKGROUND INFORMATION

Aminopyralid (4-amino-3,6-dichloropyridine-2-carboxylic acid) is the proposed common name of XDE-750, a new active ingredient developed by Dow AgroSciences. Aminopyralid is a systemic postemergence herbicide which belongs to the pyridine carboxylic acid class of herbicides. The petitioner is currently proposing food/feed uses on grasses grown in rangelands and permanent pastures and on wheat for the selective control of invasive and noxious broadleaf weeds. It is also proposed for weed control in sites such as parks, electric utility rights-of way, forestry, woodlands, and wildlife openings, with smaller amounts used in railroads, utility substations, pipelines, and pumping stations.

The proposed end-use product (EP) is a soluble concentrate liquid (SC/L) referred to by the trade name GF-871 (EPA Reg. No. 62719-LRI). The active ingredient in GF-871 is formulated as the triisopropanolammonium (TIPA) salt, with the product containing 40.6% of aminopyralid TIPA salt at an acid equivalent (ae) of 21.1% or 2 lb ae/gal (240 g ae/L). The petitioner stated that the aminopyralid TIPA salt dissociates rapidly in water to the acid (aminopyralid) at environmental pH values greater than 2.56 (the pKa).

Currently, the 2 lb ae/gal (240 g ae/L) SC/L formulation is proposed for broadcast foliar application at maximum rates of 0.11 lb ae/A (120 g ae/hectare) on rangeland and permanent pastures and 0.009 lb ae/A (10 g ae/ha) on wheat. The proposed PHIs are 0 days for wheat hay and 50 days for wheat grain and straw. No PHI is listed or proposed for grasses or wheat forage.

TABLE A.1. Test Compound Nomenclature.

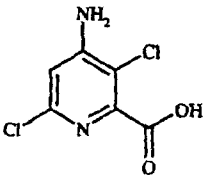
Chemical structure	
Common name	Aminopyralid
Company experimental name	XDE-750
IUPAC name	4-amino-3,6-dichloropyridine-2-carboxylic acid
CAS name	4-amino-3,6-dichloro-2-pyridinecarboxylic acid
CAS registry number	150114-71-9
End-use product (EP)	2 lb ae/gal (240 g ae/L) TIPA salt SC/L formulation (GF-871 Herbicide; EPA Reg. No. 62719-LRI; Aminopyralid Liquid Concentrate Herbicide)

TABLE A.2. Physicochemical Properties of the Aminopyralid Technical Grade Test Compound.

Parameter	Value	Reference
Melting point	163.5 °C	MRID 46235703, PMRA LS
pH	2.31 at 23.4 °C (1% solution in water)	MRID 46235703, PMRA LS
Relative density	1.72 at 20 °C	MRID 46235703, PMRA LS
Water solubility	2.48 g/L unbuffered water at 18 °C 212 g/L pH 5 buffer at 20 °C 205 g/L pH 7 buffer at 20 °C 203 g/L pH 9 Buffer at 20 °C	MRID 46235703, PMRA LS
Solvent solubility at 20 °C	methanol 52.2 g/L acetone 29.2 g/L n-octanol 3.9 g/L ethyl acetate 3.9 g/L 1,2-dichloroethane 0.2 g/L xylene 0.04 g/L heptane <10 µg/mL	MRID 46235703, PMRA LS
Vapor pressure	2.59×10^{-8} Pa at 25 °C; 9.52×10^{-9} Pa at 20 °C	MRID 46235703, PMRA LS
Dissociation constant, pK_a	2.56	MRID 46235703, PMRA LS
Octanol/water partition coefficient, $Log(K_{OW})$	0.201 unbuffered water at 19 °C; -1.76 at pH 5; -2.87 at pH 7; -2.96 at pH 9	MRID 46235703, PMRA LS
UV/visible absorption spectrum	Solution / Wavelength λ max, nm / Extinction coefficient ε, L/(mol*cm) Neutral 217 29100 Basic 220 26100 (pH 12.6) 245 10150 Acidic 217 22800 (pH 1.4) 270 9140	MRID 46235703, PMRA LS

B. EXPERIMENTAL DESIGN

The in-life phase of the feeding study was conducted at Genesis Midwest Laboratories (Neillsville, WI). Four groups of lactating dairy cows were dosed orally once per day for 28 consecutive days with gelatin capsules containing aminopyralid. Three of the groups consisted of three cows each and the fourth group, receiving the highest dose, consisted of nine cows; the six additional cows were used to evaluate residue depuration after the end of dosing. Based on the group dietary intake, the average dose levels were equivalent to 32.8, 64.5, 181.5, and 644.7 ppm in the diet. Four additional cows were dosed with empty gelatin capsules to serve as controls.

B.1. Livestock

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

Species	Breed	Age	Weight at study initiation (kg)	Health status	Description of housing/holding area
Dairy cow	Holstein	3-7 years	494-721	Cows were healthy throughout the study	Individual concrete stalls in a dairy barn; temperatures ranged ~18-23 °C during the study period.

TABLE B.1.2. Test Animal Dietary Regime.

Composition of Diet	Treatment Group	Average Feed consumption (kg/day) [1]	Water	Acclimation period	Predosing
Offered 8 kg of grain (commercial dairy ration) and 16 kg of alfalfa hay cubes daily, split in 2 feedings (am and pm); plus 2 kg of baled grass hay at the pm feeding.	T-I	17.7-19.6	*ad libitum*	14 days	None
	T-II	21.3-22.2			
	T-III	19.0-21.9			
	T-IV	20.2-22.1			

[1] Based on average daily feed consumption, reported for each week of the treatment period, of the individual cows in the group; feed consumption is expressed on a dry weight basis.

TABLE B.1.3. Dosing Regime.

Treatment group	Treatment Type	Level of administered dose (average mg/day)	Average residue intake in diet (ppm)	Vehicle	Timing/ Duration
T-I	Oral, via balling gun	618.4	32.8	Gelatin capsule	Once daily after a.m. milking for 28 consecutive days
T-II		1407.8	64.5		
T-III		3727.7	181.5		
T-IV		13654.1	644.7		

TABLE B.1.4. Sample Collection.

Milk collected	Treatment Group	Average milk production during treatment period	Average milk production during acclimation period	Urine, feces and cage wash collected	Interval from last dose to sacrifice (days)	Tissues harvested and analyzed
Milk collected twice daily	T-I	17.5-19.0 kg/day	18.2 kg/day	Not collected	within 24 hours	Liver, kidney, muscle (composite of flank, loin, and leg), and fat (composite of perinephric, abdominal, and subcutaneous)
	T-II	19.0-20.6 kg/day	20.2 kg/day			
	T-III	20.5-22.6 kg/day	19.4 kg/day			
	T-IV	20.0-21.3 kg/day	21.5 kg/day		1, 3, 7, and 14	

B.2. Sample Handling and Preparation

Whole milk samples collected the day before dosing, the first 7 days of dosing, and every third day thereafter until the end of the dosing period were reserved for residue analysis. In addition, milk samples were collected daily during the withdrawal period from cows in the depuration study. For each individual cow, the am and pm milk sample was pooled proportionally for a

daily whole milk sample. Aliquots of whole milk collected on dosing days 13 and 28, from the control and 64.5 ppm (T-II) and 644.7 ppm (T-IV) dose groups, were separated into cream and skim milk. Tissue samples collected from each cow were placed in plastic bags. All tissue, milk, cream, and skim milk samples were stored frozen (-17 °C) until shipment, 1-8 days after collection, to Dow AgroSciences (Indianapolis, IN). At Dow AgroSciences, frozen tissue samples were ground with liquid nitrogen and stored in HDPE containers. All samples were stored frozen (-20 °C) at the analytical laboratory until analysis.

B.3. Analytical Methodology

Milk and tissue samples from the cattle feeding study were analyzed for residues of aminopyralid using LC/MS/MS Method GRM 03.18, entitled "Determination of Residues of Aminopyralid in Bovine Tissues by Liquid Chromatography with Tandem Mass Spectrometry Detection." A discussion of the method was included with the study; for a complete description of the method refer to the Residue Analytical Method DER for ruminants (MRIDs 46235714 and 46235716).

Briefly, milk or ground tissue samples were extracted with methanol:sodium bicarbonate (20:1, v:v). The extract was cleaned up through an anion-exchange solid-phase extraction plate; residues were eluted with ethyl acetate:trifluoroacetic acid (99:1, v:v). The internal standard, $^{13}C_2{}^{15}N$-aminopyralid, was added to the eluate and residues reconstituted in acetonitrile:pyridine:butanol (22:2:1, v:v:v) were derivatized with butyl chloroformate to form the 1-butyl esters of aminopyralid. The derivatized solution was diluted with methanol:water:acetic acid (50:50:0.1; v:v:v) for LC/MS/MS analysis. LC/MS/MS analyses were conducted using electrospray ionization in the positive ion mode. The validated LOQ was 0.01 ppm for all matrices and the calculated LOD was 0.003 ppm.

C. RESULTS AND DISCUSSION

Concurrent method recovery data are presented in Table C.1. Samples of milk and tissues were analyzed for residues of aminopyralid using LC/MS/MS, Method GRM 03.18. The validated method LOQ was 0.01 ppm and the calculated LOD was 0.003 ppm for all matrices. This method is adequate for data collection based on acceptable concurrent method recovery data. Apparent residues of aminopyralid were below the method LOQ (<0.01 ppm) in/on all untreated samples of whole milk, skim milk, cream, fat, kidney, liver, and muscle; however, the petitioner noted that some control samples showed small interferences (≤LOD).

Sample storage conditions and intervals are summarized in Table C.2. The maximum storage intervals from sample collection to analysis were 26 days for milk, 21 days for skim milk and cream, and 7-19 days for tissue samples. No storage stability data are required because all samples from the ruminant feeding study were stored frozen from collection to analysis were analyzed within 30 days of collection.

The results of the feeding study are presented in Table C.3. and are summarized in Table C.4. Aminopyralid residues were less than the method LOQ (<0.01 ppm) in all milk samples from the

32.8 ppm dosing group. Residues ranged <0.01-0.024 ppm, 0.011-0.030 ppm, and 0.023-0.152 ppm in milk samples from the 64.5 ppm, 181.5 ppm, and 644.7 ppm dosing groups, respectively. A graph of aminopyralid residues in milk samples from all four dosing groups over the course of the study is presented in Figure C.1. Residues in milk appeared to plateau by the second dosing day. A graph of the linear regression of residues on feeding level in milk is presented in Figure C.2.1; the lowest dosing level was not included in the graph because all residues were below the LOQ. The graph indicates that residues in milk were relatively proportional to the dosing level.

Day-13 and Day-28 milk samples from the 64.5- and 644.7-ppm dosing groups were separated into skim milk and cream. Residues of aminopyralid were <0.01-0.015 ppm and 0.043-0.074 ppm in skim milk, and <0.01-0.012 ppm and 0.037-0.065 ppm in cream from the 64.5- and 644.7-ppm dosing groups, respectively. Residues of aminopyralid do not appear to preferentially partition into skim milk or cream.

Aminopyralid residues were less than the method LOQ (<0.01 ppm) in liver and muscle samples from the 32.8-ppm dosing group. Residues were <0.01-0.011 ppm in fat and 0.043-0.102 ppm in kidney from the 32.8-ppm dosing level.

In cows from the 64.5-ppm dosing group, residues of aminopyralid were <LOQ in muscle, <0.01-0.013 ppm in fat, <0.01-0.014 ppm in liver, and 0.099-0.202 ppm in kidney.

In cows from the 181.5-ppm dosing group, residues of aminopyralid were 0.010-0.046 ppm in muscle, 0.016-0.095 ppm in fat, 0.026-0.054 ppm in liver, and 0.507-1.580 ppm in kidney.

In cows from the 644.7-ppm dosing group, residues of aminopyralid were 0.012-0.029 ppm in muscle, 0.026-0.042 ppm in fat, 0.059-0.117 ppm in liver, and 0.902-2.569 ppm in kidney.

The relationship between the dose level and residues in muscle and fat was not evaluated by the petitioner because quantifiable residues were only observed consistently in these tissues at the two highest dosing levels. However, the petitioner concluded that variability of residues in muscle and fat were great enough that a linear relationship could not be established. The petitioner indicated that there were no treatment-related abnormalities.

Graphs of the linear regression of residues on feeding level in liver and kidney are presented in Figures C.2.2 and C.2.3; the lowest dosing level was not included in the graph for liver because all residues were below the LOQ. The graphs indicates that residues in liver and kidney were relatively proportional to the dosing level.

The results of the depuration study are presented in Table C.5; aminopyralid residues in cow milk and tissues from the high dosing level (644.7 ppm) declined rapidly following withdrawal from dosing. Residues in milk collected on Study Day 29 (0-day withdrawal) were 0.020-0.038 ppm and rapidly declined to below the method LOQ (<0.01 ppm) following one day withdrawal; residues were nondetectable in all milk samples from subsequent withdrawal intervals (2, 3, 6,

and 13 days). Residues in tissues collected on Study Day 29 (0-day withdrawal) were 0.012-0.029 ppm in muscle, 0.026-0.042 ppm in fat, 0.059-0.117 ppm in liver, and 0.902-2.549 ppm in kidney and rapidly declined to below the method LOQ (<0.01 ppm) following 3 days withdrawal; one cow fat sample bore residues at the LOQ (0.01 ppm) at the 3-day withdrawal period. Residues were nondetectable in all fat, kidney, liver, and muscle samples from subsequent withdrawal intervals (7 and 14 days). Depuration curves for aminopyralid residues in milk and tissues are presented in Figures C.3.1. through C.3.3.

TABLE C.1. Summary of Concurrent Recoveries of Aminopyralid from Dairy Cattle.

Matrix	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Milk	0.01	34	74, 76, 78, 78, 78, 78, 79, 79, 82, 82, 82, 83, 83, 83, 85, 86, 86, 88, 89, 90, 91, 93, 95, 95, 96, 96, 96, 97, 99, 101, 104, 106, 111, 116	89 ± 9
	0.1	15	83, 85, 87, 88, 91, 91, 92, 92, 94, 95, 95, 96, 97, 101, 103	
	1.0	8	77, 81, 83, 88, 90, 90, 90, 92	
Skim milk	0.01	4	80, 81, 87, 91	
	0.1	2	77, 89	
Cream	0.01	4	80, 82, 87, 90	
	0.1	2	82, 92	
Fat	0.01	8	70, 74, 77, 77, 81, 84, 86, 93	84 ± 8
	0.10	3	85, 88, 91	
	1.0	2	91, 92	
Kidney	0.01	6	81, 85, 93, 95, 99, 102	92 ± 7
	0.1	1	83	
	1.0	2	89, 89	
	3.0	2	92, 99	
Liver	0.01	4	73, 76, 86, 89	85 ± 8
	0.1	1	87	
	1.0	2	93, 94	
Muscle	0.01	6	68, 79, 79, 79, 79, 83	82 ± 7
	0.1	3	86, 88, 89	
	1.0	2	82, 92	

TABLE C.2. Summary of Storage Conditions.

Matrix	Storage Temp. (°C)	Actual Storage Duration	Interval of Demonstrated Storage Stability
Milk	-20	4-26 days	None required; samples were stored frozen and analyzed within 30 days of collection.
Skim milk		19-21 days	
Cream		19-21 days	
Fat		5-10 days	
Kidney		4-7 days	
Liver		5-19 days	
Muscle		5-10 days	

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

Feeding Level (ppm)	Matrix; Collection Time [1]	Aminopyralid Residues (ppm) [2]	
		Individual Cows	Group Average[3]
32.8 (T-I group)	Milk; Day 0	ND, ND, ND	(<0.003)
	Day 1	(0.006), (0.003), (0.005)	(0.005)
	Day 2	(0.006), (0.004), (0.006)	(0.005)
	Day 3	(0.003), (0.004), (0.005)	(0.004)
	Day 4	(0.004), (0.004), (0.004)	(0.004)
	Day 5	(0.004), (0.003), (0.004)	(0.004)
	Day 6	ND, (0.004), (0.006)	(<0.004)
	Day 7	ND, ND, (0.004)	(<0.003)
	Day 10	(0.004), (0.003), (0.004)	(0.004)
	Day 13	(0.003), (0.004), (0.003)	(0.003)
	Day 16	(0.003), (0.003), (0.004)	(0.003)
	Day 19	ND, (0.003), (0.004)	(<0.003)
	Day 22	ND, (0.004), (0.004)	(<0.004)
	Day 25	ND, ND, (0.003)	(<0.003)
	Day 28	ND, (0.003), (0.003)	(0.003)

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

Feeding Level (ppm)	Matrix; Collection Time [1]		Aminopyralid Residues (ppm) [2]	
			Individual Cows	Group Average[3]
64.5 (T-II group)	Milk;	Day 0	(0.007), (0.004), (0.005)	(0.005)
		Day 1	0.024, (0.007), (0.008)	0.013
		Day 2	(0.007), (0.009), (0.007)	(0.008)
		Day 3	(0.005), (0.009), (0.008)	(0.007)
		Day 4	(0.005), (0.008), (0.006)	(0.006)
		Day 5	(0.005), (0.008), (0.008)	(0.007)
		Day 6	(0.006), (0.008), (0.007)	(0.007)
		Day 7	(0.006) [4], (0.009), (0.009)	(0.008)
		Day 10	(0.005), 0.013, (0.008)	(0.009)
		Day 13	(0.005), 0.014, (0.007)	(0.009)
		Day 16	(0.005), (0.009), (0.009)	(0.008)
		Day 19	(0.004), (0.007), (0.007)	(0.006)
		Day 22	(0.003), (0.005), (0.007)	(0.005)
		Day 25	(0.004), (0.007), (0.005)	(0.005)
		Day 28	(0.004), (0.007), (0.008)	(0.006)
	Skim Milk;	Day 13	(0.005), 0.015, (0.008)	(0.009)
		Day 28	(0.004), (0.007), (0.007)	(0.006)
	Cream;	Day 13	(0.005), 0.012, (0.005)	(0.007)
		Day 28	(0.004), (0.005), (0.006)	(0.005)
181.5 (T-III group)	Milk;	Day 0	(0.005), ND, ND	(<0.004)
		Day 1	0.015, 0.011, 0.018	0.015
		Day 2	0.026, 0.021, 0.024	0.024
		Day 3	0.025, 0.021, 0.030	0.025
		Day 4	0.022, 0.016, 0.021	0.020
		Day 5	0.020, 0.018, 0.024	0.021
		Day 6	0.025 [4], 0.025, 0.026	0.025
		Day 7	0.028, 0.023, 0.026	0.026
		Day 10	0.021, 0.020, 0.025	0.022
		Day 13	0.018, 0.015, 0.021 [4]	0.018
		Day 16	0.020, 0.014, 0.018	0.017
		Day 19	0.015, 0.014, 0.015	0.015
		Day 22	0.018, 0.011, 0.016	0.015
		Day 25	0.016, 0.012, 0.016	0.015
		Day 28	0.015 [4], 0.017, 0.017	0.016

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

Feeding Level (ppm)	Matrix; Collection Time [1]		Aminopyralid Residues (ppm) [2]	
			Individual Cows	Group Average [3]
644.7 (T-IV group)	Milk;	Day 0	ND, ND, ND, ND, ND, ND, ND, ND, ND	ND
		Day 1	0.052, 0.040, 0.055, 0.023, 0.049, 0.092 [4], 0.037, 0.045, 0.042	0.048
		Day 2	0.060, 0.068 [4], 0.083, 0.047, 0.073, 0.082, 0.048, 0.085, 0.083	0.070
		Day 3	0.062, 0.070, 0.081, 0.042, 0.081, 0.152, 0.053, 0.116, 0.096	0.084
		Day 4	0.058, 0.069, 0.072 [4], 0.048, 0.073, 0.127, 0.047, 0.087, 0.097	0.075
		Day 5	0.059, 0.060, 0.084, 0.049, 0.082, 0.125, 0.051, 0.087 [4], 0.092	0.077
		Day 6	0.067, 0.069, 0.092, 0.064, 0.079, 0.127, 0.054, 0.101, 0.110	0.085
		Day 7	0.061, 0.064, 0.101, 0.058, 0.077, 0.130, 0.051, 0.096, 0.096	0.082
		Day 10	0.049, 0.067, 0.080, 0.056, 0.059 [4], 0.115, 0.047, 0.069, 0.075	0.069
		Day 13	0.056, 0.052, 0.062, 0.052, 0.057, 0.107, 0.043, 0.072, 0.069	0.063
		Day 16	0.037, 0.058 [4], 0.068, 0.047, 0.055, 0.094, 0.035, 0.062, 0.064	0.058
		Day 19	0.050, 0.051, 0.063, 0.049, 0.055, 0.107 [4], 0.041, 0.050, 0.063	0.059
		Day 22	0.052 [4], 0.063, 0.068, 0.045, 0.044, 0.094, 0.043, 0.059, 0.067	0.059
		Day 25	0.044, 0.055, 0.063, 0.039, 0.059 [4], 0.102, 0.035, 0.062, 0.078	0.060
		Day 28	0.046, 0.052, 0.077, 0.036, 0.080, 0.122, 0.036, 0.059, 0.056	0.063
	Skim Milk;	Day 13	0.058, 0.054 [4], 0.068	0.060
		Day 28	0.043, 0.048 [4], 0.074	0.055
	Cream;	Day 13	0.053 [4], 0.046, 0.059	0.053
		Day 28	0.037 [4], 0.043, 0.065	0.048
32.8 (T-I group)	Fat;	Sacrifice	ND, (0.004), 0.011	(<0.006)
	Kidney;	Sacrifice	0.043, 0.052, 0.102	0.066
	Liver;	Sacrifice	ND, (0.004), (0.007)	(<0.005)
	Muscle;	Sacrifice	ND, ND, ND	(<0.003)
64.5 (T-II group)	Fat;	Sacrifice	0.013, (0.004), (0.005)	(0.007)
	Kidney;	Sacrifice	0.099, 0.139, 0.202	0.147
	Liver;	Sacrifice	(0.007), (0.007), 0.014	(0.009)
	Muscle;	Sacrifice	(0.004), (0.003), (0.004) [4]	(0.004)
181.5 (T-III group)	Fat;	Sacrifice	0.095, 0.073, 0.016	0.061
	Kidney;	Sacrifice	0.507, 1.580 [4], 0.514 [4]	0.867
	Liver;	Sacrifice	0.054, 0.033, 0.026	0.038
	Muscle;	Sacrifice	0.046, 0.017, 0.010	0.024

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

Feeding Level (ppm)	Matrix; Collection Time [1]	Aminopyralid Residues (ppm) [2]	
		Individual Cows	Group Average[3]
644.7 (T-IV group)	Fat; Sacrifice (Day 29)	0.039, 0.026 [4], 0.042	0.036
	Kidney; Sacrifice (Day 29)	0.902, 1.247 [4], 2.569 [4]	1.573
	Liver; Sacrifice (Day 29)	0.059, 0.064, 0.117 [4]	0.080
	Muscle; Sacrifice (Day 29)	0.012, 0.021, 0.029	0.021

[1] Cows were sacrificed on Day 29 (within 24 hours after the last dose), except for six cows from the T-IV group, which were used to demonstrate residue depuration (see Table C.5).
[2] The petitioner presented both uncorrected residue values and residue values corrected for concurrent method recovery. The uncorrected values are reported here. Residues below the method LOD (<0.003 ppm) are reported as ND (nondetectable) and residues above the LOD but below the method LOQ (<0.01 ppm) are reported in parentheses. Residues are listed respectively for the individual cows (cow number 1, 2, 3, etc.) within a group.
[3] Group averages were calculated by the study reviewer using the actual values reported and the LOD (0.003) for ND residues.
[4] The highest residue of duplicate analyses is reported.

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

Matrix	Feeding Level (ppm)	Residue Levels (ppm) [1]					
		n	Min.	Max.	Median (STMdR[2])	Mean (STMR[3])	Std. Dev.
Milk	32.8	42	<0.01	<0.01	0.005	0.004	0.001
	64.5	42	<0.01	0.024	0.005	0.006	0.003
	181.5	42	0.011	0.030	0.019	0.020	0.005
	644.7	126	0.023	0.152	0.062	0.068	0.024
Milk, skim	64.5	6	<0.01	0.015	0.005	0.007	0.004
	644.7	6	0.043	0.074	0.056	0.058	0.012
Milk, cream	64.5	6	<0.01	0.012	0.005	0.006	0.003
	644.7	6	0.037	0.065	0.050	0.051	0.010
Fat	32.8	3	<0.01	0.011	0.005	0.006	0.005
	64.5	3	<0.01	0.013	0.005	0.008	0.005
	181.5	3	0.016	0.095	0.073	0.061	0.041
	644.7	3	0.026	0.042	0.039	0.036	0.009
Kidney	32.8	3	0.043	0.102	0.052	0.066	0.032
	64.5	3	0.099	0.202	0.139	0.147	0.052
	181.5	3	0.507	1.580	0.514	0.867	0.617
	644.7	3	0.902	2.569	1.247	1.573	0.880
Liver	32.8	3	<0.01	<0.01	0.005	0.004	0.002
	64.5	3	<0.01	0.014	0.005	0.008	0.005
	181.5	3	0.026	0.054	0.033	0.038	0.015
	644.7	3	0.059	0.117	0.064	0.080	0.032

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

Matrix	Feeding Level (ppm)	Residue Levels (ppm) [1]					
		n	Min.	Max.	Median (STMdR[2])	Mean (STMR[3])	Std. Dev.
Muscle	32.8	3	<0.01	<0.01	0.0015	0.0015	0.0
	64.5	3	<0.01	<0.01	0.005	0.005	0.0
	181.5	3	0.010	0.046	0.017	0.024	0.019
	644.7	3	0.012	0.029	0.021	0.021	0.009

[1] For the calculation/reporting of minimum and maximum values, the LOQ value (0.01 ppm) was used for residues reported as ND or <LOQ in Table C.3. For calculation of the median, mean, and standard deviation, ½ the LOQ (0.005 ppm) was used for residues reported between the LOQ and LOD (0.003 ppm) and ½ the LOD (0.0015 ppm) was used for residues reported as ND.
[2] STMdR = Supervised Trial Median Residue.
[3] STMR = Supervised Trial Mean Residue.

FIGURE C.1. Aminopyralid Residues in Whole Milk as a Function of Time. Residues Are Average Values for Each Treatment Group.

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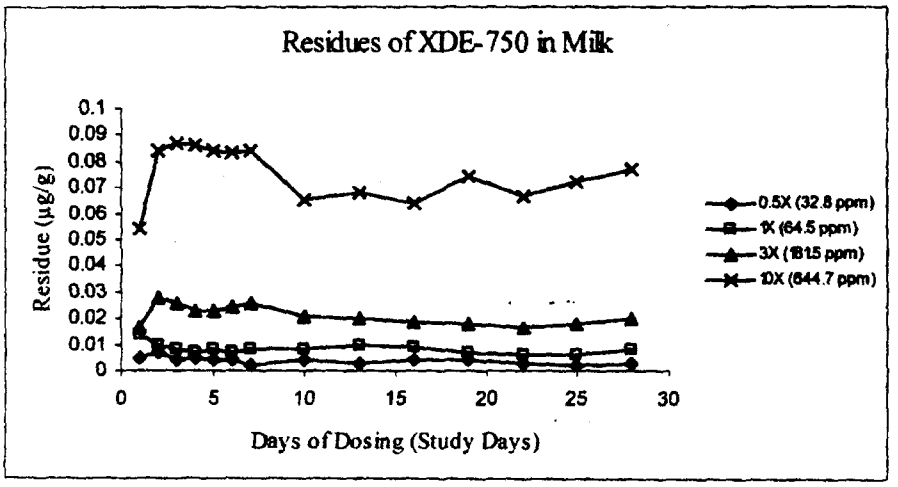

FIGURE C.2.1. Linear Regression of Residues in Milk on Feeding Level.

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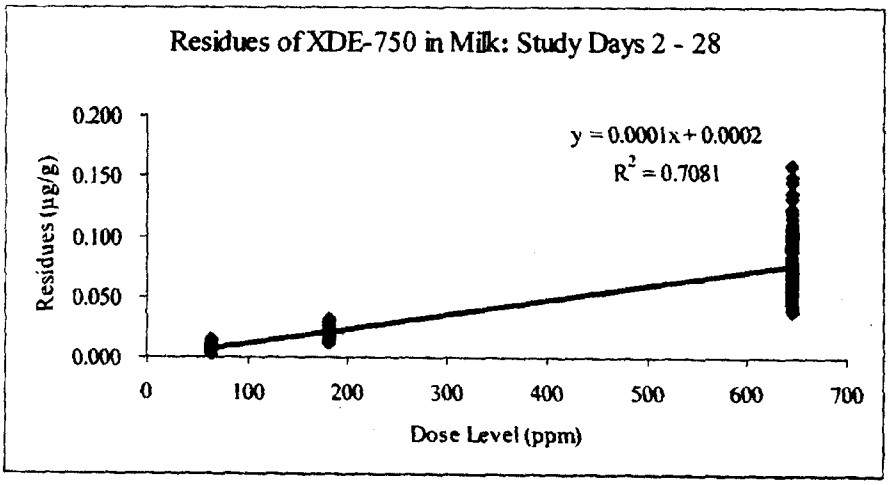

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FIGURE C.2.2. Linear Regression of Residues in Kidney on Feeding Level.
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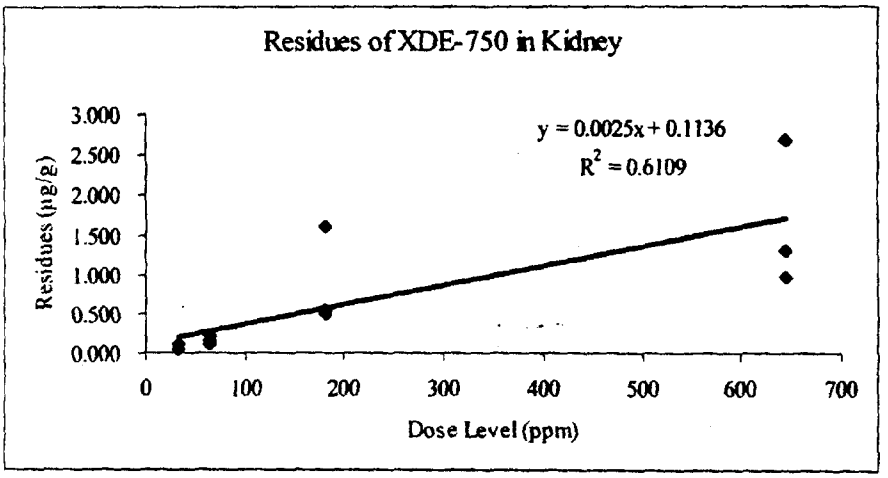

FIGURE C.2.3. Linear Regression of Residues in Liver on Feeding Level.
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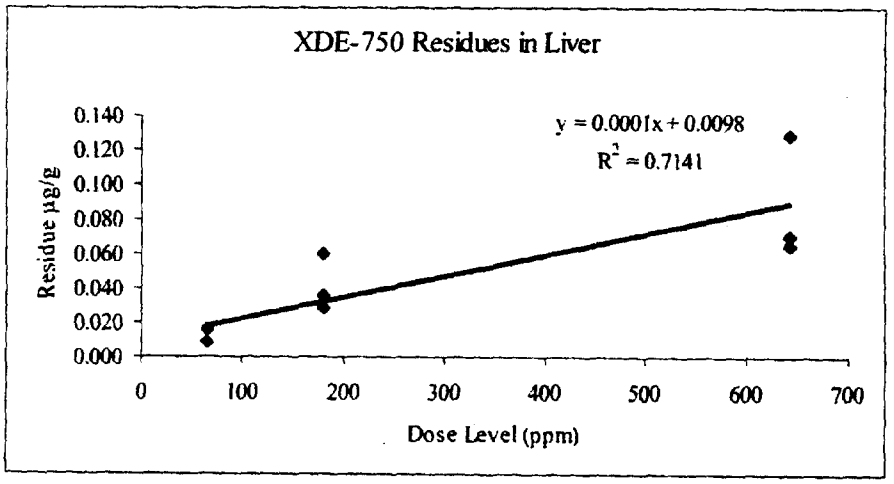

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TABLE C.5. Summary of Residues of Aminopyralid in Whole Milk and Tissues of Lactating Cows from the Depuration Study. [1]

Matrix	Study Day [2]	Residue (ppm) [3]					
		Cow #13	Cow #1	Cow #24	Cow #21	Cow #10	Cow #12
Milk	29 (0)	0.020	0.026	0.038	0.021	0.033	0.038
	30 (1)	(0.003)	ND	ND	ND	ND	(0.003)
	31 (2)	ND	ND	ND	ND	ND	ND
	32 (3)	--	--	ND	ND	ND	ND
	35 (6)	--	--	ND	ND	ND	ND
	42 (13)	--	--	--	--	ND	ND
Fat	32 (3)	(0.006)	0.010	--	--	--	--
	36 (7)	--	--	ND	ND	--	--
	43 (14)	--	--	--	--	ND	ND
Kidney	32 (3)	(0.003)	(0.004)	--	--	--	--
	36 (7)	--	--	ND	ND	--	--
	43 (14)	--	--	--	--	ND	ND
Liver	32 (3)	ND	ND	--	--	--	--
	36 (7)	--	--	ND	ND	--	--
	43 (14)	--	--	--	--	ND	ND
Muscle	32 (3)	ND	(0.004)	--	--	--	--
	36 (7)	--	--	(0.003)	ND	--	--
	43 (14)	--	--	--	--	ND	ND

[1] Cows were sacrificed on Day 29 (within 24 hours after the last dose). Two cows each from the T-IV group were sacrificed following 3, 7, and 14 days of dose withdrawal (Day 29 was considered day 0 withdrawal).

[2] Days of withdrawal are reported in parentheses.

[3] The petitioner presented both uncorrected residue values and residue values corrected for concurrent method recovery. The uncorrected values are reported here. Residues below the method LOD (<0.003 ppm) are reported as ND (nondetectable) and residues above the LOD but below the method LOQ (<0.01 ppm) are reported in parentheses.

FIGURE C.3.1. Depuration Curve for Residues of Aminopyralid in Whole Milk.
The figure below was copied without alteration from MRID 46235723.

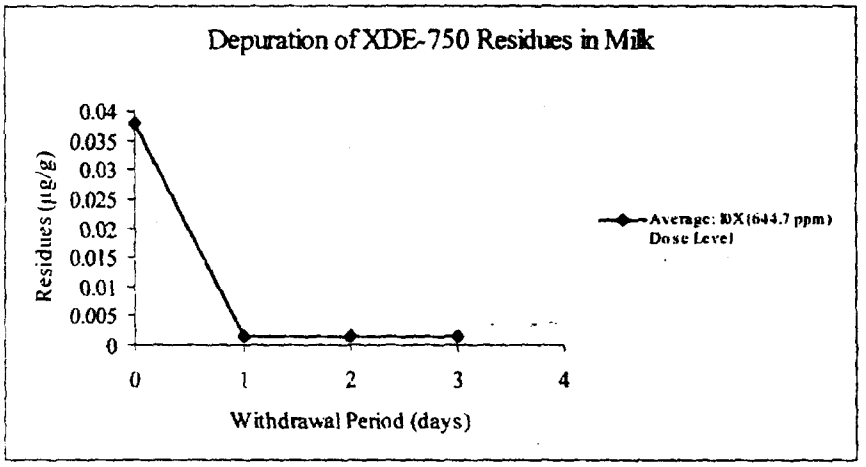


FIGURE C.3.2. Depuration Curve for Residues of Aminopyralid in Muscle, Fat, and Liver.
The figure below was copied without alteration from MRID 46235723.

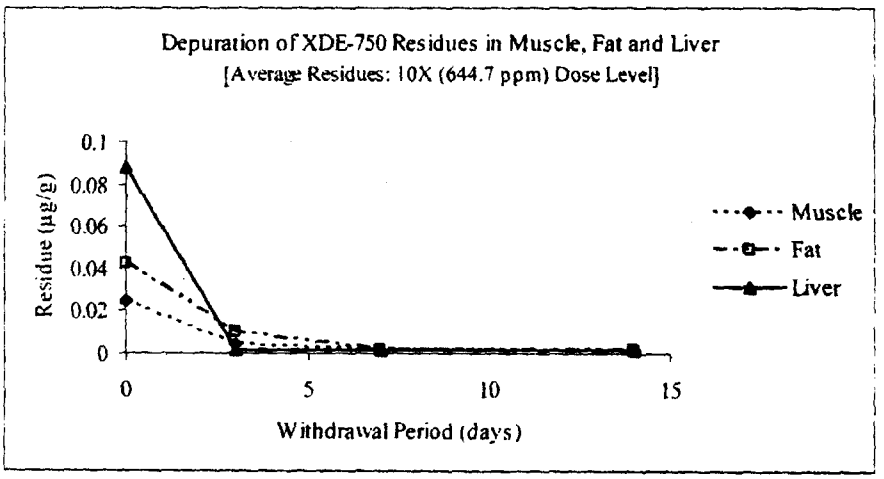
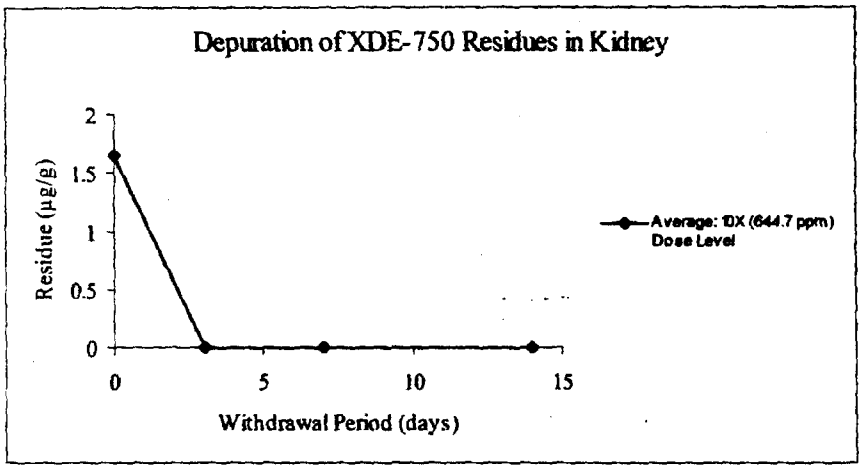


FIGURE C.3.3. Depuration Curve for Residues of Aminopyralid in Kidney.
The figure below was copied, without alteration, from MRID 46235723.



D. CONCLUSION

The submitted dairy cattle feeding study is adequate to demonstrate the magnitude of residues of aminopyralid in cattle commodities. The study indicates that there is the potential for transfer of aminopyralid residues to milk and cattle tissues, most notably to the kidneys. The feeding study reflects dietary levels of aminopyralid at 32.8, 64.5, 181.5, and 644.7 ppm. A proportional relationship between the dose level and aminopyralid residue levels in milk, kidney, and liver was demonstrated. The depuration study indicates that residues of aminopyralid generally decreased to below quantifiable levels with cessation of dosing.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: MADoherty, 06/28/05; TSheramata, 06/13/05; HBietlot, 6/13/05
Petition Number(s): PP#4F6827
DP Barcode(s): D305665
PC Code: 005100/005209

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Appendix 1. Livestock Dietary Burden Calculations

Calculation of Maximum Dietary Burdens of Aminopyralid to Livestock.				
Feedstuff	% Dry Matter [1]	% Diet [1]	Estimated Tolerance (ppm)	Dietary Contribution (ppm) [2]
Beef and Dairy Cattle				
Grass, forage	25	60	25	60
Wheat, aspirated grain fractions	85	20	0.2	0.050
TOTAL BURDEN	--	80 [3]	--	**60.05**
Poultry				
Wheat, grain	89	50	0.05	0.025
Wheat, milled byproducts (bran)	88	50	0.1	0.05
TOTAL BURDEN	--	100	--	**0.075**
Swine				
Wheat, grain	89	50	0.05	0.025
Wheat, milled byproducts (bran)	88	50	0.1	0.05
TOTAL BURDEN	--	100	--	**0.075**

[1] Table 1 (OPPTS Guideline 860.1000).

[2] Contribution = ([tolerance /% DM] x % diet) for beef and dairy cattle; contribution = (tolerance x % diet) for poultry and swine.

[3] The remainder of the diet will be composed of feedstuff derived from crops that do not have aminopyralid uses/tolerances proposed (e.g., peanut or cotton seed meal).

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Aminopyralid/XDE-750/PC Code 005100/Dow AgroSciences/62719
DACO 7.4.3/OPPTS 860.1850/OECD IIA 6.6.3. 6.8.7 and IIIA 8.6
Confined Accumulation in Rotational Crops - Lettuce, Sorghum, and Turnips

Primary Evaluator _____ Date: 6/25/05
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In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 11/08/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46235725 Magnussen, J. (2004) A Confined Rotational Crop Study with 14C XDE-750. Project Number: 030008. Unpublished study prepared by Dow AgroSciences LLC and Research for Hire. 142 p.

EXECUTIVE SUMMARY:

Dow AgroSciences has submitted a confined rotational crop study with [2,6-^{14}C]aminopyralid (specific activity 40 µCi/mg; 88,800 dpm/µg). The radiolabeled test substance was applied directly to sandy loam soil in lined wooden boxes at 0.009 lb ai/A (10 g ai/ha), and rotational lettuce, sorghum, and turnips were planted 90 and 120 days after treatment (DAT). The in-life phase of the study was conducted by Research For Hire (Porterville, CA), and the analytical phase of the study was conducted by Dow AgroSciences (Indianapolis, IN).

TRR accumulated at ≥0.01 ppm in 90- and 120-DAT early sorghum forage (0.027 ppm and 0.017 ppm, respectively), 90-day sorghum stover (0.027 ppm), and 120-DAT mature turnip tops (0.010 ppm); residues in all other rotational crop commodities ranged <0.001-0.007 ppm. TRR were generally found to decrease from the 90-day plantback interval (PBI) to the 120-day PBI.

Analysis of soil samples at planting of the rotational crops, indicated that ≤30% of the applied aminopyralid was present in the soil following the fallow periods. Between planting and harvest, aminopyralid residues in soil slowly declined.

Only 90-DAT sorghum early forage and stover, and 120-DAT sorghum early forage and turnip tops contained radioactivity ≥0.01 ppm and were extracted for metabolite characterization.

Extraction with acetonitrile (ACN)/water released the majority of the TRR (66-98% TRR). Additional radioactivity was released by ACN/acid reflux of sorghum stover (24% TRR). Nonextractable residues in 90-DAT sorghum early forage and stover, and 120-DAT sorghum early forage and turnip tops were 2.3-15.8% TRR (≤0.003 ppm). The extraction procedures extracted sufficient residues from rotational crop matrices from the 90- and 120-day PBIs. Because the petitioner normalized extraction results, reported accountabilities were 100%; extraction/hydrolysis recoveries prior to normalization were 80.2-114.5%.

Total identified residues ranged 17-44% TRR in sorghum early forage and stover and in turnip tops and consisted entirely of free aminopyralid; residue profiles were similar between the matrices. Free aminopyralid was the major residue identified in rotational crop matrices, at 44.2% TRR (0.012 ppm) and 26.9% TRR (0.005 ppm) in 90- and 120-DAT sorghum early forage, respectively, 18.1% TRR (0.005 ppm) in 90-DAT sorghum stover, and 17.2% TRR (0.002 ppm) in 120-DAT turnip tops. Two metabolite fractions were also characterized in each matrix. C-1 Fraction, which accounted for 5.4-20.5% TRR (0.001-0.006 ppm) was more polar than aminopyralid, and C-2 Fraction, which accounted for 23.1-67.9% TRR (0.006-0.010 ppm) was slightly less polar than aminopyralid. These metabolites were further characterized as base-labile conjugates of aminopyralid following base hydrolysis of aqueous-soluble residues, which demonstrated significant conversion of the metabolite fractions to aminopyralid. In conjunction with the results of the primary plant metabolism studies (see DERs for MRIDs 46235709 and 46235710), the petitioner indicated that these conjugates are believed to consist primarily of N-glucoside and glucose ester conjugates of aminopyralid.

The results of the confined rotational crop study indicate that residues of aminopyralid are metabolized in the same manner in rotated crops as in primary crops. The petitioner noted that initial uptake of residues resulted in aminopyralid-related residues >0.01 ppm in commodities used exclusively as animal feedstock items, but stated that based on the ruminant metabolism study (see DER for MRID 46235708), residue levels of this magnitude would not result in detectable residues in either meat or milk. Uptake of residues appeared to be more limited (≤0.01 ppm) in rotated crop commodities used for direct human consumption.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the confined rotational crop residue data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D305665 and in Canada's Regulatory Decision Document.

COMPLIANCE:

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

A. BACKGROUND INFORMATION

Aminopyralid (4-amino-3,6-dichloropyridine-2-carboxylic acid) is the proposed common name of XDE-750, a new active ingredient developed by Dow AgroSciences. Aminopyralid is a systemic postemergence herbicide which belongs to the pyridine carboxylic acid class of herbicides. The petitioner is currently proposing food/feed uses on grasses grown in rangelands and permanent pastures and on wheat for the selective control of invasive and noxious broadleaf weeds. It is also proposed for weed control in sites such as parks, electric utility rights-of way, forestry, woodlands, and wildlife openings, with smaller amounts used in railroads, utility substations, pipelines, and pumping stations.

The proposed end-use product (EP) is a soluble concentrate liquid (SC/L) referred to by the trade name GF-871 (EPA Reg. No. 62719-LRI). The active ingredient in GF-871 is formulated as the triisopropanolammonium (TIPA) salt, with the product containing 40.6% of aminopyralid TIPA salt at an acid equivalent (ae) of 21.1% or 2 lb ae/gal (240 g ae/L). The petitioner stated that the aminopyralid TIPA salt dissociates rapidly in water to the acid (aminopyralid) at environmental pH values greater than 2.56 (the pKa).

Currently, the 2 lb ae/gal (240 g ae/L) SC/L formulation is proposed for broadcast foliar application at maximum rates of 0.11 lb ae/A (120 g ae/hectare) on rangeland and permanent pastures and 0.009 lb ae/A (10 g ae/ha) on wheat. The proposed PHIs are 0 days for wheat hay and 50 days for wheat grain and straw. No PHI is listed or proposed for grasses or wheat forage.

TABLE A.1. Test Compound Nomenclature.

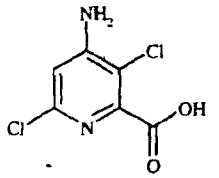
Chemical structure	
Common name	Aminopyralid
Company experimental name	XDE-750
IUPAC name	4-amino-3,6-dichloropyridine-2-carboxylic acid
CAS name	4-amino-3,6-dichloro-2-pyridinecarboxylic acid
CAS registry number	150114-71-9
End-use product (EP)	2 lb ae/gal (240 g ae/L) TIPA salt SC/L formulation (GF-871 Herbicide; EPA Reg. No. 62719-LRI; Aminopyralid Liquid Concentrate Herbicide in Canada)

TABLE A.2. Physicochemical Properties of the Aminopyralid Technical Grade Test Compound.

Parameter	Value	Reference
Melting point	163.5 °C	MRID 46235703
pH	2.31 at 23.4 °C (1% solution in water)	MRID 46235703
Relative density	1.72 at 20 °C	MRID 46235703

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TABLE A.2. Physicochemical Properties of the Aminopyralid Technical Grade Test Compound.

Parameter	Value	Reference
Water solubility	2.48 g/L unbuffered water at 18 °C 212 g/L pH 5 buffer at 20 °C 205 g/L pH 7 buffer at 20 °C 203 g/L pH 9 Buffer at 20 °C	MRID 46235703, PMRA LS
Solvent solubility at 20 °C	methanol 52.2 g/L acetone 29.2 g/L n-octanol 3.9 g/L ethyl acetate 3.9 g/L 1,2-dichloroethane 0.2 g/L xylene 0.04 g/L heptane <10 µg/mL	MRID 46235703, PMRA LS
Vapor pressure	2.59 x 10^{-8} Pa at 25 °C; 9.52 x 10^{-9} Pa at 20 °C	MRID 46235703, PMRA LS
Dissociation constant, pK_a	2.56	MRID 46235703, PMRA LS
Octanol/water partition coefficient, $Log(K_{OW})$	0.201 unbuffered water at 19 °C; -1.76 at pH 5; -2.87 at pH 7; -2.96 at pH 9	MRID 46235703, PMRA LS
UV/visible absorption spectrum	Solution: Neutral; Basic; (pH 12.6); Acidic; (pH 1.4) Wavelength λ max, nm: 217; 220; 245; 217; 270 Extinction coefficient ε, L/(mol*cm): 29100; 26100; 10150; 22800; 9140	MRID 46235703, PMRA LS

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

TABLE B.1.1. Test Site Information.

Testing Environment and location	Soil characteristics						
	Type	% Sand	% Silt	% Clay	%OM	pH	CEC (meq/100 g)
Wooden boxes (3' x 5') lined with plastic, maintained in outdoor plots at Research For Hire (Porterville, CA)	Sandy loam	71	20	9	2.3	6.1	9.5

The petitioner stated that the treated soil was irrigated throughout the fallow period with up to 0.75 acre inches of water per week depending on rainfall. Before planting, the soil was tilled to a depth of 7-8 cm. Plants were irrigated after planting as necessary to produce normal crops. The petitioner provided monthly minimum and maximum temperature and humidity data and monthly rainfall data for the duration of the in-life phase of the study. No extreme values for temperature or rainfall were reported. No comparison was made to historical conditions.

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TABLE B.1.2. Crop Information.

Crop; crop group	Variety	Plantback intervals (days)	Growth stage at harvest	Harvested RAC	Harvesting procedure
Lettuce; leafy vegetable, except brassica, group 4	New Red Fire M1	90, 120	Immature (45-50 DAP) Mature (58-61 DAP)	Leaves	Plants were cut with scissors ~ 1 inch above the soil surface
Sorghum; cereal grain, group 15; and forage, fodder, and straw of cereal grain, group 16	Sordan 79	90, 120	Immature --Before onset of heading (29-34 DAP) --After development of seed heads (soft to hard dough stage; 80-97 DAP)	Immature -Early forage --Late forage	Early forage was cut ~ 1 inch above the soil surface; late forage was cut from the upper 2/3 to 3/4 of the plant (including seed heads)
			Mature (110-127 DAP)	Grain and stover	The seed head was cut from the plant, and the grain was pulled from the seed head; the remaining 2/3 to 3/4 of the plant was cut for stover
Turnips; root and tuber vegetable, group 1; and leaves of root and tuber vegetables, group 2	Purple Top White Globe	90, 120	Immature (39-42 DAP) Mature (73-82 DAP)	Immature tops Mature tops and roots	Immature plants were cut ~ 1 inch above the soil surface; mature plants were pulled from the ground, and the tops were cut from the roots; loose soil was lightly brushed from the roots

[1] DAP = Days after planting.

Sorghum was substituted for wheat as the representative cereal grain, because of concerns that wheat would not produce a sizeable grain crop during the hot summer months. Immature sorghum was collected at intervals intended to simulate wheat forage (early sorghum forage) and hay (late sorghum forage); late sorghum forage samples were not subjected to drying.

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics.

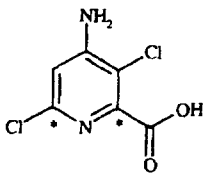
Chemical structure	
Radiolabel position	2- and 6-positions of the pyridine ring
Lot No.	SPS Reference No. DE3-E1004-77; Inventory No. INV1893
Purity	98.2%

TABLE B.2.1. Test Material Characteristics.	
Specific activity	Test substance: 28.6 mCi/mmol: 138.2 µCi/mg Isotopically diluted test substance: 40 µCi/mg (88,800 dpm/µg)

B.3. Study Use Pattern

TABLE B.3.1. Use Pattern Information.	
Chemical name	Aminopyralid
Application method	Prior to application, the isotopically diluted test substance was formulated as a potassium salt by the addition of 0.1 N potassium hydroxide, and diluted with water. Application to bare ground was made using a hand-held CO_2-powered sprayer
Application rate	0.009 lb ai/A (10 g ai/ha)
Number of applications	One
Timing of applications	Application was made to the soil; rotated crops were planted 90 and 120 days after application.
PHI (days)	Not applicable, soil application

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Lettuce, sorghum, and turnip top samples were bagged and stored frozen after collection; turnip root samples were bagged and refrigerated. Samples were then shipped as stored to Dow AgroSciences (Indianapolis, IN). At Dow, all rotational crop samples except turnip root were cut into small pieces and cryogenically ground in the presence of liquid nitrogen and dry ice. Turnip root samples were washed by hand with water to remove surface dirt, rinsed, then cut into small pieces, and frozen; the frozen turnip root samples were then cryogenically ground as described above. All samples were returned to the freezer (-20 °C) until analysis.

Only 90- and 120-day early sorghum forage, 90-day sorghum stover, and 120-day mature turnip tops had radioactivity ≥0.01 ppm; therefore, only these samples were subjected to extraction for metabolite characterization/identification. The extraction procedures are detailed in Figures B.4.1.1 (90-day sorghum early forage), B.4.1.2 (120-day sorghum early forage), B.4.1.3 (90-day sorghum stover), and B.4.1.4 (120-day turnip tops).

Sorghum forage samples were extracted (2x) with acetonitrile (ACN):water (70:30, v:v) and vacuum filtered. The combined extracts were partitioned (3x) with hexane, and the resulting ACN/water phase was acidified to <pH 2 with 1.0 N HCl and partitioned (2x) with ACN:dichloromethane (DCM; 1:1, v:v). The resulting ACN/DCM and aqueous phases were reserved for HPLC analysis. An aliquot of the aqueous phase was refluxed with 1.0 N sodium hydroxide, acidified to pH <2 with concentrated HCl after cooling, and partitioned with ACN/DCM. The ACN/DCM phase was reserved for HPLC analysis.

Sorghum stover was extracted (2x) with ACN:water (70:30, v:v) and vacuum filtered. The combined extracts were acidified to <pH 2 with 1.0 N HCl and partitioned (2x) with ACN:DCM

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(1:1, v:v). The resulting ACN/DCM and aqueous phases were reserved for HPLC analysis. An aliquot of the aqueous phase was refluxed with 1.0 N sodium hydroxide, acidified to <pH 2 with concentrated HCl after cooling, and partitioned with ACN/DCM. The ACN/DCM phase was reserved for HPLC analysis. Nonextractable solids were refluxed with ACN:1.0 N HCl (1:1, v:v), vacuum filtered, and partitioned (2x) with ACN/DCM. The ACN/DCM phase was reserved for HPLC analysis, while the aqueous phase was subjected to base hydrolysis as described above for the aqueous phase of the initial extract. The resulting ACN/DCM phase was also reserved for HPLC analysis.

Turnip tops were extracted as described for stover, except the ACN/water extracts were not combined; only the first extract was partitioned and hydrolyzed. The ACN/acid extract following reflux with nonextractable solids was not further partitioned or hydrolyzed as described for stover because of low radioactivity.

Figure B.4.1.1. **Extraction Flowchart for 90-day Sorghum Early Forage.** Copied, without alteration, from MRID 46235725.

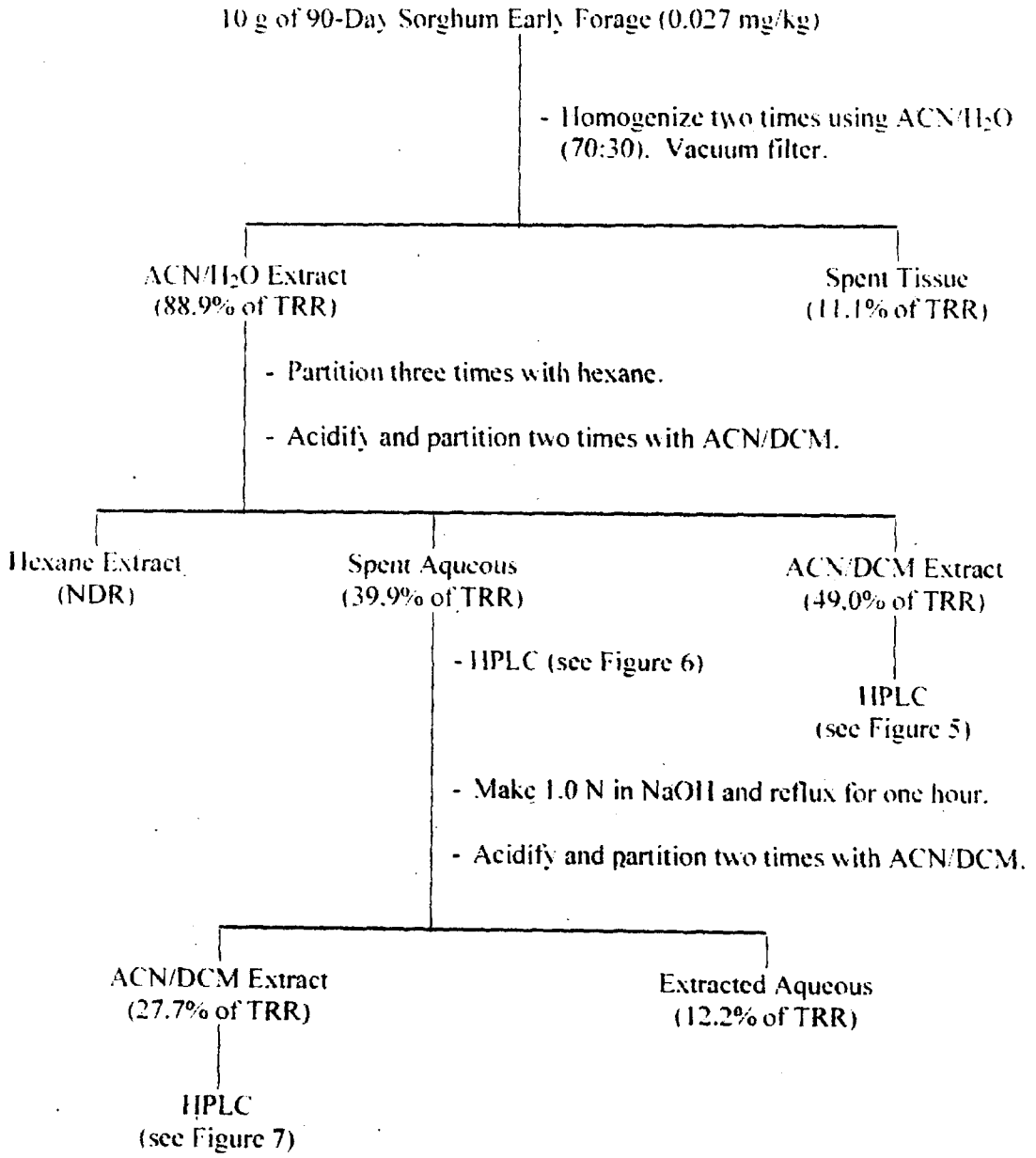


Figure B.4.1.2. **Extraction Flowchart for 120-day Sorghum Early Forage.** Copied, without alteration, from MRID 46235725.

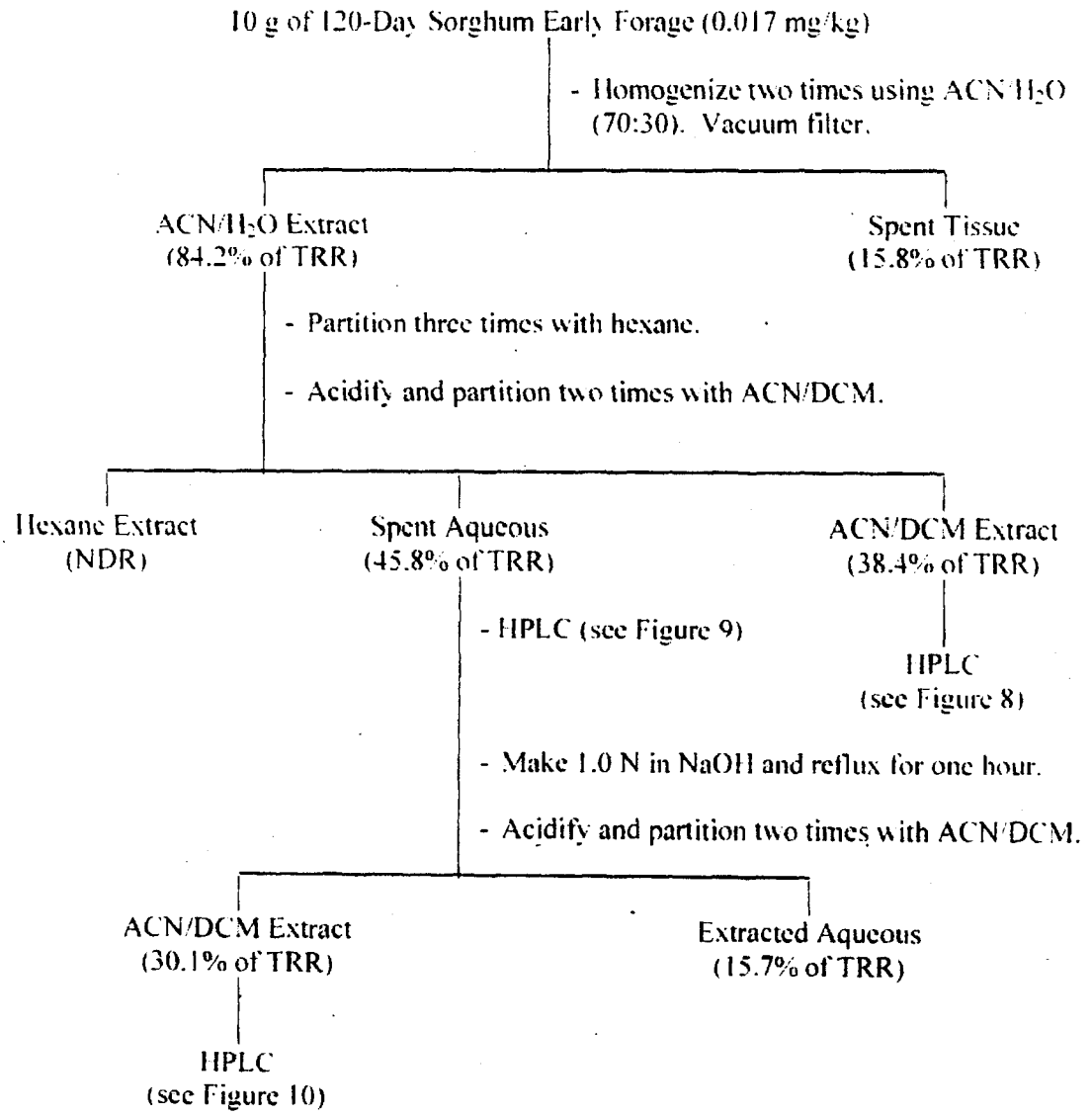


Figure B.4.1.3. **Extraction Flowchart for 90-day Sorghum Stover.** Copied, without alteration, from MRID 46235725.

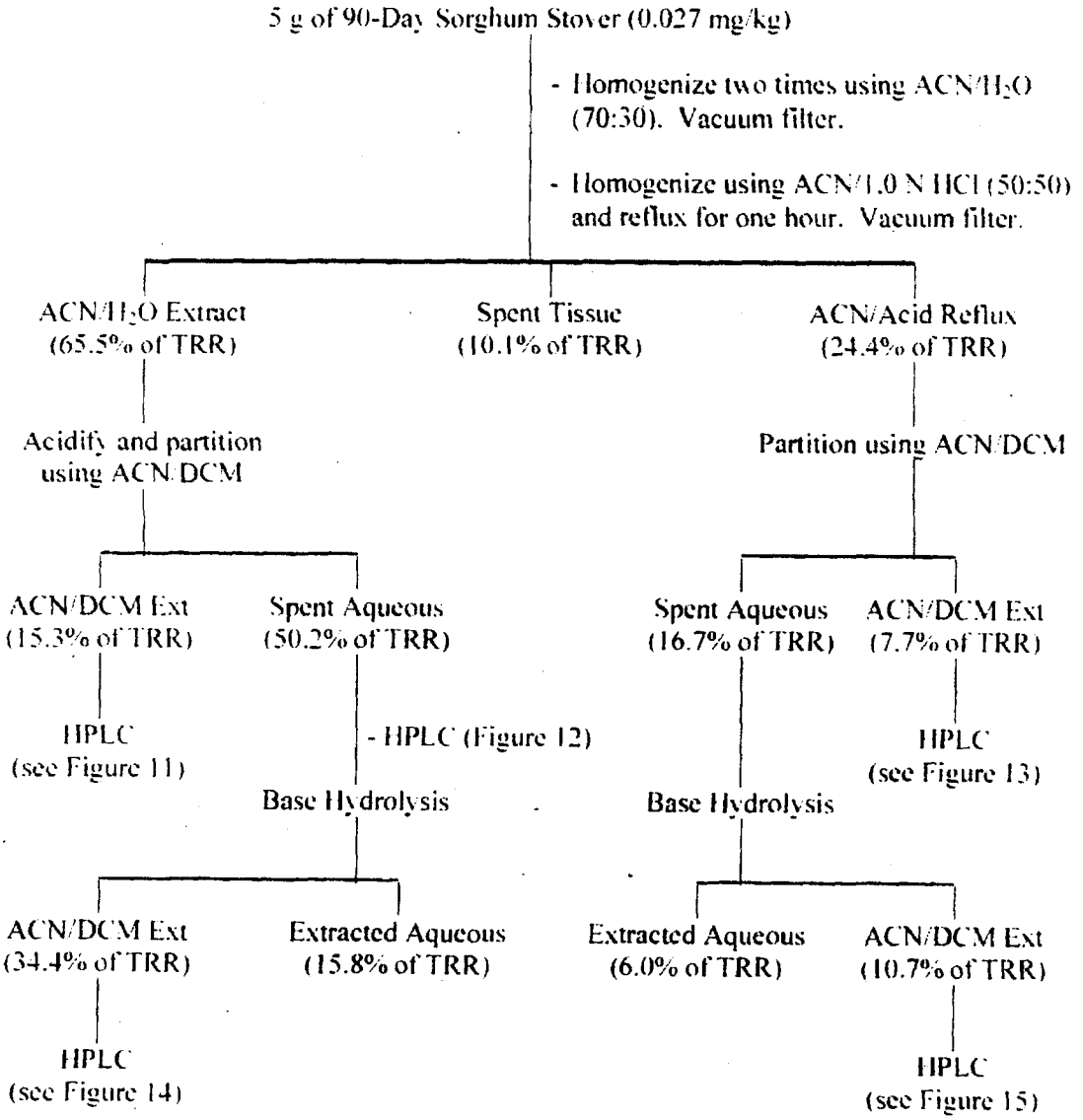
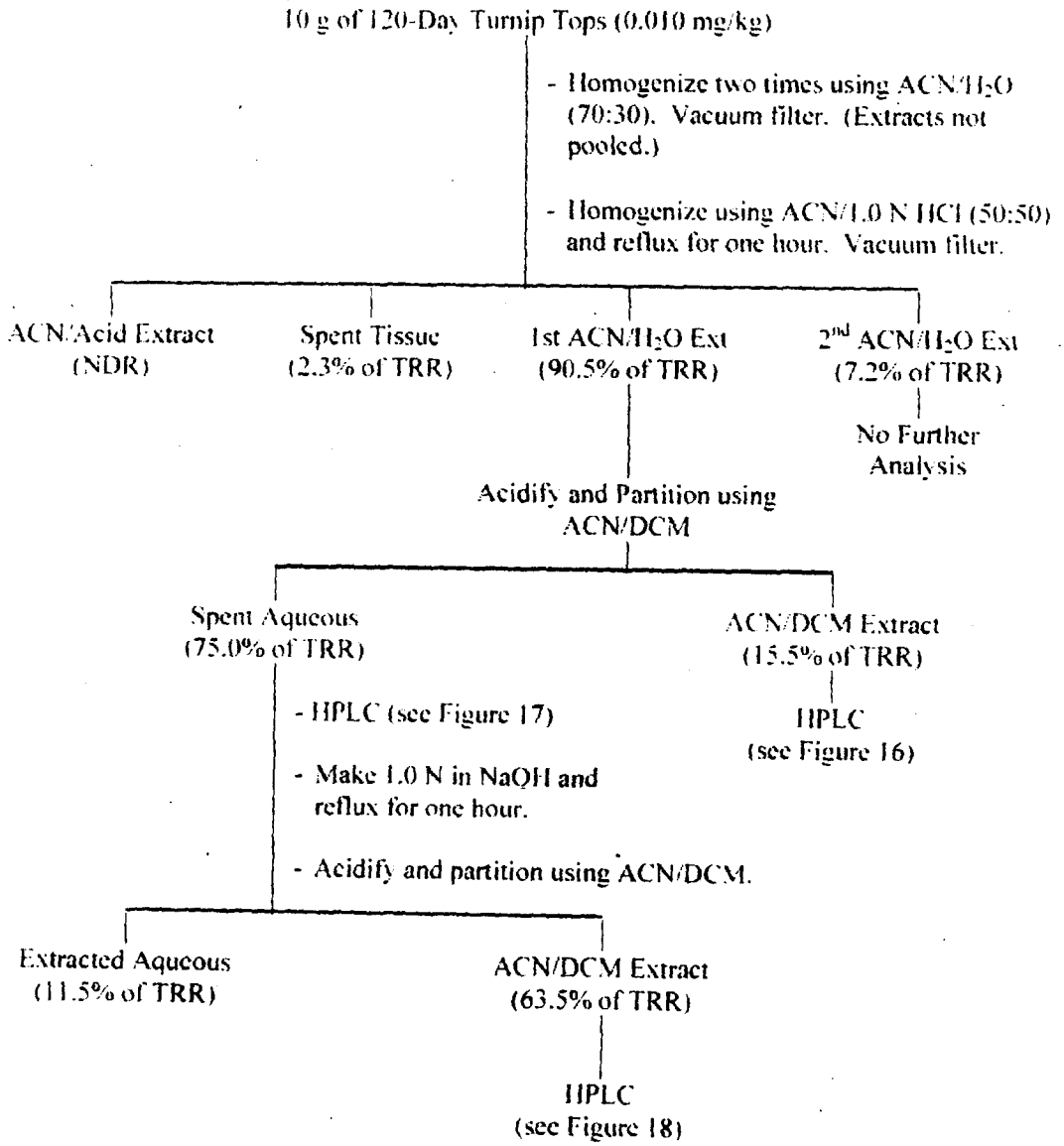


Figure B.4.1.4. **Extraction Flowchart for 120-day Turnip Tops.** Copied, without alteration, from MRID 46235725.



B.4.2. Analytical Methodology

Total radioactive residues (TRR) in rotated crop matrices and nonextractable residue fractions were determined in triplicate by combustion/LSC. Radioactivity in the extracts was determined directly by LSC. The reported limit of detection (LOD) and limit of quantitation (LOQ) were 0.0005 ppm and 0.002-0.003 ppm.

Extracts of rotational crop matrices were analyzed by HPLC using a system equipped with a reverse phase C18 column, a UV detector (270 nm), and fraction collection/LSC for radiodetection; a gradient mobile phase of water and ACN, each containing 0.5% trifluoroacetic acid (TFAA) was used. Aminopyralid was identified by co-chromatography and retention time comparison with the reference standard. The two metabolite fractions detected in the extracts were characterized by comparison with the HPLC chromatogram of aminopyralid standard.

C. RESULTS AND DISCUSSION

The storage intervals and conditions for rotational crop samples are presented in Table C.1. Based on the provided harvest, radioanalysis, and characterization dates, all samples were stored frozen for less than 2 months from collection to analysis; therefore, no storage stability data are required to support the confined rotational crop study.

Total radioactive residues (TRR) in the matrices of rotational lettuce, sorghum, and turnip planted 90 and 120 days following a single soil application of [2,6-^{14}C]aminopyralid at 0.009 lb ai/A (10 g ai/ha) are reported in Table C.2.1. TRR accumulated at ≥0.01 ppm in 90- and 120-day early sorghum forage (0.027 ppm and 0.017 ppm, respectively), 90-day sorghum stover (0.027 ppm), and 120-day mature turnip tops (0.010 ppm); residues in all other rotational crop commodities ranged <0.001-0.007 ppm.

Analysis of soil samples at planting of the rotational crops indicated that ≤30% of the applied aminopyralid was present in the soil at planting. Between planting and harvest, residues of aminopyralid slowly declined.

The extraction profiles and distribution of the radioactivity in the rotational crop commodities having TRR ≥0.01 ppm are presented in Table C.2.2. Extraction with acetonitrile (ACN)/water released the majority of the TRR (66-98% TRR). Additional radioactivity was released by ACN/acid reflux of sorghum stover (24% TRR). Nonextractable residues in 90-DAT sorghum early forage and stover, and 120-DAT sorghum early forage and turnip tops were 2.3-15.8% TRR (≤0.003 ppm). The extraction procedures extracted sufficient residues from rotational crop matrices from the 90- and 120-day PBIs.

The characterization and identification of residues in rotational crop matrices are summarized in Table C.2.3. Total identified residues ranged 17-44% TRR in sorghum early forage and stover and in turnip tops and consisted entirely of free aminopyralid; residue profiles were similar between the matrices. Free aminopyralid was the major residue identified in rotational crop

matrices, at 44.2% TRR (0.012 ppm) and 26.9% TRR (0.005 ppm) in 90- and 120-DAT sorghum early forage, respectively, 18.1% TRR (0.005 ppm) in 90-DAT sorghum stover, and 17.2% TRR (0.002 ppm) in 120-DAT turnip tops. Two metabolite fractions were also characterized in each matrix. C-1 Fraction, which accounted for 5.4-20.5% TRR (0.001-0.006 ppm) was more polar than aminopyralid, and C-2 Fraction, which accounted for 23.1-67.9% TRR (0.006-0.010 ppm) was slightly less polar than aminopyralid. These metabolites were further characterized as base-labile conjugates of aminopyralid on the following base hydrolysis of aqueous-soluble residues, which demonstrated significant conversion of the metabolite fractions to aminopyralid. In conjunction with the results of the primary plant metabolism studies (see DERs for MRIDs 46235709 and 46235710), the petitioner indicated that these conjugates are believed to consist primarily of N-glucoside and glucose ester conjugates of aminopyralid.

C.1. Storage Stability

Samples of rotational crop matrices were stored refrigerated (turnip root) or frozen following harvest and were shipped within 3-17 days of harvest to Dow Agrosciences, where they were homogenized in liquid nitrogen and dry ice and stored at ~-20°C until analysis. The petitioner provided sampling, shipping, and sample combustion dates for all rotational crop samples, as well as residue characterization dates for relevant samples. Based on the submitted information, samples were stored frozen for less than 2 months from collection to analysis; therefore, no storage stability data are required to support the confined rotational crop study.

TABLE C.1. Summary of Storage Conditions.

Matrix (RAC or Extract)	Plantback interval (days)	Storage Temp. (°C)	Actual Storage Duration [1]	Interval of Demonstrated Storage Stability
Lettuce	90, 120	-20	6-27 days (<1 month)	None required; samples stored <6 months
Sorghum, early and late forage, stover and grain			18-52 days (0.6-1.7 months)	
Turnip, tops and roots			11-33 days (0.4-1.1 months)	

[1] Storage intervals reflect collection to residue characterization for sorghum and turnip and collection to combustion for lettuce.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRR) in Lettuce, Sorghum, and Turnip Matrices.

Matrix	[2,6-^{14}C]Aminopyralid, ppm [1]	
	90-Day Plantback Interval	120-Day Plantback Interval
Lettuce, leaf, immature	(0.002)	(0.001)
Lettuce, leaf, mature	(<0.002)	(<0.001)
Sorghum, early forage	0.027	0.017
Sorghum, late forage	0.003	0.003
Sorghum, stover	0.027	0.003
Sorghum, grain	0.006	0.003

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TABLE C.2.1. Total Radioactive Residues (TRR) in Lettuce, Sorghum, and Turnip Matrices.

Matrix	[2,6-^{14}C]Aminopyralid, ppm [1]	
	90-Day Plantback Interval	120-Day Plantback Interval
Turnip tops, immature	0.007	0.007
Turnip, tops, mature	0.004	0.010
Turnip, roots, mature	(<0.001)	(<0.001)

[1] Expressed as aminopyralid equivalents. For values reported in parentheses one or more combustion values may have been less than the LOD of 0.0005 ppm, and all combustion values were below the LOQ (0.002-0.003 ppm).

TABLE C.2.2. Distribution of the Parent and the Metabolites in Rotational Crop Matrices Following Application of ^{14}C-labeled Aminopyralid to Bare Soil at 0.009 lb ai/A (10 g ai/ha). [1]

Metabolite Fraction [2]	90-Day Sorghum Early Forage		120-Day Sorghum Early Forage		90-Day Sorghum Stover		120-Day Turnip Tops	
	TRR = 0.027 ppm		TRR = 0.017 ppm		TRR = 0.027 ppm		TRR = 0.010 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN/water	88.9	0.024	84.2	0.014	65.5	0.018	90.5	0.009
Hexane phase	ND [3]	ND	ND	ND				
ACN/DCM phase	49.0	0.013	38.4	0.006	15.3	0.004	15.5	0.002
Aminopyralid	39.6	0.011	23.7	0.004	5.4	0.001	4.4	<0.001
C-1 Fraction	--	--	--	--	1.4	<0.001	--	--
C-2 Fraction	9.4	0.003	14.2	0.002	8.5	0.002	11.1	0.001
Aqueous phase	39.9	0.011	45.8	0.008	50.2	0.014	75.0	0.007
Aminopyralid	4.6	0.001	3.2	<0.001	7.2	0.002	12.6	0.001
C-1 Fraction	20.5	0.006	17.0	0.003	15.9	0.004	5.4	<0.001
C-2 Fraction	13.7	0.004	24.0	0.004	27.2	0.007	56.8	0.006
Base hydrolysis, aqueous	12.2	0.004	15.7	0.003	15.8	0.004	11.5	0.001
Base hydrolysis, ACN/DCM	27.7	0.007	30.1	0.005	34.4	0.009	63.5	0.006
Aminopyralid	27.7	0.007	29.7	0.005	32.2	0.009	61.5	0.006
C-1 Fraction	--	--	--	--	0.8	<0.001	--	--
C-2 Fraction	--	--	0.4	<0.001	1.4	<0.001	2.0	<0.001
2nd ACN/water							7.2	0.001
ACN/acid reflux					24.4	0.006	ND	ND
ACN/DCM phase					7.7	0.002		
Aminopyralid					5.5	0.002		
C-2 Fraction					2.2	<0.001		
Aqueous phase					16.7	0.004		
Base hydrolysis, aqueous					6.0	0.002		
Base hydrolysis, ACN/DCM					10.7	0.003		

TABLE C.2.2. Distribution of the Parent and the Metabolites in Rotational Crop Matrices Following Application of ^{14}C-labeled Aminopyralid to Bare Soil at 0.009 lb ai/A (10 g ai/ha). [1]

Metabolite Fraction [2]	90-Day Sorghum Early Forage		120-Day Sorghum Early Forage		90-Day Sorghum Stover		120-Day Turnip Tops	
	TRR = 0.027 ppm		TRR = 0.017 ppm		TRR = 0.027 ppm		TRR = 0.010 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Aminopyralid					7.7	0.002		
C-2 Fraction					3.0	0.001		
Nonextractable solids	11.1	0.003	15.8	0.003	10.1	0.003	2.3	<0.001

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question. Values were normalized by the petitioner; actual extraction/hydrolysis recoveries were 80.2-114.5%.
[2] Refer to the extraction flowcharts for a full description of the extract fractions.
[3] ND = None detected.

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Rotational Crop Matrices Following Application of Radiolabeled Aminopyralid to Bare Soil at 0.009 lb ai/A (10 g ai/ha).

Compound	90-Day Sorghum Early Forage		120-Day Sorghum Early Forage		90-Day Sorghum Stover		120-Day Turnip Tops	
	TRR = 0.027 ppm		TRR = 0.017 ppm		TRR = 0.027 ppm		TRR = 0.010 ppm	
	%TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Aminopyralid	44.2	0.012	26.9	0.005	18.1	0.005	17.2	0.002
C-1 Fraction [1]	20.5	0.006	17.0	0.003	17.3	0.005	5.4	0.001
C-2 Fraction [1]	23.1	0.006	38.2	0.006	37.9	0.010	67.9	0.007
Reflux, aqueous	--	--	--	--	16.7 [2]	0.004	--	--
ACN/water (2nd extract)	--	--	--	--	--	--	7.2	0.001
Total identified	44.2	0.012	26.9	0.005	18.1	0.005	17.2	0.002
Total characterized	43.6	0.012	55.2	0.009	71.9	0.019	80.5	0.009
Total extractable	88.9	0.024	84.2	0.014	89.9	0.024	97.7	0.010
Unextractable (PES) [3]	11.1	0.003	15.8	0.003	10.1	0.003	2.3	<0.001
Accountability [4]	100		100		100		100	

[1] The petitioner stated that these fractions consist primarily of glucose conjugates of aminopyralid based on base hydrolysis of the aqueous fraction and characterization work performed in conjunction with primary plant metabolism studies.
[2] Following base hydrolysis, additional aminopyralid 7.7% TRR, 0.002 ppm) and C-2 Fraction (3.0% TRR, 0.001 ppm) residues were released.
[3] Residues remaining after exhaustive extractions.
[4] Values were normalized by the petitioner; therefore, accountabilities were 100% for all matrices.

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C.3. Proposed Metabolic Profile

FIGURE C.3.1. Proposed Metabolic Profile of Aminopyralid in Rotational Crops

(Copied without alteration from MRID 46235725)

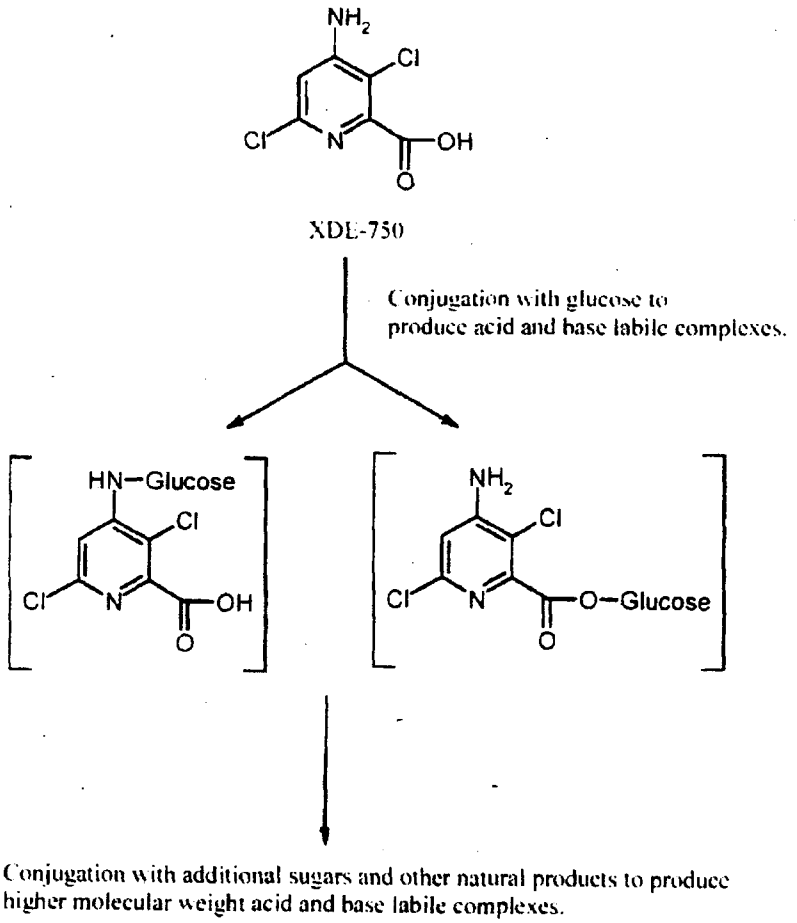
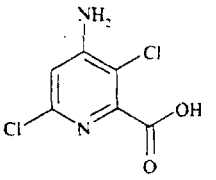


TABLE C.3.1. Identification of Compounds from the Confined Rotational Crop Study.		
Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
Aminpyralid/XDE-750	4-amino-3,6-dichloro-2-pyridinecarboxylic acid	

D. CONCLUSION

TRR accumulated at ≥0.01 ppm in selected matrices of rotational lettuce, sorghum, and turnip planted 90 and 120 days following a single soil application of [2,6-^{14}C]aminopyralid at 0.009 lb ai/A (10 g ai/ha). TRR were ≥0.01 ppm in 90- and 120-day early sorghum forage (0.027 ppm and 0.017 ppm, respectively), 90-day sorghum stover (0.027 ppm), and 120-day mature turnip tops (0.010 ppm); residues in all other rotational crop commodities ranged <0.001-0.007 ppm.

Only 90-DAT sorghum early forage and stover, and 120-DAT sorghum early forage and turnip tops contained radioactivity ≥0.01 ppm and were extracted for metabolite characterization. Total identified residues ranged 17-44% TRR in rotated sorghum early forage and stover, and turnip tops and consisted entirely of free aminopyralid; residue profiles were similar between the matrices. Two metabolite fractions, which represented up to 67.9% TRR, were characterized as base-labile N-glucoside and glucose ester conjugates of aminopyralid.

The results of the confined rotational crop study indicate that residues of aminopyralid are metabolized in the same manner as in primary crops.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: MADoherty, 06/28/05; TSheramata, 06/13/05; HBietlot, 6/13/05
Petition Number(s): PP#4F6827
DP Barcode(s): D305665
PC Code: 005100/005209

Template Version September 2003

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Aminopyralid (including TIPA salt)/XDE-750/PC Codes 005100 & 005209/Dow AgroSciences/62719
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Wheat

Primary Evaluator _____ Date: 6/28/05
Michael A. Doherty, Ph.D., Chemist, RAB2

Peer Reviewer _____ Date: June 7/05
Tamara Sheremata, Ph.D.
Evaluation Officer, FREAS, HED, PMRA

Approved by _____ Date: Jun 13/05
Henri Bietlot, Ph.D.
A/Section Head, FREAS, HED, PMRA

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 11/08/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46235721 Roberts, D; Schelle, G.; Knuteson, J. (2004) Magnitude of Residue of XDE-750 in Wheat Agricultural Commodities: Amended Report. Project Number: 030042. Unpublished study prepared by Dow AgroSciences LLC. 232 p.

EXECUTIVE SUMMARY:

Dow AgroSciences has submitted field trial data depicting the magnitude of the residue of aminopyralid in/on wheat commodities. A total of 22 wheat field trials were conducted in Canada and the U.S. during the 2003 growing season. For the 0.08 lb ae/gal (9.6 g ae/L) water emulsion in oil (EO) formulation, two Canadian wheat field trials were conducted in Region 7 (SK; 2 trials), and twenty U.S. wheat field trials were conducted in Regions 2 (VA; 1 trial), 4 (AR; 1 trial), 5 (IN, MN, NE, ND, and SD; 5 trials), 6 (OK; 1 trial), 7 (NE, ND, and SD; 5 trials), 8 (KS and TX; 6 trials), and 11 (WA; 1 trial). For the 2 lb ae/gal (240 g ae/L) SC/L triisopropanolammonium (TIPA) salt formulation and 2.1 lb ae/gal (252 g ae/L) SC/L K salt formulation, a total of seven trials were conducted with each formulation in Region 7 (two trials in Canada and five trials in the U.S.). The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 for the EO formulation but are not in accordance for the SC/L formulations. The petitioner is limiting the use of the end-use product (SC/L TIPA salt formulation) to Region 7 of Western Canada. In accordance with PMRA Directive 98-02, Section 9, seven trials are required, which the petitioner has submitted.

At each test location, wheat plants at the 3-leaf to heading stage were treated with a single broadcast foliar application of a 0.08 lb ae/gal (9.6 g ae/L) aminopyralid TIPA salt as an EO coformulated with fluroxypyr 1-methylheptyl (Starane). Application was made at ~0.009 lb ae/A

(~10 g ae/ha). Wheat forage and hay were sampled 0 and 6-7 days post application, and wheat grain and straw were sampled at maturity, 49-80 days post application. For data bridging purposes, at seven test locations (Region 7; 2 trials in Canada and 5 trials in the U.S.) two soluble concentrate liquid (SC/L) formulations, a 2 lb ae/gal (240 g ae/L) SC/L aminopyralid TIPA salt formulation and a 2.1 lb ae/gal (252 g ae/L) SC/L aminopyralid potassium (K) salt formulation, were applied to separate plots at the same rate and timing as stated above. Applications at all test sites were made using ground equipment in ~11-20 gal/A (~103-187 L/ha) of water. No spray adjuvant was used. Additional forage and hay samples were collected from three U.S. test locations at 14, 21, and 28 days after application to evaluate residue decline.

Samples of wheat forage, hay, grain, and straw were analyzed for residues of aminopyralid using LC/MS/MS Method GRM 02.31. The validated limit of quantitation (LOQ) was 0.01 ppm. This method is adequate for data collection based on acceptable concurrent method recovery and radiovalidation data.

The maximum storage interval of crop samples from harvest to analysis was 167 days (5.5 months) for wheat forage, hay, grain, and straw. In support of the crop field trial study, the petitioner cited storage stability data (refer to the DER for MRID 46235719) submitted in conjunction with the current petition; these interim data indicate that residues of aminopyralid are stable under frozen storage conditions in grass forage and hay for up to 187 days (6.2 months), wheat grain for up to 168 days (5.5 months), and wheat straw for up to 175 days (5.8 months). The available storage stability data support the storage conditions and intervals of samples from the submitted wheat field trials.

The maximum aminopyralid residues wheat forage, hay, grain, and straw from the submitted wheat field trials are reported below.

Commodity	PHI (days)	Maximum Aminopyralid Residue Levels (ppm)		
		0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt formulation	2 lb ae/gal (240 g ae/L) SC/L TIPA salt formulation	2 lb ae/gal (240 g ae/L) SC/L K salt formulation
Canadian Trials				
Wheat forage	0	0.494	0.777	0.883
	7	0.093	0.189	0.221
Wheat hay	0	1.375	2.377	2.608
	7	0.217	0.620	0.648
Wheat grain	49-55	0.012	0.013	0.013
Wheat straw	49-55	0.080	0.069	0.145
U.S. Trials				
Wheat forage	0	0.858	0.440	0.666
	6/7	0.269	0.171	0.182
Wheat hay	0	2.121	1.441	1.393
	6/7	1.031	0.368	0.355

Commodity	PHI (days)	Maximum Aminopyralid Residue Levels (ppm)		
		0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt formulation	2 lb ae/gal (240 g ae/L) SC/L TIPA salt formulation	2 lb ae/gal (240 g ae/L) SC/L K salt formulation
Wheat grain	50-80	0.026	0.018	0.026
Wheat straw	50-80	0.170	0.076	0.092

Residue decline data showed that aminopyralid residues generally decreased in wheat forage and hay with increasing sampling intervals, with maximum residues occurring at the 0-day sampling interval.

In the side-by-side trials, the SC/L formulations were found to generally yield higher residues in forage and hay than the EO formulation in the Canadian trials, and the EO formulation was found to generally yield higher residues in forage and hay than the SC/L formulations in the U.S. trials. However, the differences in residue levels were not significant. Residue levels in wheat grain and straw samples from the side-by-side trials were found to be similar across formulations.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the residue data for wheat are classified as scientifically acceptable.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D305665, and in Canada's Regulatory Decision Document.

COMPLIANCE:

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

A. BACKGROUND INFORMATION

Aminopyralid (4-amino-3,6-dichloropyridine-2-carboxylic acid) is the proposed common name of XDE-750, a new active ingredient developed by Dow AgroSciences. Aminopyralid is a systemic postemergence herbicide which belongs to the pyridine carboxylic acid class of herbicides. The petitioner is currently proposing food/feed uses on grasses grown in rangelands and permanent pastures and on wheat for the selective control of invasive and noxious broadleaf weeds. It is also proposed for weed control in sites such as parks, electric utility rights-of way, forestry, woodlands, and wildlife openings, with smaller amounts used in railroads, utility substations, pipelines, and pumping stations.

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The proposed end-use product (EP) is a soluble concentrate liquid (SC/L) referred to by the trade name GF-871 (EPA Reg. No. 62719-LRI). The active ingredient in GF-871 is formulated as the triisopropanolammonium (TIPA) salt, with the product containing 40.6% of aminopyralid TIPA salt at an acid equivalent (ae) of 21.1% or 2 lb ae/gal (240 g ae/L). The petitioner stated that the aminopyralid TIPA salt dissociates rapidly in water to the acid (aminopyralid) at environmental pH values greater than 2.56 (the pKa).

Currently, the 2 lb ae/gal (240 g ae/L) SC/L formulation is proposed for broadcast foliar application at maximum rates of 0.11 lb ae/A (120 g ae/hectare) on rangeland and permanent pastures and 0.009 lb ae/A (10 g ae/ha) on wheat. The proposed PHIs are 0 days for wheat hay and 50 days for wheat grain and straw. No PHI is listed or proposed for grasses or wheat forage.

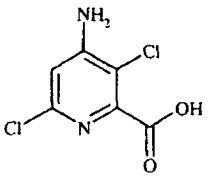
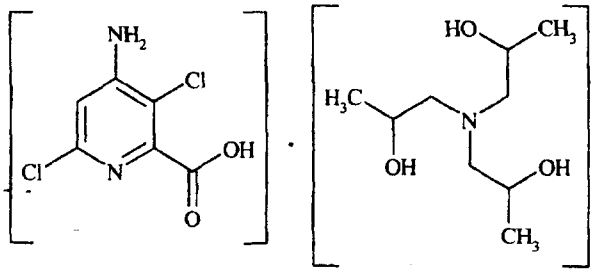
TABLE A.1. Test Compound Nomenclature.	
Chemical structure	
Common name	Aminopyralid
Company experimental name	XDE-750
IUPAC name	4-amino-3,6-dichloropyridine-2-carboxylic acid
CAS name	4-amino-3,6-dichloro-2-pyridinecarboxylic acid
CAS registry number	150114-71-9
End-use product (EP)	2 lb ae/gal (240 g ae/L) TIPA salt SC/L formulation (GF-871 Herbicide; EPA Reg. No. 62719-LRI; Aminopyralid Liquid Concentrate Herbicide in Canada) 2.1 lb ae/gal (252 g ae/L) K salt SC/L formulation (GF-389)
Chemical structure	
Common name	Aminopyralid, triisopropanolammonium (TIPA) salt
Company experimental name	XDE-750 TIPA salt
IUPAC name	Not provided
CAS name	Not provided
CAS registry number	Not provided
End-use product (EP)	0.08 lb ae/gal (9.6 g ae/L) TIPA salt water emulsion in oil (EO) formulation (GF-982) 2 lb ae/gal (240 g ae/L) TIPA salt SC/L formulation (GF-871 Herbicide; EPA Reg. No. 62719-LRI; Aminopyralid Liquid Concentrate Herbicide in Canada)

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TABLE A.2. Physicochemical Properties of the Aminopyralid Technical Grade Test Compound.

Parameter	Value	Reference
Melting point	163.5 °C	MRID 46235703, PMRA LS
pH	2.31 at 23.4 °C (1% solution in water)	MRID 46235703, PMRA LS
Relative density	1.72 at 20 °C	MRID 46235703, PMRA LS
Water solubility	2.48 g/L unbuffered water at 18 °C 212 g/L pH 5 buffer at 20 °C 205 g/L pH 7 buffer at 20 °C 203 g/L pH 9 Buffer at 20 °C	MRID 46235703, PMRA LS
Solvent solubility at 20 °C	methanol 52.2 g/L acetone 29.2 g/L n-octanol 3.9 g/L ethyl acetate 3.9 g/L 1,2-dichloroethane 0.2 g/L xylene 0.04 g/L heptane <10 µg/mL	MRID 46235703, PMRA LS
Vapor pressure	2.59 x 10^{-8} Pa at 25 °C; 9.52 x 10^{-9} Pa at 20 °C	MRID 46235703, PMRA LS
Dissociation constant, pK_a	2.56	MRID 46235703, PMRA LS
Octanol/water partition coefficient, $Log(K_{OW})$	0.201 unbuffered water at 19 °C; -1.76 at pH 5; -2.87 at pH 7; -2.96 at pH 9	MRID 46235703, PMRA LS
UV/visible absorption spectrum	Solution: Neutral; Wavelength λ max, nm: 217; Extinction coefficient ε, L/(mol*cm): 29100 Basic: 220; 26100 (pH 12.6): 245; 10150 Acidic: 217; 22800 (pH 1.4): 270; 9140	MRID 46235703, PMRA LS

TABLE A.3. Physicochemical Properties of the Aminopyralid TIPA Salt 2 lb/gal (240 g ae/L) SC/L Formulation.

Parameter	Value	Reference
Melting point	Not provided	-
pH	7.33 at 19.8 °C	MRID 46235704, PMRA LS
Density	1.1401 g/mL at 20.0 °C	MRID 46235704, PMRA LS
Water solubility	Not provided	-
Solvent solubility at 20 °C	Not provided	-
Vapor pressure	Not provided	-
Dissociation constant, pK_a	Not provided	-
Octanol/water partition coefficient, $Log(K_{OW})$	Not provided	-
UV/visible absorption spectrum	Not provided	-

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B. EXPERIMENTAL DESIGN

B.1. Study Site Information

TABLE B.1.1. Trial Site Conditions.

Trial Identification: City, State/Province; Year (Study ID)	Soil characteristics				Meteorological data	
	Type	%OM	pH	CEC	Overall monthly rainfall range (inches) [1]	Overall temperature range (°C)
Canadian Trials						
Vanscoy, SK; 2003 (SK76)	Loam	Optional			0.0-1.4	9.4-30.6
Delisle, SK; 2003 (SK77)	Silt loam	Optional			0.0-1.4	9.4-30.6
U.S. Trials						
Suffolk, VA; 2003 (VA2)	Loamy sand	Optional			0.7-5.4	7.2-28.3
Stuttgart, AR; 2003 (AR4)	Silt loam	Optional			1.0-9.4	13.3-29.4
Fowler, IN; 2003 (IN51)	Silty clay loam	Optional			0.1-7.6	8.3-30.0
Paynesville, MN; 2003 (MN52)	Sandy loam	Optional			1.0-5.3	12.8-28.9
Carpenter, SD; 2003 (SD53)	Silt loam	Optional			0.2-3.6	12.2-31.7
Oakes, ND; 2003 (ND54)	Loam	Optional			0.5-5.5	13.3-30
York, NE; 2003 (NE55)	Silt loam	Optional			0.0-3.8	8.3-33.3
Colony, OK; 2003 (OK6)	Sandy loam	Optional			1.3-4.8	9.4-28.3
Redfield, SD; 2003 (SD71)	Loam	Optional			0.5-10.0	12.2-31.7
Edgeley, ND; 2003 (ND72)	Loam	Optional			0.6-9.7	12.2-31.1
Eldridge, ND; 2003 (ND73)	Loam	Optional			0.0-1.7	11.1-30.0
Grand Island, NE; 2003 (NE74)	Silt Loam	Optional			0.3-3.7	10.0-34.4
Leola, SD; 2003 (SD75)	Loam	Optional			0.0-2.9	12.2-30.0
Levelland, TX; 2003 (TX81)	Sandy loam	Optional			0.0-1.7	11.1-31.1
Wolfforth, TX; 2003 (TX82)	Clay	Optional			0.3-1.5 (I)	10.0-31.1
Farwell, TX; 2003 (NM83)	Fine sandy loam	Optional			0.0-2.8 (I)	4.4-28.9
Groom, TX; 2003 (TX84)	Clay loam	Optional			0.3-3.2	5.0-27.8
Claude, TX; 2003 (TX85)	Silt loam	Optional			0.4-3.5	5.6-27.8
Garden City, KS; 2003 (KS86)	Silt loam	Optional			1.8-2.7 (I)	7.2-28.3
Moses Lake, WA; 2003 (WA11)	Fine sandy loam	Optional			0.0 (I)	11.1-31.1

[1] (I) indicates that supplemental irrigation was received.

The petitioner provided average maximum and minimum air temperatures and rainfall data for all of the wheat field trials and historical values for temperatures and rainfall. The actual temperature recordings were within the average historical values for the residue study period. The actual rainfall average was within the historical rainfall average, except at sites 042SD71 and 042SD72, where on-site rainfall was 400% and 358% of normal for the first month of the study (June 2003) due to a tornado event. At those sites, some hail damage was observed, but not enough to affect the amount of plant commodity needed for the study. Irrigation was used to

supplement rainfall as needed. In general, variations in weather conditions were not considered by the petitioner to have an impact on the results of the study.

TABLE B.1.2. Study Use Pattern.

Location: City, State/Province; Year (Study ID)	EP [1]	Application					Tank Mix Adjuvants
		Method; Timing	Vol. (GPA[2]) [L/ha]	Rate (lb ae/A) [g ae/ha]	RTI[3] (days)	Total Rate (lb ae/A) [g ae/ha]	
Canadian Trials							
Vanscoy, SK; 2003 (SK76)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 30 cm, 30 BBCH	11.3 [107]	0.009 [9.7]	N/A	0.009 [9.7]	None
	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	Single broadcast foliar application; crop height 30 cm, 30 BBCH	11.6 [109]	0.009 [10]	N/A	0.009 [10]	None
	2.1 lb ae/gal (252 g ae/L) SC/L K salt	Single broadcast foliar application; crop height 30 cm, 30 BBCH	11.3 [107]	0.008 [9.4]	N/A	0.008 [9.4]	None
Delisle, SK; 2003 (SK77)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 30-40 cm, 37 BBCH	11.6 [109]	0.009 [9.9]	N/A	0.009 [9.9]	None
	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	Single broadcast foliar application; crop height 30-40 cm, 37 BBCH	11.8 [111]	0.009 [10.1]	N/A	0.009 [10.1]	None
	2.1 lb ae/gal (252 g ae/L) SC/L K salt	Single broadcast foliar application; crop height 30-40 cm, 37 BBCH	12.0 [113]	0.009 [9.9]	N/A	0.009 [9.9]	None
U.S. Trials							
Suffolk, VA; 2003 (VA2)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 25 cm, 32 BBCH	14.3 [135]	0.009 [10.1]	N/A	0.009 [10.1]	None
Stuttgart, AR; 2003 (AR4)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 79 cm, 41-45 BBCH	12.3 [116]	0.009 [10.0]	N/A	0.009 [10.0]	None
Fowler, IN; 2003 (IN51)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 25 cm, 31 BBCH	19.9 [188]	0.009 [10.0]	N/A	0.009 [10.0]	None
Paynesville, MN; 2003 (MN52)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 41 cm, 37 BBCH	15.9 [150]	0.009 [10.0]	N/A	0.009 [10.0]	None
Carpenter, SD; 2003 (SD53)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 43 cm, 37 BBCH	16.1 [152]	0.009 [10.1]	N/A	0.009 [10.1]	None
Oakes, ND; 2003 (ND54)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 43 cm, 37 BBCH	16.0 [151]	0.009 [10.0]	N/A	0.009 [10.0]	None
York, NE; 2003 (NE55)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 25 cm, 31 BBCH	19.8 [187]	0.009 [10.0]	N/A	0.009 [10.0]	None

TABLE B.1.2. Study Use Pattern.

Location: City, State/Province; Year (Study ID)	EP [1]	Application					Tank Mix Adjuvants
		Method; Timing	Vol. (GPA[2]) [L/ha]	Rate (lb ae/A) [g ae/ha]	RTI[3] (days)	Total Rate (lb ae/A) [g ae/ha]	
Colony, OK; 2003 [4] (OK6)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 31-36 cm, 33 BBCH	12.6 [119]	0.009 [10.0]	N/A	0.009 [10.0]	None
Redfield, SD; 2003 (SD71)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 44 cm, 37 BBCH	16.1 [152]	0.009 [10.1]	N/A	0.009 [10.1]	None
	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	Single broadcast foliar application; crop height 44 cm, 37 BBCH	16.1 [152]	0.009 [9.7]	N/A	0.009 [9.7]	None
	2.1 lb ae/gal (252 g ae/L) SC/L K salt	Single broadcast foliar application; crop height 45 cm, 38 BBCH	16.1 [152]	0.009 [10.4]	N/A	0.009 [10.4]	None
Edgeley, ND; 2003 (ND72)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 44 cm, 37 BBCH	16.1 [152]	0.009 [10.1]	N/A	0.009 [10.1]	None
	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	Single broadcast foliar application; crop height 44 cm, 37 BBCH	16.1 [152]	0.009 [9.7]	N/A	0.009 [9.7]	None
	2.1 lb ae/gal (252 g ae/L) SC/L K salt	Single broadcast foliar application; crop height 44 cm, 38 BBCH	16.1 [152]	0.009 [10.4]	N/A	0.009 [10.4]	None
Eldridge, ND; 2003 (ND73)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 40 cm, 37 BBCH	15.6 [147]	0.009 [10.4]	N/A	0.009 [10.4]	None
	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	Single broadcast foliar application; crop height 40 cm, 37 BBCH	15.5 [146]	0.009 [10.5]	N/A	0.009 [10.5]	None
	2.1 lb ae/gal (252 g ae/L) SC/L K salt	Single broadcast foliar application; crop height 40 cm, 37 BBCH	15.5 [146]	0.009 [10.5]	N/A	0.009 [10.5]	None
Grand Island, NE; 2003 (NE74)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 24 cm, 31 BBCH	19.8 [187]	0.009 [10.0]	N/A	0.009 [10.0]	None
	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	Single broadcast foliar application; crop height 24 cm, 31 BBCH	19.9 [188]	0.009 [10.0]	N/A	0.009 [10.0]	None
	2.1 lb ae/gal (252 g ae/L) SC/L K salt	Single broadcast foliar application; crop height 24 cm, 31 BBCH	19.8 [187]	0.009 [10.3]	N/A	0.009 [10.3]	None

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TABLE B.1.2. Study Use Pattern.

Location: City, State/Province; Year (Study ID)	EP [1]	Application					Tank Mix Adjuvants
		Method; Timing	Vol. (GPA[2]) [L/ha]	Rate (lb ae/A) [g ae/ha]	RTI[3] (days)	Total Rate (lb ae/A) [g ae/ha]	
Leola, SD; 2003 (SD75)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 25-30 cm, 30-31 BBCH	14.8 [140]	0.009 [10.0]	N/A	0.009 [10.0]	None
	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	Single broadcast foliar application; crop height 30-31 cm, 30-31 BBCH	14.8 [140]	0.009 [10.1]	N/A	0.009 [10.1]	None
	2.1 lb ae/gal (252 g ae/L) SC/L K salt	Single broadcast foliar application; crop height 23-31 cm, 30-31 BBCH	14.8 [140]	0.009 [10.1]	N/A	0.009 [10.1]	None
Levelland, TX; 2003 (TX81)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 30 cm, 39 BBCH	15.2 [143]	0.009 [10.1]	N/A	0.009 [10.1]	None
Wolfforth, TX; 2003 (TX82)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 30 cm, 37 BBCH	15.2 [143]	0.009 [10.1]	N/A	0.009 [10.1]	None
Farwell, TX; 2003 (NM83)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 30 cm, 37 BBCH	14.9 [141]	0.009 [9.9]	N/A	0.009 [9.9]	None
Groom, TX; 2003 (TX84)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 18 cm, 33 BBCH	18.8 [177]	0.009 [10.2]	N/A	0.009 [10.2]	None
Claude, TX; 2003 (TX85)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 43 cm, 37 BBCH	18.4 [174]	0.009 [10.0]	N/A	0.009 [10.0]	None
Garden City, KS; 2003 (KS86)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 20 cm, 30 BBCH	11.7 [110]	0.009 [9.8]	N/A	0.009 [9.8]	None
Moses Lake, WA; 2003 (WA11)	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	Single broadcast foliar application; crop height 58 cm, 39 BBCH	14.9 [141]	0.009 [10.1]	N/A	0.009 [10.1]	None

[1] EP = End-use Product. The EO formulation additionally contained fluroxypyr 1-methylheptyl ester (Starane).
[2] GPA = Gallons per acre [Liters per hectare]
[3] RTI = Retreatment Interval; Not applicable (N/A) because only one application was made.
[4] A second plot was treated at an exaggerated rate to produce samples for a processing study; refer to the 860.1520 DER for MRID 46235721.

TABLE B.1.3. Trial Numbers and Geographical Locations.

NAFTA Growing Region	Wheat			
	Submitted		Requested	
	Canada	US	Canada [1]	US [2]
1				
1A				
2		1		1
3				
4		1		1
5		5	2	5
5A				
5B				
6		1		1
7	2	5	7	5
7A			1	
8		6		6
9				
10				
11		1		1
12				
13				
14			10	
15				
16				
17				
18				
19				
20				
21				
Total	2	20	20	20

[1] Directive 98-02, Section 9, Table 2.
[2] OPPTS 860.1500, Table 5.

B.2. Sample Handling and Preparation

One untreated and two treated samples of wheat forage, hay, grain, and straw were collected from each trial site. Wheat forage and hay were harvested 0 and 6-7 days after application and wheat grain and straw samples were harvested at maturity, 49-80 days after application. Hay samples were allowed to dry for 2 to 7 days prior to collection to obtain the proper moisture. Additional forage and hay samples were collected from three trials 14, 21, and 28 days after application to evaluate residue decline. Wheat forage samples were frozen within 5 hours of sampling, and wheat hay samples were allowed to dry prior to freezing. The petitioner did not provide

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sampling procedures for wheat grain and straw. Samples were shipped to Dow AgroSciences (Indianapolis, IN), where samples were stored frozen (<-20 °C). Prior to analysis, samples were frozen using liquid nitrogen and then ground using a hammer mill.

B.3. Analytical Methodology

Samples of wheat forage, hay, grain, and straw were analyzed for residues of aminopyralid by Dow AgroSciences (Indianapolis, IN) using LC/MS/MS Method GRM 02.31. A description of the method was included in the submission. For a complete description of the method, refer to the Residue Analytical Method DER for plants (MRID 46235712).

Briefly, ground samples of wheat forage, hay, grain, and straw were extracted with 0.1 N sodium hydroxide, releasing bound residues and hydrolyzing base-labile conjugates to free aminopyralid. Acid-labile conjugates were hydrolyzed by the acidification of the extract with hydrochloric acid and heating. Following hydrolysis, the extract was cleaned up through a polymeric solid-phase extraction column. The eluate was evaporated to dryness after the addition of the internal standard, $^{13}C_2{}^{15}N$-aminopyralid, and the residues reconstituted with the derivitization coupling reagent. The solution was derivatized with butyl chloroformate and diluted with methanol:water:acetic acid (50:49.9:0.1, v:v:v) for LC/MS/MS analysis. The validated LOQ was 0.01 ppm, and the calculated limit of detection (LOD) was 0.003 ppm. This method is adequate for data collection based on acceptable concurrent method recovery data.

C. RESULTS AND DISCUSSION

Concurrent method recovery data are presented in Table C.1. Apparent residues of aminopyralid were below the method LOQ (<0.01 ppm) in/on all untreated samples of wheat forage, hay, grain, and straw except in/on one untreated wheat hay sample (0.017 ppm) where detectable residues of aminopyralid were observed. The petitioner did not provide an explanation for the residues in the untreated hay sample, but these residues were insignificant in comparison with residues in the treated samples.

Sample storage conditions and intervals are summarized in Table C.2. The maximum storage interval of crop samples from harvest to analysis was 167 days (5.5 months) for wheat forage, hay, grain, and straw. In support of the crop field trial study, the petitioner cited storage stability data (refer to the DER for MRID 46235719) submitted in conjunction with the current petition; these interim data indicate that residues of aminopyralid are stable under frozen storage conditions in grass forage and hay for up to 187 days (6.2 months), wheat grain for up to 168 days (5.5 months), and wheat straw for up to 175 days (5.8 months). The available storage stability data support the storage conditions and intervals of samples from the submitted wheat field trials.

Residue data from the wheat field trials are reported in Table C.3. A summary of residue data for wheat forage, hay, grain, and straw is presented in Table C.4. Based on residue levels and for summary purposes, samples with PHIs of 49 days and greater have been grouped together.

0.08 lb/gal (9.6 g ae/L) EO TIPA salt formulation: In the Canadian field trials, residues of aminopyralid were 0.105-0.494 ppm in/on wheat forage and 0.371-1.375 ppm in/on wheat hay harvested on the day (0-day PHI) of a single broadcast foliar application of the 0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt formulation (containing fluroxypyr 1-methylheptyl) at 0.009 lb ae/A (9.7-9.9 g ae/ha). Residues of aminopyralid were 0.052-0.093 ppm in/on wheat forage and 0.121-0.217 ppm in/on wheat hay harvested 7 days following treatment with the 0.08 lb ae/gal EO TIPA salt formulation. In the U.S. field trials, residues of aminopyralid were 0.112-0.858 ppm in/on wheat forage and 0.358-2.121 ppm in/on wheat hay harvested on the day (0-day PHI) of a single broadcast foliar application of the 0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt formulation at 0.009 lb ae/A (9.9-10.4 g ae/ha). Residues of aminopyralid were 0.022-0.269 ppm in/on wheat forage and 0.058-1.031 ppm in/on wheat hay harvested 6/7 days following treatment with the EO formulation.

In the Canadian field trials, residues of aminopyralid were less than the method LOQ (<0.01 ppm) to 0.012 ppm in/on wheat grain and 0.067-0.080 ppm in/on wheat straw harvested at maturity, 49-55 days following treatment with the EO formulation. In the U.S. field trials, residues of aminopyralid were less than the method LOQ (<0.01 ppm) to 0.026 ppm in/on wheat grain and less than the method LOQ (<0.01 ppm) to 0.170 ppm in/on wheat straw harvested at maturity, 49-80 days following treatment with the EO formulation.

2 lb ae/gal (240 g ae/L) SC/L TIPA salt formulation: In the Canadian field trials, residues of aminopyralid were 0.523-0.777 ppm in/on wheat forage and 1.318-2.377 ppm in/on wheat hay harvested on the day (0-day PHI) of a single broadcast foliar application of the 2 lb ae/gal (240 g ae/L) SC/L TIPA salt formulation at 0.009 lb ae/A (10-10.1 g ae/ha). Residues of aminopyralid were 0.034-0.189 ppm in/on wheat forage and 0.047-0.620 ppm in/on wheat hay harvested 7 days following treatment with the SC/L TIPA salt formulation. In the U.S. field trials, residues of aminopyralid were 0.158-0.440 ppm in/on wheat forage and 0.259-1.441 ppm in/on wheat hay harvested on the day (0-day PHI) of a single broadcast foliar application of the 2 lb ae/gal (240 g ae/L) SC/L TIPA salt formulation at 0.009 lb ae/A (9.7-10.5 g ae/ha). Residues of aminopyralid were 0.044-0.171 ppm in/on wheat forage and 0.151-0.368 ppm in/on wheat hay harvested 7 days following treatment with the SC/L TIPA salt formulation.

In the Canadian field trials, residues of aminopyralid were less than the method LOQ (<0.01 ppm) to 0.013 ppm in/on wheat grain and 0.046-0.069 ppm in/on wheat straw harvested at maturity, 49-54 days following treatment with the SC/L TIPA salt formulation. In the U.S. field trials, residues of aminopyralid were less than the method LOQ (<0.01 ppm) to 0.018 ppm in/on wheat grain and 0.020-0.076 ppm in/on wheat straw harvested at maturity, 50-72 days following treatment with the SC/L TIPA salt formulation.

2.1 lb ae/gal (252 g ae/L) SC/L K salt formulation: In the Canadian field trials, residues of aminopyralid were 0.409-0.883 ppm in/on wheat forage and 1.390-2.608 ppm in/on wheat hay harvested on the day (0-day PHI) of a single broadcast foliar application of the 2.1 lb ae/gal (252 g ae/L) SC/L K salt formulation at 0.008-0.009 lb ae/A (9.4-9.9 g ae/ha). Residues of aminopyralid were 0.042-0.221 ppm in/on wheat forage and 0.117-0.648 ppm in/on wheat hay

harvested 7 days following treatment with the SC/L K salt formulation. In the U.S. field trials, residues of aminopyralid were 0.165-0.666 ppm in/on wheat forage and 0.329-1.393 ppm in/on wheat hay harvested on the day (0-day PHI) of a single broadcast foliar application of the 2.1 lb ae/gal (252 g ae/L) SC/L K salt formulation at 0.009 lb ae/A (10.1-10.5 g ae/ha). Residues of aminopyralid were less than the method LOQ (<0.01 ppm) to 0.182 ppm in/on wheat forage and less than the method LOQ (<0.01 ppm) to 0.355 ppm in/on wheat hay harvested 7 days following treatment with the SC/L K salt formulation.

In the Canadian field trials, residues of aminopyralid were 0.011-0.013 ppm in/on wheat grain and 0.066-0.145 ppm in/on wheat straw harvested at maturity, 49-54 days following treatment with the SC/L K salt formulation. In the U.S. field trials, residues of aminopyralid were less than the method LOQ (<0.01 ppm) to 0.026 ppm in/on wheat grain and 0.021-0.092 ppm in/on wheat straw harvested at maturity, 50-65 days following treatment with the SC/L K salt formulation.

In the side-by-side trials, the SC/L formulations were found to generally yield higher residues in forage and hay than the EO formulation in the Canadian trials, and the EO formulation was found to generally yield higher residues in forage and hay than the SC/L formulations in the U.S. trials. However, the differences in residue levels were not significant. Residue levels in wheat grain and straw samples from the side-by-side trials were found to be similar across formulations.

Residue decline data show that aminopyralid residues generally decrease in/on wheat forage and hay with increasing sampling intervals, with the maximum residues occurring at the 0-day sampling interval. In the three U.S. decline trials, in samples receiving application of the 0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt formulation, aminopyralid residues declined in/on forage from 0.338-0.858 ppm at the 0-day PHI to 0.020-0.130 ppm at the 28-day PHI. Residues declined in/on hay from 0.802-2.121 ppm at the 0-day PHI to 0.042-0.431 ppm at the 28-day PHI. In the one side-by-side U.S. decline trial, in samples treated with the 2 lb ae/gal (240 g ae/L) SC/L TIPA salt formulation or 2.1 lb ae/gal (252 g ae/L) SC/L K salt formulation, aminopyralid residues in/on forage declined from 0.200-0.290 ppm at the 0-day PHI to 0.057-0.161 ppm at the 28-day PHI. Residues declined in/on hay from 0.329-0.378 ppm at the 0-day PHI to 0.038-0.088 ppm at the 28-day PHI.

A total of 22 wheat field trials were conducted in Canada and the U.S. during the 2003 growing season. The petitioner stated that trials were conducted at sites growing either winter or early-spring wheat varieties, but did not specify the type of wheat used in each trial. The wheat types (winter or spring) reported in Table C.3 were determined by the study reviewer based on the wheat variety and the planting date.

For the 0.08 lb ae/gal (9.6 g ae/L) EO formulation, two Canadian wheat field trials were conducted in Region 7 (SK; 2 trials), and twenty U.S. wheat field trials were conducted in Regions 2 (VA; 1 trial), 4 (AR; 1 trial), 5 (IN, MN, NE, ND, and SD; 5 trials), 6 (OK; 1 trial), 7 (NE, ND, and SD; 5 trials), 8 (KS and TX; 6 trials), and 11 (WA; 1 trial). The number and locations of field trials are in accordance with OPPTS Guideline 860.1500 for the EO formulation but are not in accordance with PMRA Directive 98-02, Section 9. To satisfy PMRA

geographic representation requirements for the EO formulation, eleven additional crop field trials are needed, in Regions 7A (1 trial) and 14 (10 trials).

For the 2 lb ae/gal (240 g ae/L) SC/L TIPA salt formulation and 2.1 lb ae/gal (252 g ae/L) SC/L K salt formulation, seven trials were conducted with each formulation in Region 7 (two trials in Canada and five trials in the U.S.). A reduced number of trials are needed for these formulations (if a full set of trials are available for the EO formulation). To satisfy geographic representation requirements for both EPA and PMRA for the SC/L formulations, an additional 19 field trials are needed, from Regions 2 (1 trial), 4 (1 trial), 5 (3 trials), 6 (1 trial), 7A (1 trial), 8 (4 trials), 11 (1 trial), and 14 (7 trials). This distribution is based on interpretation of DACO 7.4.1 and OPPTS 860.1500 guidelines, combined. The appropriateness of bridging between the available SC/L and EO data is addressed in the forthcoming aminopyralid residue chemistry summary document (EPA, DP Barcode D305665) and the Canadian Regulatory Decision Document

TABLE C.1. Summary of Concurrent Recoveries of Aminopyralid from Wheat Forage, Hay, Grain, and Straw.

Matrix	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
Wheat forage	0.01	23	75, 75, 79, 82, 85, 85, 86, 86, 86, 87, 88, 88, 90, 90, 92, 93, 94, 94, 95, 95, 99, 101, 105	90 ± 7
	0.10	2	85, 97	
	1.0	9	82, 86, 88, 89, 94, 94, 95, 98, 101	
Wheat hay	0.01	23	70, 70, 75, 76, 76, 77, 78, 78, 79, 79, 80, 83, 85, 86, 87, 87, 88, 89, 91, 92, 96, 101, 107	84 ± 9
	0.10	1	90	
	1.0	6	81, 81, 84, 84, 87, 99	
	5.0	5	68, 75, 79, 82, 84	
Wheat grain	0.01	12	80, 85, 88, 89, 90, 90, 92, 95, 96, 96, 97, 102	93 ± 6
	0.10	6	88, 94, 96, 98, 100, 103	
Wheat straw	0.01	12	75, 77, 80, 82, 82, 83, 84, 86, 87, 87, 93, 93	83 ± 6
	0.10	1	82	
	1.0	4	72, 79, 87, 90	

TABLE C.2. Summary of Storage Conditions.

Matrix	Storage Temp. (°C)	Actual Storage Duration	Interval of Demonstrated Storage Stability [1]
Wheat forage	-20	≤167 days (5.5 months)	Interim storage stability data indicate that residues of aminopyralid are stable under frozen storage conditions in/on fortified samples of grass forage and hay for up to 187 days, wheat grain for up to 168 days, and wheat straw for up to 175 days.
Wheat hay			
Wheat grain			
Wheat straw			

[1] Refer to the DER for MRID 46235719.

TABLE C.3. Residue Data from Crop Field Trials with Aminopyralid.

Trial ID: City, State/Province; Year (Study ID)	Region	Wheat Type; Variety	Formulation	Total Rate (lb ae/A) [g ae/ha]	Commodity or Matrix	PHI (days)	Aminopyralid Residues (ppm) [1]
Canadian Field Trials							
Vanscoy, SK; 2003 (SK76)	7	Spring; ACEatonia	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [9.7]	forage	0	0.105, 0.112
						7	0.083, 0.093
					hay	0	0.371, 0.379
						7	0.171, 0.217
					grain	55	*0.008*, 0.012 [2]
					straw	55	0.072, 0.074
Vanscoy, SK; 2003 (SK76)	7	Spring; ACEatonia	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	0.009 [10]	forage	0	0.661, 0.777
						7	0.034, 0.189
					hay	0	2.259, 2.377
						7	0.617, 0.620
					grain	54	*0.006, 0.007*
					straw	54	0.068, 0.069
Vanscoy, SK; 2003 (SK76)	7	Spring; ACEatonia	2.1 lb ae/gal (252 g ae/L) SC/L K salt	0.008 [9.4]	forage	0	0.816, 0.883
						7	0.210, 0.221
					hay	0	2.107, 2.608
						7	0.626, 0.648
					grain	54	0.011, 0.011
					straw	54	0.131, 0.145
Delisle, SK; 2003 (SK77)	7	Spring; ACEatonia	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [9.9]	forage	0	0.490 [2], 0.494
						7	0.052, 0.054
					hay	0	1.236 [2], 1.375
						7	0.121, 0.130
					grain	49	*0.005*, 0.011
					straw	49	0.067, 0.080 [2]
Delisle, SK; 2003 (SK77)	7	Spring; ACEatonia	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	0.009 [10.1]	forage	0	0.523, 0.529
						7	0.043, 0.052
					hay	0	1.318, 1.649
						7	0.047, 0.117
					grain	49	0.013, 0.013
					straw	49	0.046, 0.056
Delisle, SK; 2003 (SK77)	7	Spring; ACEatonia	2.1 lb ae/gal (252 g ae/L) SC/L K salt	0.009 [9.9]	forage	0	0.409, 0.433
						7	0.042, 0.047
					hay	0	1.390, 1.526
						7	0.117, 0.121
					grain	49	0.013, 0.013
					straw	49	0.066, 0.071

TABLE C.3. Residue Data from Crop Field Trials with Aminopyralid.

Trial ID: City, State/Province; Year (Study ID)	Region	Wheat Type; Variety	Formulation	Total Rate (lb ae/A) [g ae/ha]	Commodity or Matrix	PHI (days)	Aminopyralid Residues (ppm) [1]
U.S. Field Trials							
Suffolk, VA; 2003 (VA2)	2	Winter; Coker 9835	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.1]	forage	0	0.748, 0.858 [2]
						7	0.253, 0.269
						14	0.174, 0.211
						21	0.156, 0.165
						28	0.116 [2], 0.121
					hay	0	1.888 [2], 2.121
						7	0.958, 1.031
						14	0.586, 0.636
						21	0.437, 0.462
						28	0.396, 0.431
					grain	73	0.025 [2], 0.025
					straw	73	0.026 [2], 0.090
Stutgart, AR; 2003 (AR4)	4	Winter; Natchez	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.0]	forage	0	0.296, 0.310
						7	0.022, 0.115
					hay	0	0.549, 0.962
						7	0.278, 0.291 [2]
					grain	50	0.020, 0.026
					straw	50	0.036, 0.043
Fowler, IN; 2003 (IN51)	5	Winter; Bravo	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.0]	forage	0	0.388, 0.642
						7	0.122, 0.144
					hay	0	1.263, 1.367
						7	0.326, 0.331
					grain	80	*0.007, 0.009*
					straw	80	0.034, 0.036
Payneville, MN; 2003 (MN52)	5	Spring; Oxen	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.0]	forage	0	0.193 [2], 0.204
						7	0.162, 0.176
					hay	0	0.435, 0.471
						7	0.394
					grain	72	*0.006, 0.006*
					straw	72	*0.008*, 0.010
Carpenter, SD; 2003 (SD53)	5	Spring; Marshall	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.1]	forage	0	0.284, 0.291
						7	0.181, 0.192
					hay	0	0.645, 0.745
						7	0.439 [2], 0.697
					grain	72	*0.007, 0.007*
					straw	72	*0.006, 0.008*

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TABLE C.3. Residue Data from Crop Field Trials with Aminopyralid.

Trial ID: City, State/Province; Year (Study ID)	Region	Wheat Type; Variety	Formulation	Total Rate (lb ae/A) [g ae/ha]	Commodity or Matrix	PHI (days)	Aminopyralid Residues (ppm) [1]
Oakes, ND; 2003 (ND54)	5	Spring; Oxen	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.0]	forage	0	0.311, 0.321
						7	0.144, 0.179
					hay	0	0.680, 0.714
						7	0.447, 0.472
					grain	72	*0.006, 0.007*
					straw	72	*0.006, 0.007*
York, NE; 2003 (NE55)	5	Winter; VNS HRW	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.0]	forage	0	0.535, 0.553
						7	0.108, 0.125
					hay	0	0.950, 1.009
						7	0.206, 0.257
					grain	61	0.024, 0.025
					straw	61	0.101 [2], 0.170
Colony, OK; 2003 (OK6)	6	Winter; Coker 9663	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.0]	forage	0	0.520, 0.630
						7	0.109, 0.125
						14	0.094 [2], 0.112
						21	0.096, 0.112
						28	0.109, 0.130
					hay	0	0.918, 1.239
						7	0.288, 0.311 [2]
						14	0.273, 0.412
						21	0.238, 0.252
						28	0.214, 0.225
					grain	69	0.012, 0.014 [2]
					straw	69	0.059, 0.059
Redfield, SD; 2003 (SD71)	7	Spring; Marshall	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.1]	forage	0	0.338 [2], 0.371
						7	0.164, 0.182
						14	0.068, 0.083
						21	0.039, 0.050 [2]
						28	0.020, 0.037 [2]
					hay	0	0.802 [2], 0.932
						7	0.427, 0.477
						14	0.227, 0.270
						21	0.034, 0.056 [2]
						28	0.042, 0.047
					grain	72	*0.007, 0.007*
					straw	72	*0.005, 0.009*

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TABLE C.3. Residue Data from Crop Field Trials with Aminopyralid.

Trial ID: City, State/Province; Year (Study ID)	Region	Wheat Type; Variety	Formulation	Total Rate (lb ae/A) [g ae/ha]	Commodity or Matrix	PHI (days)	Aminopyralid Residues (ppm) [1]
Redfield, SD; 2003 (SD71)	7	Spring; Marshall	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	0.009 [9.7]	forage	0	0.209, 0.290
						7	0.052, 0.057
						14	0.104, 0.125
						21	0.116, 0.178
						28	0.057, 0.103
					hay	0	0.373, 0.377
						7	0.222, 0.265
						14	0.223, 0.298
						21	0.172, 0.180
						28	0.038, 0.088
					grain	72	*0.008*, 0.010
					straw	72	0.022, 0.023
Redfield, SD; 2003 (SD71)	7	Spring; Marshall	2.1 lb ae/gal (252 g ae/L) SC/L K salt	0.009 [10.4]	forage	0	0.200, 0.226
						7	0.083, 0.169
						14	0.157, 0.157
						21	0.228, 0.327
						28	0.059, 0.161
					hay	0	0.329, 0.378
						7	0.178, 0.355
						14	0.188, 0.229
						21	0.241, 0.246
						28	0.057, 0.065
					grain	64	0.010, 0.014
					straw	64	0.021, 0.092
Edgeley, ND; 2003 (ND72)	7	Spring; Oxen	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.1]	forage	0	0.303, 0.304
						7	0.136, 0.192
					hay	0	0.740 [2], 0.801
						7	0.291, 0.347
					grain	72	*0.006, 0.007*
					straw	72	*0.008*, 0.011 [2]
Edgeley, ND; 2003 (ND72)	7	Spring; Oxen	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	0.009 [9.7]	forage	0	0.158, 0.172
						7	0.142, 0.171
					hay	0	0.259, 0.423
						7	0.346, 0.368
					grain	72	*0.009, 0.009*
					straw	72	0.020, 0.030

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TABLE C.3. Residue Data from Crop Field Trials with Aminopyralid.

Trial ID: City, State/Province; Year (Study ID)	Region	Wheat Type; Variety	Formulation	Total Rate (lb ae/A) [g ae/ha]	Commodity or Matrix	PHI (days)	Aminopyralid Residues (ppm) [1]
Edgeley, ND; 2003 (ND72)	7	Spring; Oxen	2.1 lb ae/gal (252 g ae/L) SC/L K salt	0.009 [10.4]	forage	0	0.165, 0.260
						7	ND, 0.182
					hay	0	0.508, 0.580
						7	ND, ND
					grain	65	*0.008*, 0.012
					straw	65	0.033, 0.039
Eldridge, ND; 2003 (ND73)	7	Spring; Alsen	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.4]	forage	0	0.388 [2], 0.427
						7	0.095, 0.144
					hay	0	1.603, 1.740
						7	0.270 [2], 0.297
					grain	50	0.012, 0.014 [2]
					straw	50	0.121, 0.135
Eldridge, ND; 2003 (ND73)	7	Spring; Alsen	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	0.009 [10.5]	forage	0	0.416, 0.440
						7	0.054, 0.057
					hay	0	1.195, 1.441
						7	0.164, 0.216
					grain	50	0.012, 0.015
					straw	50	0.065, 0.069
Eldridge, ND; 2003 (ND73)	7	Spring; Alsen	2.1 lb ae/gal (252 g ae/L) SC/L K salt	0.009 [10.5]	forage	0	0.378, 0.391
						7	0.056, 0.056
					hay	0	0.435, 1.315
						7	0.157, 0.161
					grain	50	0.013, 0.015
					straw	50	0.049, 0.085
Grand Island, NE; 2003 (NE74)	7	Spring; Forge HRS	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.0]	forage	0	0.448, 0.523
						7	0.105, 0.121
					hay	0	1.207, 1.244
						7	0.329, 0.404
					grain	56	0.020, 0.024
					straw	56	0.100 [2], 0.103
Grand Island, NE; 2003 (NE74)	7	Spring; Forge HRS	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	0.009 [10.0]	forage	0	0.356, 0.386
						7	0.044, 0.055
					hay	0	0.646, 0.774
						7	0.151, 0.190
					grain	56	*0.008*, 0.018
					straw	56	0.054, 0.075

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TABLE C.3. Residue Data from Crop Field Trials with Aminopyralid.

Trial ID: City, State/Province; Year (Study ID)	Region	Wheat Type; Variety	Formulation	Total Rate (lb ae/A) [g ae/ha]	Commodity or Matrix	PHI (days)	Aminopyralid Residues (ppm) [1]
Grand Island, NE; 2003 (NE74)	7	Spring; Forge HRS	2.1 lb ae/gal (252 g ae/L) SC/L K salt	0.009 [10.3]	forage	0	0.595, 0.666
						7	0.079, 0.083
					hay	0	1.100, 1.383
						7	0.224, 0.236
					grain	56	0.020, 0.026
					straw	56	0.073, 0.082
Leola, SD; 2003 (SD75)	7	Spring; Walworth	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.0]	forage	0	0.522, 0.529
						7	0.126, 0.129
					hay	0	1.761, 1.995
						7	0.418, 0.476 [2]
					grain	64	0.010, 0.011
					straw	64	0.096, 0.103
Leola, SD; 2003 (SD75)	7	Spring; Walworth	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	0.009 [10.1]	forage	0	0.399, 0.415
						7	0.046, 0.050
					hay	0	1.335, 1.416
						7	0 183, 0.197
					grain	64	0.010, 0.010
					straw	64	0.068, 0.076
Leola, SD; 2003 (SD75)	7	Spring; Walworth	2.1 lb ae/gal (252 g ae/L) SC/L K salt	0.009 [10.1]	forage	0	0.400, 0.403
						7	0.045, 0.050
					hay	0	1.262, 1.393
						7	0.141, 0.173
					grain	64	0.010, 0.010
					straw	64	0.053, 0.062
Levelland, TX; 2003 (TX81)	8	Winter; TAM 105	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.1]	forage	0	0.333, 0.399
						6	0.111, 0.128
					hay	0	0.889, 1.070
						6	0.314, 0.343
					grain	57	*0.007, 0.009*
					straw	57	0.035, 0.043
Wolfforth, TX; 2003 (TX82)	8	Winter; TAM 105	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.1]	forage	0	0.112, 0.406 [2]
						7	0.024, 0.029
					hay	0	0.764, 0.906 [2]
						7	0.058, 0.062
					grain	69	ND, *0.003*
					straw	69	0.020, 0.022

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TABLE C.3. Residue Data from Crop Field Trials with Aminopyralid.

Trial ID: City, State/Province: Year (Study ID)	Region	Wheat Type; Variety	Formulation	Total Rate (lb ae/A) [g ae/ha]	Commodity or Matrix	PHI (days)	Aminopyralid Residues (ppm) [1]
Farwell, TX; 2003 (NM83)	8	Winter; Jagger	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [9.9]	forage	0	0.419, 0.491
						7	0.105, 0.110
					hay	0	0.950, 1.086
						7	0.314, 0.320
					grain	62	*0.006* [2], *0.006*
					straw	62	0.103, 0.129
Groom, TX; 2003 (TX84)	8	Winter; TAM 200	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.2]	forage	0	0.494, 0.759
						7	0.095, 0.105
					hay	0	0.532, 0.539
						7	0.172, 0.212
					grain	67	*0.008*, 0.014
					straw	67	0.040, 0.044
Claude, TX; 2003 (TX85)	8	Winter; Jagger	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.0]	forage	0	0.328, 0.394
						7	0.056, 0.062
					hay	0	0.413, 0.450 [2]
						7	0.101, 0.181
					grain	67	*0.008*, *0.009*
					straw	67	0.031, 0.050 [2]
Garden City, KS; 2003 (KS86)	8	Winter; Ike	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [9.8]	forage	0	0.625 [2], 0.737
						7	0.050, 0.053
					hay	0	1.420, 1.496
						7	0.121, 0.127
					grain	80	*0.008*, 0.010
					straw	80	0.029, 0.042
Moses Lake, WA; 2003 (WA11)	11	Winter; Declo	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [10.1]	forage	0	0.130, 0.184
						7	0.048, 0.052
					hay	0	0.358, 0.408
						7	0.156, 0.200
					grain	57	0.020, 0.022
					straw	57	0.032, 0.050

[1] The petitioner presented both uncorrected residue values and residue values corrected for concurrent method recovery. The uncorrected values are reported here. Residues below the method LOD (<0.003 ppm) are reported as ND; residues reported between the method LOQ (<0.01 ppm) and LOD are *italicized*.

[2] The highest residue of duplicate analyses is reported.

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TABLE C.4. Summary of Residue Data from Crop Field Trials with Aminopyralid.

Commodity	Formulation	Total Applic. Rate (lb ae/A) [g ae/ha]	PHI (days)	Residue Levels (ppm) [1]						
				n	Min.	Max.	HAFT[2]	Median (STMdR[3])	Mean (STMR[4])	Std. Dev.
Canadian Trials										
Wheat forage	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [9.7-9.9]	0	4	0.105	0.494	0.492	0.301	0.300	0.221
			7	4	0.052	0.093	0.088	0.069	0.071	0.021
	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	0.009 [10-10.1]	0	4	0.523	0.777	0.719	0.595	0.623	0.121
			7	4	0.034	0.189	0.112	0.048	0.080	0.073
	2.1 lb ae/gal (252 g ae/L) SC/L K salt	0.008-0.009 [9.4-9.9]	0	4	0.409	0.883	0.850	0.625	0.635	0.249
			7	4	0.042	0.221	0.216	0.129	0.130	0.099
Wheat hay	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [9.7-9.9]	0	4	0.371	1.375	1.306	0.808	0.840	0.540
			7	4	0.121	0.217	0.194	0.151	0.160	0.044
	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	0.009 [10-10.1]	0	4	1.318	2.377	2.318	1.954	1.901	0.503
			7	4	0.047	0.620	0.619	0.367	0.350	0.311
	2.1 lb ae/gal (252 g ae/L) SC/L K salt	0.008-0.009 [9.4-9.9]	0	4	1.390	2.608	2.358	1.817	1.908	0.561
			7	4	0.117	0.648	0.637	0.374	0.378	0.299
Wheat grain	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [9.7-9.9]	49-55	4	<0.01	0.012	0.012	0.008	0.008	0.004
	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	0.009 [10-10.1]	49-54	4	<0.01	0.013	0.013	0.009	0.009	0.005
	2.1 lb ae/gal (252 g ae/L) SC/L K salt	0.008-0.009 [9.4-9.9]	49-54	4	0.011	0.013	0.013	0.012	0.012	0.001
Wheat straw	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [9.7-9.9]	49-55	4	0.067	0.080	0.074	0.073	0.073	0.005
	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	0.009 [10-10.1]	49-54	4	0.046	0.069	0.069	0.062	0.060	0.011
	2.1 lb ae/gal (252 g ae/L) SC/L K salt	0.008-0.009 [9.4-9.9]	49-54	4	0.066	0.145	0.138	0.101	0.103	0.041

TABLE C.4. Summary of Residue Data from Crop Field Trials with Aminopyralid.

Commodity	Formulation	Total Applic. Rate (lb ae/A) [g ae/ha]	PHI (days)	Residue Levels (ppm) [1]						
				n	Min.	Max.	HAFT[2]	Median (STMdR[3])	Mean (STMR[4])	Std. Dev.
U.S. Trials										
Wheat forage	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [9.9-10.4]	0	40	0.112	0.858	0.803	0.397	0.426	0.176
			6/7	40	0.022	0.269	0.261	0.125	0.121	0.057
	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	0.009 [9.7-10.5]	0	10	0.158	0.440	0.428	0.371	0.324	0.108
			7	10	0.044	0.171	0.157	0.055	0.073	0.045
	2.1 lb ae/gal (252 g ae/L) SC/L K salt	0.009 [10.1-10.5]	0	10	0.165	0.666	0.631	0.385	0.368	0.165
			7	10	<0.01	0.182	0.126	0.068	0.081	0.056
Wheat hay	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [9.9-10.4]	0	40	0.358	2.121	2.005	0.925	1.002	0.469
			6/7	39	0.058	1.031	0.995	0.314	0.336	0.203
	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	0.009 [9.7-10.5]	0	10	0.259	1.441	1.376	0.710	0.824	0.477
			7	10	0.151	0.368	0.357	0.207	0.230	0.074
	2.1 lb ae/gal (252 g ae/L) SC/L K salt	0.009 [10.1-10.5]	0	10	0.329	1.393	1.328	0.840	0.868	0.457
			7	10	<0.01	0.355	0.267	0.167	0.163	0.105
Wheat grain	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [9.9-10.4]	50-80	40	<0.01	0.026	0.025	0.005	0.011	0.008
	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	0.009 [9.7-10.5]	50-72	10	<0.01	0.018	0.014	0.010	0.010	0.005
	2.1 lb ae/gal (252 g ae/L) SC/L K salt	0.009 [10.1-10.5]	50-65	10	<0.01	0.026	0.023	0.013	0.014	0.006
Wheat straw	0.08 lb ae/gal (9.6 g ae/L) EO TIPA salt	0.009 [9.9-10.4]	50-80	40	<0.01	0.170	0.136	0.038	0.051	0.044
	2 lb ae/gal (240 g ae/L) SC/L TIPA salt	0.009 [9.7-10.5]	50-72	10	0.020	0.076	0.072	0.060	0.050	0.024
	2.1 lb ae/gal (252 g ae/L) SC/L K salt	0.009 [10.1-10.5]	50-65	10	0.021	0.092	0.078	0.058	0.059	0.024

[1] For the calculation/reporting of minimum and maximum values, the LOQ value (0.01 ppm) was used for residues reported as ND or <LOQ in Table C.3. For calculation of the median, mean, and standard deviation, ½ the LOQ (0.005 ppm) was used for residues reported between the LOQ and LOD (0.003 ppm) and ½ the LOD (0.0015 ppm) was used for residues reported as ND.
[2] HAFT = Highest Average Field Trial.
[3] STMdR = Supervised Trial Median Residue.
[4] STMR = Supervised Trial Mean Residue.

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D. CONCLUSION

Aminopyralid residue trials conducted on wheat in the U.S. and Canada indicate that residues of aminopyralid are fairly consistent across geographic region. Furthermore, side-by-side trials conducted with emulsion-in-oil (TIPA salt) and soluble concentrate (TIPA salt and K salt) show that these formulations have very little impact on residues of aminopyralid in those commodities showing quantifiable residues (i.e., forage, hay, and straw). In forage and hay, residues decline with increasing PHI, with a rapid decline noted between pre-harvest intervals of 0 and 7 days. In the few cases where residues increase at a longer PHI, there is no trend for this and the apparent increase is probably insignificant, since it falls within the variability of the PHI-specific residue data. Higher than normal rainfall was noted at four trial locations. The high rainfall does not appear to have significantly affected residue levels based on comparisons to trials located in other areas.

E. REFERENCES

None.

F. DOCUMENT TRACKING

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Nature of the Residues in Plants - Wheat

Primary Evaluator _____ Date: 6/28/05
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This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 11/08/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46235709 Graper, L.; Smith, K.; Hilla, S. (2003) A Nature of the Residue Study with (Carbon 14)-Labeled XDE-750 Applied to Spring Wheat. Project Number: 020022. Unpublished study prepared by Dow AgroSciences LLC and Research For Hire. 197 p.

EXECUTIVE SUMMARY:

Dow AgroSciences has submitted a wheat metabolism study with aminopyralid. The aminopyralid test substance used in the study was labeled at the 2- and 6-positions of the pyridine ring and formulated as the potassium salt. The test substance was foliarly applied once to spring wheat when plants were at the BBCH 26-28 stage (6 to 8 tillers) using two treatment rates: a low rate (0.036 lb ai/A or 40.1 g ai/ha) and a high rate (0.072 lb ai/A or 80.3 g ai/ha). The wheat plants were grown to maturity outdoors. Plant samples were collected 0 days after treatment (DAT), 14 DAT (forage), 35 DAT (hay) and 86 DAT (straw and grain). The in-life phase of the study was conducted by Research For Hire (Porterville, CA), and the analytical phase was performed by Dow AgroSciences (Indianapolis, Indiana). Following sample preparation, the total radioactive residues (TRR) in/on treated samples of wheat matrices were determined by combustion/LSC. The TRR in wheat matrices are tabulated below.

Wheat Matrix	Sample Collection Timing	TRR (ppm) (Expressed as [2,6-^{14}C]aminopyralid equivalents)	
		Low rate (0.036 lb ai/A or 40.1 g ai/ha)	High rate (0.072 lb ai/A or 80.3 g ai/ha)
Wheat, early forage	0 DAT	2.022	4.121
Wheat, forage	14 DAT	0.418	0.874
Wheat, hay	35 DAT	0.284	0.691
Wheat, grain	86 DAT	0.039	0.084
Wheat, straw	86 DAT	0.281	0.623

All wheat samples were homogenized and then extracted, and for some samples also refluxed, with acetonitrile/water (70:30, v:v). The aqueous extracts and nonextractable residues from straw and grain were subjected to base and/or acid hydrolysis to release conjugated radioactivity. [For the purpose of brevity, the results for only the high-rate samples are included in this Data Evaluation Record; the distribution of radioactive residues observed in the chromatographic profiles for low and high rate samples were similar.] For all wheat matrices, the extractability of radioactive residues was high and ranged from 75.0% to 98.7% of TRR. The nonextractable residues following solvent extraction and acid/base hydrolysis were 0.7-9.4% of TRR.

Parent aminopyralid, either conjugated or free, was identified as the major residue component and comprised approximately 90% of TRR in 0-DAT forage, 38% of TRR in 14-DAT forage, 15% of TRR in 35-DAT hay, 79% of TRR in 86-DAT straw, and 60% of TRR in 96-DAT grain. In addition, the following metabolites were found in 35-DAT wheat hay: the glucose conjugate of aminopyralid (15.6% TRR) and the glucose conjugate of hydroxylated aminopyralid (4.8% TRR).

Based on the available data, the petitioner concluded that the major metabolic pathway of aminopyralid in wheat proceeds by conjugation of aminopyralid and hydroxylated aminopyralid with glucose. The petitioner further stated that, while only two metabolites were isolated as glucose conjugates, any other metabolites present in wheat which were not identified are believed to be conjugates of glucose or similar endogenous compounds, based on the fact that most of the radioactivity in the wheat samples that was not initially detected as the parent could be hydrolyzed to aminopyralid.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the spring wheat metabolism data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D305665 and in Canada's Regulatory Decision Document.

COMPLIANCE:

All aspects of this study were conducted in accordance with the requirements for Good Laboratory Practice Standards, 40 CFR 160, with the following exceptions: off-site climatic data for the in-life phase of the study; field history records; and some raw data entries were recorded at a date later than when the original operation was performed. There appeared to be no negative impact to the study from any of these exceptions.

A. BACKGROUND INFORMATION

Aminopyralid (4-amino-3,6-dichloropyridine-2-carboxylic acid) is the proposed common name of XDE-750, a new active ingredient developed by Dow AgroSciences. Aminopyralid is a systemic postemergence herbicide which belongs to the pyridine carboxylic acid class of herbicides. The petitioner is currently proposing food/feed uses on grasses grown in rangelands and permanent pastures and on wheat for the selective control of invasive and noxious broadleaf weeds. It is also proposed for weed control in sites such as parks, electric utility rights-of way, forestry, woodlands, and wildlife openings, with smaller amounts used in railroads, utility substations, pipelines, and pumping stations.

The proposed end-use product (EP) is a soluble concentrate liquid (SC/L) referred to by the trade name GF-871 (EPA Reg. No. 62719-LRI). The active ingredient in GF-871 is formulated as the triisopropanolammonium (TIPA) salt, with the product containing 40.6% of aminopyralid TIPA salt at an acid equivalent (ae) of 21.1% or 2 lb ae/gal (240 g ae/L). The petitioner stated that the aminopyralid TIPA salt dissociates rapidly in water to the acid (aminopyralid) at environmental pH values greater than 2.56 (the pKa).

Currently, the 2 lb ae/gal (240 g ae/L) SC/L formulation is proposed for broadcast foliar application at maximum rates of 0.11 lb ae/A (120 g ae/hectare) on rangeland and permanent pastures and 0.009 lb ae/A (10 g ae/ha) on wheat. The proposed PHIs are 0 days for wheat hay and 50 days for wheat grain and straw. No PHI is listed or proposed for grasses or wheat forage.

TABLE A.1. Test Compound Nomenclature.

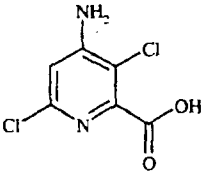
Chemical structure	
Common name	Aminopyralid
Company experimental name	XDE-750
IUPAC name	4-amino-3,6-dichloropyridine-2-carboxylic acid
CAS name	4-amino-3,6-dichloro-2-pyridinecarboxylic acid
CAS registry number	150114-71-9

TABLE A.1. Test Compound Nomenclature.	
End-use product (EP)	2 lb ae/gal (240 g ae/L) TIPA salt SC/L formulation (GF-871 Herbicide; EPA Reg. No. 62719-LRI; Aminopyralid Liquid Concentrate Herbicide in Canada)

TABLE A.2. Physicochemical Properties of the Aminopyralid Technical Grade Test Compound.

Parameter	Value	Reference
Melting point	163.5 °C	MRID 46235703, PMRA LS
pH	2.31 at 23.4 °C (1% solution in water)	MRID 46235703, PMRA LS
Relative density	1.72 at 20 °C	MRID 46235703, PMRA LS
Water solubility	2.48 g/L unbuffered water at 18 °C 212 g/L pH 5 buffer at 20 °C 205 g/L pH 7 buffer at 20 °C 203 g/L pH 9 Buffer at 20 °C	MRID 46235703, PMRA LS
Solvent solubility at 20 °C	methanol 52.2 g/L acetone 29.2 g/L n-octanol 3.9 g/L ethyl acetate 3.9 g/L 1,2-dichloroethane 0.2 g/L xylene 0.04 g/L heptane <10 µg/mL	MRID 46235703, PMRA LS
Vapor pressure	2.59×10^{-8} Pa at 25 °C; 9.52×10^{-9} Pa at 20 °C	MRID 46235703, PMRA LS
Dissociation constant, pK_a	2.56	MRID 46235703, PMRA LS
Octanol/water partition coefficient, $Log(K_{OW})$	0.201 unbuffered water at 19 °C; -1.76 at pH 5; -2.87 at pH 7; -2.96 at pH 9	MRID 46235703, PMRA LS
UV/visible absorption spectrum	Solution: Neutral; Wavelength λ max, nm: 217; Extinction coefficient ε, L/(mol*cm): 29100 Basic: 220; 26100 (pH 12.6): 245; 10150 Acidic: 217; 22800 (pH 1.4): 270; 9140	MRID 46235703, PMRA LS

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

TABLE B.1.1. Test Site Information

Testing Environment	Soil characteristics			
	Type	%OM	pH	CEC
Outdoor test plots at Research For Hire in Porterville, CA.	Loamy sand (87% sand, 8% silt, and 5% clay)	4.3	5.3	9.3 meq/100 g

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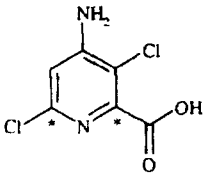
Average minimum and maximum temperatures and precipitation were reported from a nearby weather station for the study period. No unusual weather conditions were noted.

TABLE B.1.2. Crop Information.

Crop: crop group	Variety	Growth stage at application	Growth stage at harvest	Harvested RAC	Harvesting procedure
Spring wheat: cereal grain, group 15; and forage, fodder, and straw of cereal grain, group 16	Brooks	BBCH 26-28 (6-8 tillers detectable)	0 DAT (forage) 14 DAT (forage) 35 DAT (hay) 86-DAT (straw and grain)	forage, hay, straw, and grain	Hay was dried for 13 days. Grain was separated from chaff, and chaff was included with the straw sample.

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics.

Chemical structure	
Radiolabel position	In the 2- and 6-positions of the pyridinyl ring
Lot No.	Inventory No. INV1590 (radiolabeled lot no.); TSN 102298 (non-radiolabeled lot no.)
Purity	98.6% when applied
Specific activity	27.4 mCi/mmol; 2.258 Bq/μg (135.488 dpm/μg) when applied

B.3. Study Use Pattern

TABLE B.3.1. Use Pattern Information.

Chemical name	Aminopyralid
Application method	Prior to application, isotopically diluted test substance was formulated as a potassium salt by the addition of an equimolar amount of potassium hydroxide, and diluted with water. The formulated test substance was then applied foliarly to spring wheat using a CO_2 pressurized sprayer with a flat fan nozzle.
Application rates	Low rate (0.036 lb ai/A or 40.1 g ai/ha); High rate (0.072 lb ai/A or 80.3 g ai/ha)
Number of applications	1
Timing of applications	At BBCH 26-28 growth stage of wheat (6-8 tillers detectable)
PHI (days)	0 for early forage, 14 for forage, 35 for hay, and 86 for straw and grain

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Plant samples were collected 0, 14, 35 and 86 days after application (DAT). The 0- and 14-DAT samples were forage, the 35-DAT samples were hay, and the 86-DAT samples were mature grain and straw. The 35-DAT hay samples were allowed to dry in a greenhouse for 13 days in order to produce a dried hay sample. Grain was separated from chaff, and the straw sample consisted of the straw plus chaff. Samples were cut up, if necessary, frozen, and either ground or milled. Dry ice and/or liquid nitrogen were used to keep the samples frozen during grinding or milling.

Because of similar extraction ratios and residue profiles between the respective matrices from low and high treated wheat, but higher TRR in high rate samples, more extensive work was performed using the high rate samples; only these results are presented herein. The general extraction procedures for wheat matrices are summarized in the flow charts depicted in Figures 4-7, which were copied without alteration from MRID 46235709. Note that the figure numbers from the MRID were included for reference through the flowchart steps.

Extraction of Radioactive Residues: Residues in wheat matrices were extracted by blending with 70/30 acetonitrile/water (v/v) for approximately 5 to 10 minutes using a Polytron homogenizer. Extracted tissue was separated from the extraction solvent by centrifugation or vacuum filtration using Whatman #1 paper. For the 0-DAT forage, this extraction step was repeated twice, the three filtrates or supernatants were combined and the extracted tissue was allowed to air dry. The combined filtrates were concentrated and reserved for HPLC analysis. For all other samples, the extraction was repeated once, the two filtrates were combined and the extracted tissue was refluxed with stirring for approximately one hour in an additional volume of 70/30 acetonitrile/water (v/v). After reflux, the extracted tissue was separated from the reflux solution by vacuum filtration, the filtrate was combined with the two filtrates from Polytron extraction, and the extracted tissue was allowed to air dry. For the 14-DAT forage and the 35-DAT hay samples, the combined filtrates were concentrated and prepared for HPLC. For the 86-DAT grain samples, the combined filtrates were concentrated and partitioned once with dichloromethane (DCM). For the 86-DAT straw samples, the combined filtrates were concentrated and partitioned once with a ratio of dichloromethane/acetonitrile of approximately 2/1 (v/v). For both grain and straw samples, the aqueous phase from partition was prepared for HPLC. The organic phase from both grain and straw was concentrated, and the residue was partitioned between dichloromethane and water (back-extracted). For some samples, the resulting aqueous phase from this back extraction of the organic phase was concentrated and prepared for HPLC. The organic phase from the back extraction was not analyzed further.

Total radioactive residues in all tissues both before and after extraction were determined by oxidative combustion using a Harvey Oxidizer, OX-500, or a Packard Model 307 and entrapment of the evolved $^{14}CO_2$ in an alkaline trapping reagent. TRR levels in all liquid samples (extracts, oxidizer samples, etc,) were determined by direct counting (LSC) for up to 20 minutes using Packard liquid scintillation counters. The reported limit of detection and limit of quantitation was 0.001 ppm and 0.003 ppm, respectively, for all matrices.

Hydrolysis of Radioactive Residues in Aqueous Phase and Extracted Tissue: The aqueous phase and extracted tissue samples (aqueous soluble and nonextractable radioactivity, respectively) resulting from extraction of 86-DAT straw and grain samples were subjected to individual base and acid treatments or sequential base and acid treatment. Sample treatment was conducted by heating the samples in 50/50 acetonitrile/water (v/v) which was prepared to a concentration of 0.1 N NaOH or 1 N HCl. Samples were refluxed or heated for 1 hour using a heating mantle or block. Temperatures during heating or reflux ranged from 70 to 75°C. When base and acid treatment were used sequentially for a sample, base treatment was done first and was followed by acid treatment. After base and/or acid treatment of the aqueous phase samples, they were concentrated, if necessary, and assayed by HPLC. After base and/or acid treatment of the extracted tissue samples, they were vacuum filtered using Whatman #1 paper to separate the remaining tissue from the filtrate. The extracted tissue was allowed to air dry. The filtrates were kept at a pH of approximately 1 or less, and then partitioned three times with approximately 1/1 dichloromethane/acetonitrile (v/v). The resulting aqueous and organic phases were concentrated, and the concentrated aqueous and organic phases were then subjected to a precipitation of organic solubles method or a C18 solid phase extraction (SPE) method. Fractions resulting from these methods were assayed by HPLC.

Isolation and Identification of Metabolites: An extract from the 35-DAT hay was concentrated, acidified to a pH of approximately 1 and subjected to C18 SPE analysis. Three main fractions from SPE were neutralized, concentrated, re-acidified and filtered and then assayed by HPLC to determine the nature of the radioactive components. The fraction containing the most radioactivity was subjected to additional neutralization, concentration, re-acidification and filtration prior to HPLC isolation using a Synergi Hydro-RP column. Selected fractions from this first preparative HPLC attempt were combined, concentrated and assayed by HPLC to determine the success of the isolation effort. Then selected fractions were neutralized, concentrated further, filtered, re-acidified and subjected to a second HPLC isolation attempt. Selected fractions from this second attempt were concentrated, combined, and analyzed by C18 SPE. Selected fractions from SPE were combined, neutralized, concentrated, filtered, and re-acidified prior to LC/MS analysis.

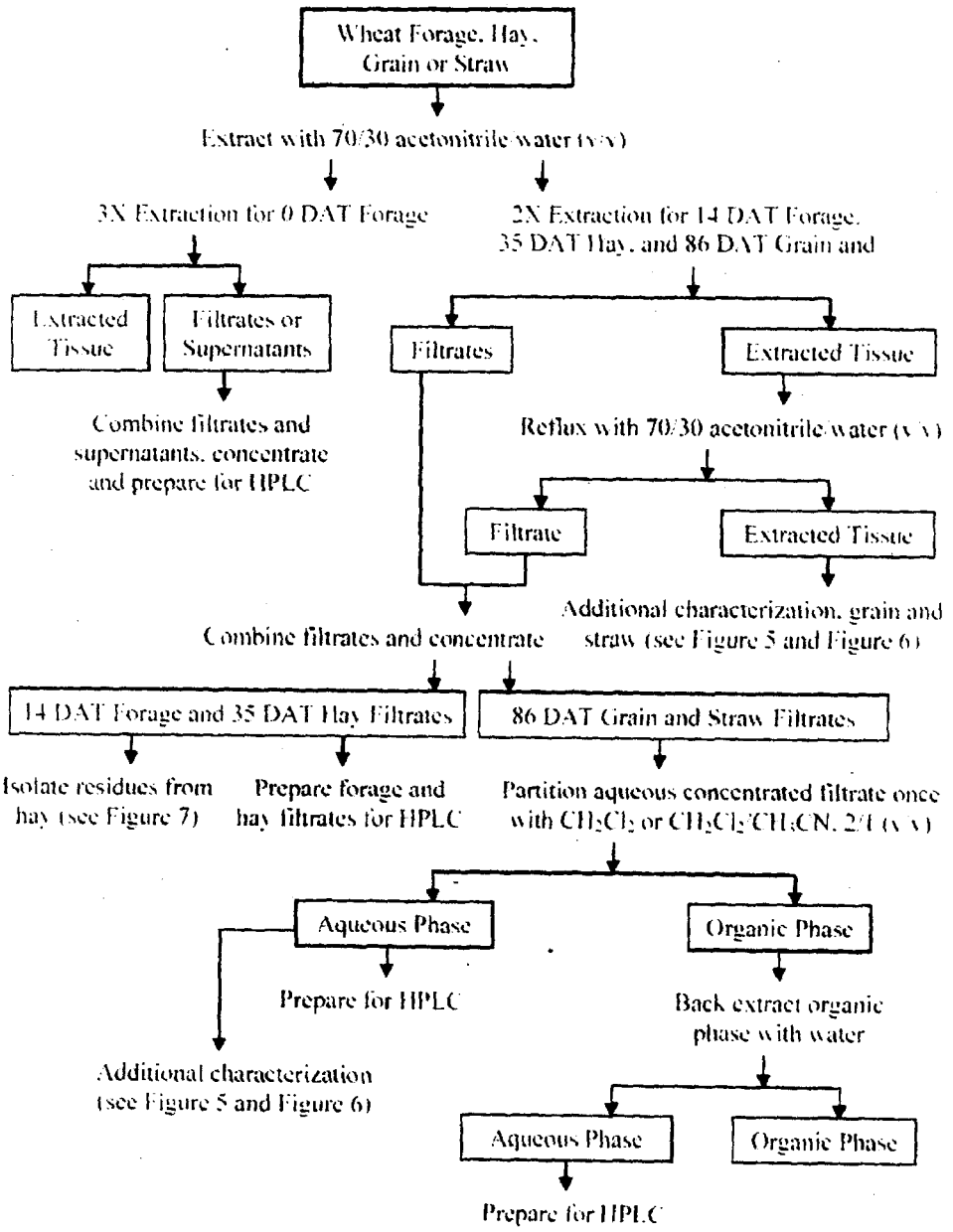
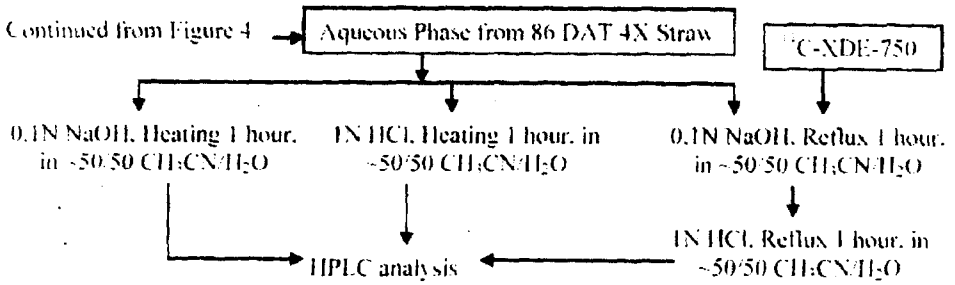
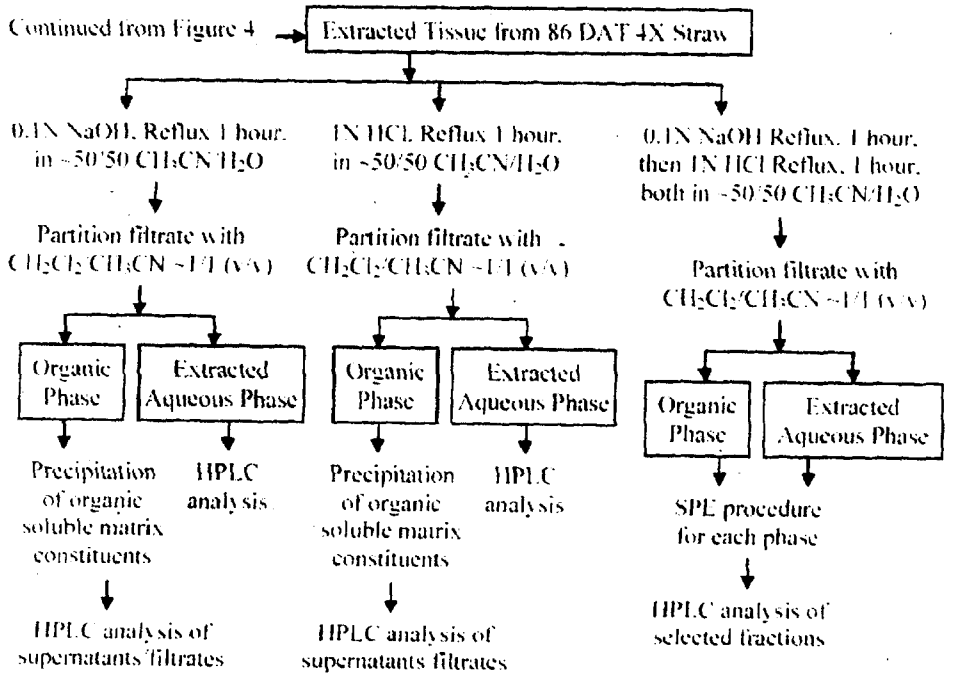


Figure 4. Flow Diagram for Initial Extraction of Wheat Forage, Hay, Grain or Straw

Note: In Figures 4-7, 4X refers to the low application rate and 8X refers to the high application rate.

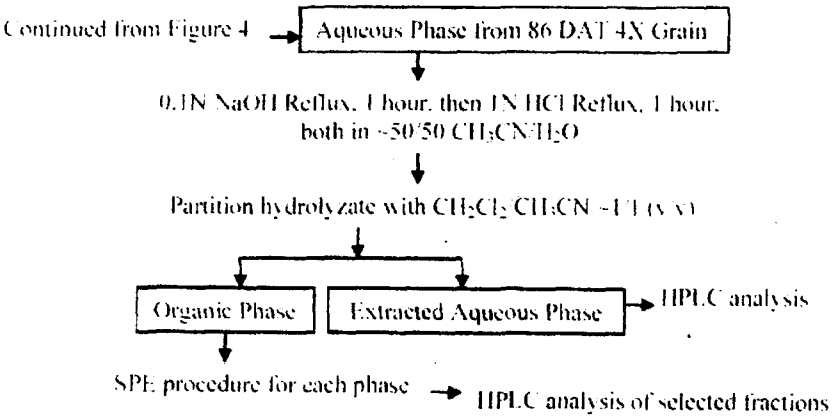


a) Flow Diagram for 86 DAT 4X Straw Aqueous Phase and ^{14}C-XDE-750

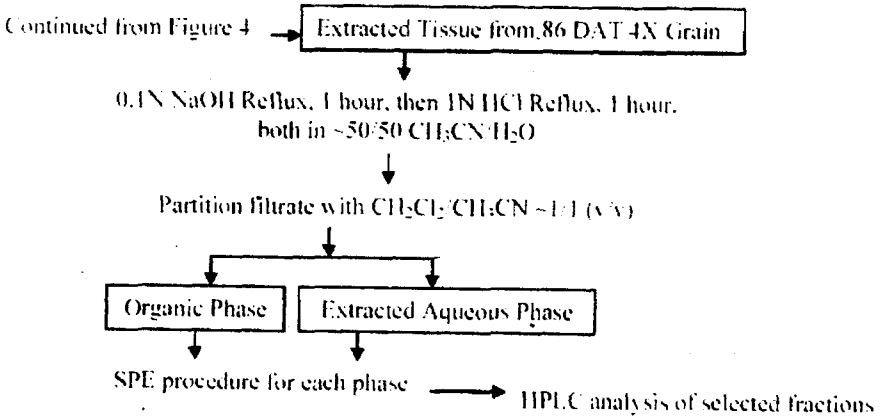


b) Flow Diagram for 86 DAT 4X Straw Extracted Tissue

Figure 5. Flow Diagrams for a) 86 DAT 4X Straw Aqueous Phase, ^{14}C-XDE-750 and b) 86 DAT 4X Straw Extracted Tissue



a) Flow Diagram for 86 DAT 4X Grain Aqueous Phase



b) Flow Diagram for 86 DAT 4X Grain Extracted Tissue

Figure 6. Flow Diagrams for a) 86 DAT 4X Grain Aqueous Phase and b) 86 DAT 4X Grain Extracted Tissue

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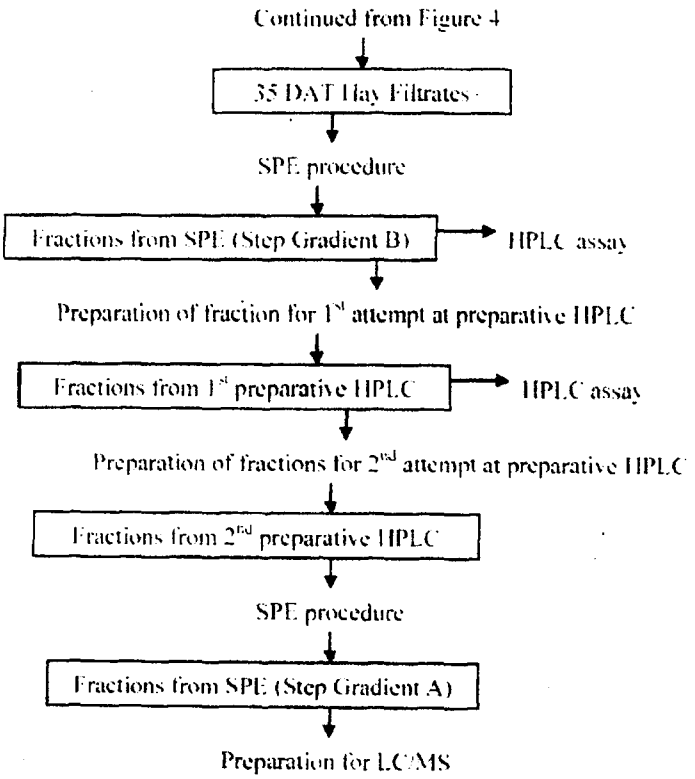

Figure 7. Flow Diagram for Isolation of Radioactive Residues from Combined 70/30 Extract and Reflux Filtrates of 35 DAT 4X Wheat Hay

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B.4.2. Analytical Methodology

High Performance Liquid Chromatography (HPLC): Concentrates of samples were dissolved with water or with water and acetonitrile. Some samples were also acidified (typically pH 0 to 2) by addition of HCl. Samples were filtered prior to HPLC, if necessary. Most of the HPLC was done using an Inertsil ODS-2 column eluted with gradients of acetonitrile and water. Some HPLC was done using YMC ODS-AQ C18 and Synergi Hydro-RP columns. Both acetonitrile and water solvents contained 0.5% trifluoroacetic acid or 0.1% formic acid. For radiolabeled samples, elution of radioactivity was monitored either by an inline flow detector or by collection of one-minute fractions followed by LSC analysis. Elution of non-radiolabeled reference standards was monitored using a variable wavelength detector set at 270 nm.

Liquid Chromatography-Mass Spectrometry (LC-MS): Nominal and accurate mass spectrometry analyses were conducted using a Micromass QTOF Micro, s/n YA091. The HPLC used was a Hewlett Packard/Agilent 1100 system with radiochemical detection using a Berthold LB509, with a 150 µLYG solid cell.

C. RESULTS AND DISCUSSION

The storage intervals for wheat samples are presented in Table C.1. The study submission reported that all samples were extracted within 15-42 days of harvest, and the analysis dates indicate that all initial HPLC analyses were completed within ~3 months. No supporting storage stability data are required because samples were stored frozen ≤3 months from harvest to initial analysis.

Total radioactive residues (TRR) in the harvested wheat samples are summarized in Table C.2.1. TRR of 2.022 and 4.121 ppm in 0-DAT forage samples declined to 0.418 and 0.874 ppm in the 14-DAT forage samples. TRR in the 35-DAT hay samples of 0.284 and 0.691 ppm were roughly the same as the 0.281 and 0.623 ppm residues found in 86-DAT straw. The 86-DAT grain TRR of 0.039 and 0.084 ppm were approximately seven times less than residues in corresponding straw samples.

Aliquots of the homogenized plant tissue samples were extracted with neutral solvent (70/30 acetonitrile/water, v/v) either by blending and/or by refluxing. At 0 DAT, greater than 98% TRR was extracted. The amount extracted declined to approximately 83% TRR in the straw and 75% TRR in the grain. The extractable radioactivity was analyzed by HPLC, and the results are shown in Tables C.2.2.1 (forage and hay) and C.2.2.2 (straw and grain). Also shown in Table C.2.2.2 are results from sequential base and acid treatment of the extractable radioactivity from the straw and grain. The results for untreated samples (not treated with base and acid) demonstrate that samples at later time points contained very little parent and that most of the radioactivity was found in two regions of the HPLC chromatogram (Regions 1 and 5) separate from aminopyralid.

In order to determine if the residues in Regions 1 and 5 might represent conjugates, sequential base and acid treatments were conducted with some of the 86-DAT straw and grain aqueous soluble extracts. The results for sequential base and acid treatment that are shown in Table C.2.2.2 demonstrate that most of the aqueous soluble radioactivity was converted to the parent aminopyralid. The extracted tissue from the straw and grain samples was subjected to base and/or acid treatment, and the liberated radioactivity was analyzed by HPLC. Prior to base and/or acid treatment, the straw and grain contained 17.0 and 25.0% TRR, respectively, as total bound residues. After base treatment, 6.6% TRR was converted to aminopyralid from the extracted tissue of straw. After sequential base and acid treatment, 11.0%TRR was converted to aminopyralid from the extracted tissue of grain. These data represented an approximately 39 to 44% conversion of bound radioactivity in the extracted tissue to the parent. No greater than 3.5% and 0.7%TRR remained in the non-extractable portion of straw and grain, respectively, after base and/or acid treatment. When results from base and/or acid treatment of both extractable radioactivity and extracted tissue were totalled, over 78% TRR in 86-DAT straw and almost 60% TRR in 86-DAT grain were found in the aminopyralid region of the chromatogram.

An aliquot of a solution of test substance was subjected to sequential base and acid treatment as described previously and analyzed by HPLC. The recovery of radioactivity after base and acid treatment was greater than 94%, and greater than 97% of the radioactivity was recovered as aminopyralid (in HPLC Region 3). These results demonstrate that parent molecule is stable to the base and acid treatments used to analyse aqueous soluble and nonextractable radioactivity.

Radioactive residues were isolated for structural identity determination from a 35-DAT hay sample that had not been base and/or acid treated. During the process of isolation, radioactivity in Region 5 that had appeared as one peak was found to be composed of at least five minor metabolites. Aminopyralid and two metabolites were isolated primarily from HPLC Regions 1 and 3. Using liquid chromatography-mass spectrometry (LC-MS), structural identity of parent compound was confirmed, plus glucose conjugates of parent and of hydroxylated aminopyralid were identified. Hydroxylated aminopyralid appeared to be a minor conjugated metabolite and comprised no greater than 5% TRR in the 35-DAT hay sample (see Table C.2.3).

Proposed Metabolic Pathway

The proposed metabolic pathway is presented in Figure C.3.1. As shown in the diagram, the metabolism of test substance is thought to proceed by conjugation of aminopyralid and hydroxylated aminopyralid with glucose. The hydroxylated aminopyralid appears to be a minor component and was not isolated in the non-conjugated form. While only two metabolites were isolated as glucose conjugates, any other metabolites present in wheat samples from this study but not identified were thought to be conjugates of glucose or similar endogenous compounds. This assessment was based on the fact that most of the radioactivity in the samples not initially found as aminopyralid could be hydrolyzed to aminopyralid. The study author reported that in studies conducted with picloram, water soluble and glucose conjugates of the parent compound were formed. Because of the structural similarity between picloram and aminopyralid, the

petitioner believes that it is likely that aminopyralid would have formed the same types of conjugates.

C.1. Storage Stability

TABLE C.1. Summary of Storage Conditions.

Matrix	Storage Temp. (°C)	Actual Study Duration [1]	Interval of Demonstrated Storage Stability [2]
Wheat, early forage	Frozen	52 days (1.7 months)	329 days (10.8 months)
Wheat, forage		38 days (1.3 months)	315 days (10.4 months)
Wheat, hay		93 days (3.1 months)	294 days (9.7 months)
Wheat, grain		55 days (1.8 months)	243 days (8.0 months)
Wheat, straw		55 days (1.8 months)	70 days (2.3 months)

[1] Storage interval from harvest to initial HPLC analysis; samples were analyzed within 13-36 days of extraction initiation, except hay samples which were analyzed 52 days after extraction.
[2] Based on the re-analysis of high dose extracts.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRR) in Wheat Matrices Following A Foliar Application of [2,6-^{14}C]Aminopyralid to Spring Wheat at the BBCH 26-28 Stage (6 to 8 Tillers) Using Two Treatment Rates.

Wheat Matrix	TRR (expressed as aminopyralid equivalents) in ppm	
	Low Rate (0.036 lb ai/A or 40.1 g ai/ha)	High Rate (0.072 lb ai/A or 80.3 g ai/ha)
0-DAT Forage	2.022	4.121
14-DAT Forage	0.418	0.874
35-DAT Hay	0.284	0.691
86-DAT Grain	0.039	0.084
86-DAT Straw	0.281	0.623

TABLE C.2.2.1. Distribution of the Parent and the Metabolites in Wheat Forage and Hay Following Application of ^{14}C-labeled Aminopyralid at a High Dose Rate (0.072 lb ai/A; 80.3 g ai/ha).

Metabolite Fraction	0-DAT Forage		14-DAT Forage		35-DAT Hay	
	TRR = 4.121 ppm		TRR = 0.874 ppm		TRR = 0.691 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
Organosoluble [1]						
Aqueous soluble (ACN/water)	98.7	4.069	90.7	0.793	88.4	0.610
Aminopyralid [2]	89.9	3.703				
Region 1 (6-8 min Rt) [2]	4.6	0.190				
Region 5 (20-21 min Rt) [2]	2.1	0.087				
Other Regions [2]	2.2	0.089	--	--	<LOQ	<LOQ
Aqueous soluble (ACN/water reflux)			1.8	0.016	2.2	0.016
Total aqueous soluble			92.5	0.809	90.6	0.626
Aminopyralid [2]			37.9	0.332	12.7	0.088
Region 1 (6-8 min Rt) [2]			36.0	0.314	50.0	0.345
Region 5 (20-21 min Rt) [2]			18.6	0.163	25.7	0.178
Total extractable (Aqueous + Organic)	98.7	4.069	92.5	0.809	90.6	0.626
Total bound (nonextractable) residues	1.3	0.052	7.5	0.066	9.4	0.065
Total identified as aminopyralid [3]	89.9	3.703	37.9	0.332	12.7	0.088
Total unidentified [4]	10.1	0.418	62.1	0.542	87.3	0.603
% Accountability	For the reported data, recoveries ranged from 78.2 to 115.9%, although most of the recoveries were in the range of 90 to 110 percent.					

[1] There was only one extract for the 0, 14 and 35 DAT samples, and it was not partitioned into organosoluble and aqueous soluble radioactivity. The HPLC results for this one extract were reported under the aqueous soluble section for these samples.

[2] Radioactivity eluting in HPLC Region 1 was more polar than parent aminopyralid, radioactivity eluting in HPLC Region 3 co-eluted with parent aminopyralid, radioactivity eluting in HPLC Region 5 was less polar than parent aminopyralid, and other Regions were composed of all other radioactivity in the HPLC profile.

[3] The totals listed for the 0 to 35 DAT samples were merely a repeat of the values for radioactivity found in Region 3 (aminopyralid) from the HPLC analysis of the aqueous soluble portion of these samples. If the 0 to 35 DAT samples had been subjected to base and/or acid treatment, the total identified for these samples would be expected to be as high or higher than the results obtained for the straw (in terms of %TRR).

[4] These totals are merely the total identified subtracted from 100%TRR or from the total ppm for that sample.

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TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Wheat Straw and Grain Following Application of ^{14}C-labeled Aminopyralid at a High Dose Rate (0.072 lb ai/A; 80.3 g ai/ha).

Metabolite Fraction	86-DAT Straw TRR = 0.623 ppm		86-DAT Grain TRR = 0.084 ppm	
	%TRR	ppm	%TRR	ppm
Organosoluble [1]	1.7	0.011	13.4	0.011
Aminopyralid [2]	0.1	<0.001	0.3	<0.001
Region 1 (6-8 min Rt) [2]	<0.1	<0.001	<LOQ	<LOQ
Region 5 (20-21 min Rt) [2]	0.3	0.002	6.9	0.006
Other Regions [2]	<LOQ	<LOQ	<LOQ	<LOQ
Aqueous soluble [1]	81.3	0.506	61.6	0.052
Aminopyralid [2]	11.3	0.071	15.9	0.013
Region 1 (6-8 min Rt) [2]	32.9	0.205	12.3	0.010
Region 5 (20-21 min Rt) [2]	37.0	0.230	33.3	0.028
Other Regions [2]	--	--	--	--
HPLC of aqueous soluble after base and acid treatment [3]				
Aminopyralid [2]	71.9	0.448	48.6	0.041
Region 1 (6-8 min Rt) [2]	6.1	0.038	1.8	0.002
Region 5 (20-21 min Rt) [2]	--	--	0.5	<0.001
Other Regions [2]	3.3	0.020	6.5	0.006
Total extractable (Aqueous + Organic)	83.0	0.517	75.0	0.063
Nonextractable residues	17.0	0.106	25.0	0.021
Total bound residues after base and/or acid treatment [4]	3.5	0.022	0.7	0.001
HPLC of extractable radioactivity released after base and/or acid treatment [5]				
Aminopyralid [2]	6.6	0.041	11.0	0.009
Region 1 (6-8 min Rt) [2]	1.5	0.009	<LOQ	<LOQ
Region 5 (20-21 min Rt) [2]	0.7	0.004	<LOQ	<LOQ
Other Regions [2]	0.9	0.005	1.6	0.001
Total identified as aminopyralid [6]	78.5	0.489	59.6	0.050
Total unidentified [7]	21.5	0.134	40.4	0.034
% Accountability	For the reported data, recoveries ranged from 78.2 to 115.9%, although most of the recoveries were in the range of 90 to 110 percent.			

[1] For the 86-DAT samples, the extract was partitioned into organosoluble and aqueous soluble radioactivity.
[2] Radioactivity eluting in HPLC Region 1 was more polar than parent aminopyralid, radioactivity eluting in HPLC Region 3 co-eluted with parent aminopyralid, radioactivity eluting in HPLC Region 5 was less polar than parent aminopyralid, and other Regions were composed of all other radioactivity in the HPLC profile.
[3] HPLC results obtained from analyses of samples of the aqueous soluble radioactivity after sequential base and acid treatment.
[4] Bound residues remaining after base and/or acid treatment of the extracted tissue (bound residues).
[5] HPLC results obtained from analyses of samples of extractable radioactivity released after base and/or acid treatment of extracted tissue (bound residues) from grain and straw.
[6] The totals identified for the straw and grain samples were the sum of radioactivity found as aminopyralid after base and/or acid treatment of the aqueous soluble radioactivity and the extracted tissue.
[7] These totals are merely the total identified subtracted from 100%TRR or from the total ppm for that sample.

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Wheat Matrices Following Application of ^{14}C-labeled Aminopyralid at a High Dose Rate (0.072 lb ai/A; 80.3 g ai/ha).

Compound	0-DAT Forage		14-DAT Forage		35-DAT Hay		86-DAT Straw		86-DAT Grain	
	TRR=4.121 ppm		TRR=0.874 ppm		TRR=0.691 ppm		TRR = 0.623 ppm		TRR = 0.084 ppm	
	% TRR	ppm	%TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Aminopyralid [1]	89.9	3.703	37.9	0.332	15.0	0.103	78.5	0.489	59.6	0.050
Aminopyralid-glucose [1]					15.6	0.108				
Hydroxylated aminopyralid-glucose [1]					4.8	0.033				
Total identified [2]	89.9	3.703	37.9	0.332	35.4	0.244	78.5	0.489	59.6	0.050
Total characterized [3]										
Total extractable [4]	98.7	4.069	92.5	0.809	90.6	0.626	96.5	0.601	99.3	0.083
Total bound [5]	1.3	0.052	7.5	0.066	9.4	0.0605	3.5	0.022	0.7	0.001

[1] The values listed for the 0- and 14-DAT samples were merely a repeat of the values for radioactivity found in Region 3 (aminopyralid) from the HPLC analysis of the aqueous soluble. It was not feasible to reliably estimate %TRR or ppm values for aminopyralid-glucose and hydroxylated aminopyralid-glucose for the 0- and 14-DAT samples. Identification of aminopyralid, aminopyralid-glucose and hydroxylated aminopyralid for the 35-DAT hay residues was established by LC-MS analysis of radioactivity isolated from solvent extracts that had not been treated with base and/or acid. Identification of aminopyralid in 86- DAT straw and grain was established by co-chromatography with reference standard for aminopyralid. The aminopyralid identified in the 86-DAT straw and grain samples was liberated using base and/or acid treatment of the extractable and bound (extracted tissue) radioactivity.

[2] If the 0 to 35 DAT samples had been subjected to base and/or acid treatment, the total identified for these samples would be expected to be as high or higher than the results obtained for the straw (in terms of %TRR).

[3] The total characterized would be essentially the same as the total extractable, since most of the extractable radioactivity was subjected to characterization by partition, SPE, HPLC, or other methods.

[4] The total extractable for 0 to 35 DAT was radioactivity that was extractable by solvent extraction and/or reflux. The total extractable for 86 DAT straw and grain was the difference between the total matrix TRR and the total bound. In this way, the total extractable for straw and grain included that radioactivity released after base and/or acid treatment.

[5] The total bound for 0 to 35 DAT was radioactivity that remained in extracted tissue after solvent extraction and/or reflux. The total bound for 86-DAT straw and grain was the radioactivity remaining in extracted tissue after base and/or acid treatment.

C.3. Proposed Metabolic Profile

FIGURE C.3.1. Proposed Metabolic Profile of Aminopyralid in Wheat

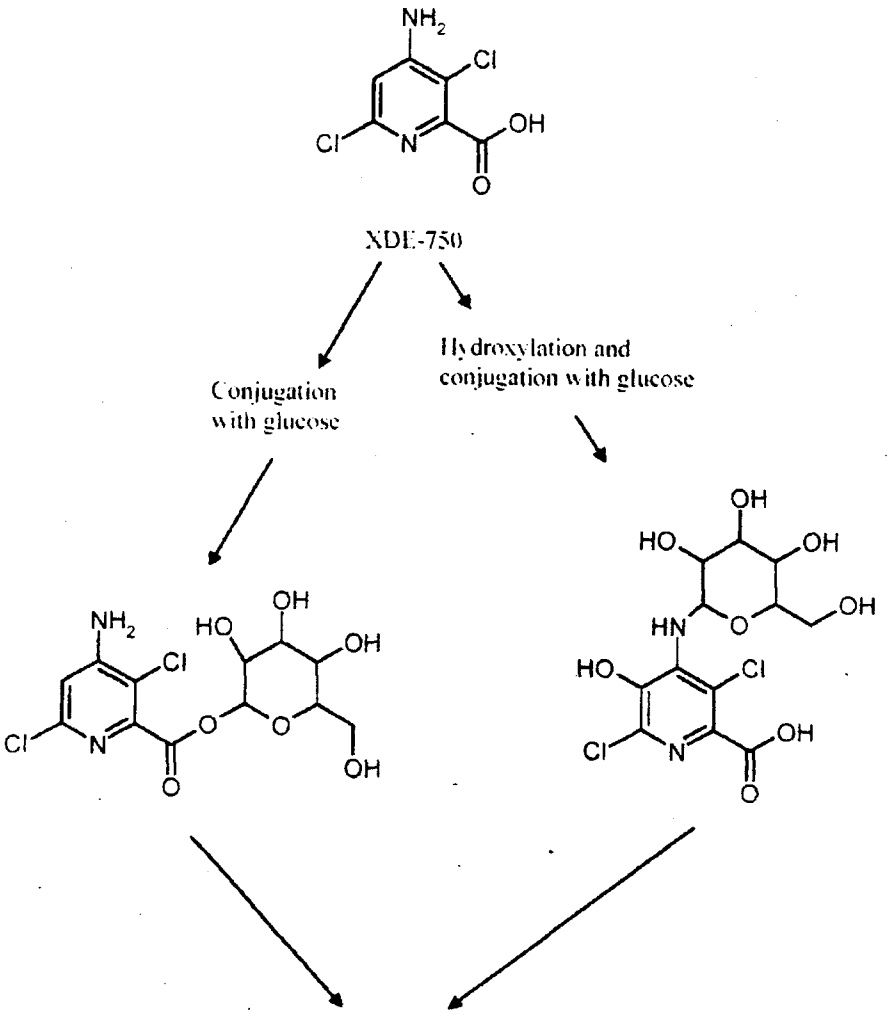
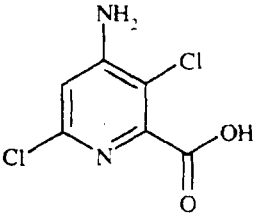
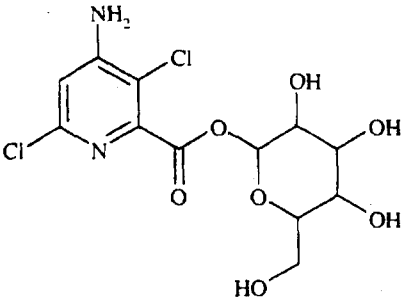
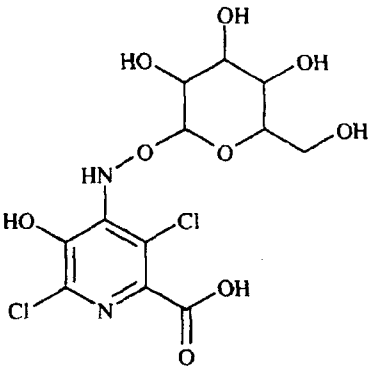


TABLE C.3.1. Identification of Compounds from Wheat Metabolism Study.		
Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
Aminopyralid/XDE-750	4-amino-3,6-dichloro-2-pyridinecarboxylic acid	
Aminopyralid - glucose conjugate	glucose conjugate of 4-amino-3,6-dichloro-2-pyridinecarboxylic acid	
Hydroxylated aminopyralid - glucose conjugate	glucose conjugate of 4-amino-3,6-dichloro-5-hydroxypyridine -2-carboxylic acid	

D. CONCLUSION

Residue characterization/identification of wheat matrices treated at high rate show that the parent aminopyralid, either conjugated or free, as the predominant residue component. The parent comprised approximately 90% of TRR in 0-DAT forage, 38% of TRR in 14-DAT forage, 15% of TRR in 35-DAT hay, 79% of TRR in 86-DAT straw, and 60% of TRR in 96-DAT grain. The identity of aminopyralid in wheat extracts was confirmed by mass spectrometry.

Most of the extractable radioactivity not found as aminopyralid in wheat hay could be converted to aminopyralid after sequential base and acid treatment and was thought to be parent compound conjugated to glucose or other natural plant constituents. When isolated and analyzed prior to

base and acid treatment, the radioactivity less polar than aminopyralid appeared to consist of at least five minor components. Two radioactive components that were more polar than or of similar polarity to aminopyralid were identified as a glucose conjugate of aminopyralid (15.6% TRR) and a glucose conjugate of a hydroxylated metabolite of aminopyralid (4.8% TRR).

Based on previous studies with picloram and on the results from base and acid treatment of water-soluble radioactivity in this study, the petitioner concluded that the primary metabolites of aminopyralid in wheat are conjugates of glucose or other endogenous compounds with parent compound. The fact that a substantial percentage of the radioactivity in extracted tissue is released as aminopyralid after base and/or acid treatment indicates that the conjugates are being incorporated into non-extractable compartments.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: MADoherty, 06/28/05; TSheramata, 06/13/05; HBietlot, 6/13/05
Petition Number(s): PP#4F6827
DP Barcode(s): D305665
PC Code: 005100/005209

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Aminopyralid/XDE-750/PC Code 005100/Dow AgroScienc... ...719
DACO 6.2/OPPTS 860.1300/OECD II 6.2.2, 6.2.3 & IIIA 8.2, 8.4.1, 8.4.2
Nature of the Residues in Livestock - Goat

Primary Evaluator Michael A Doherty Date: 6/28/05
Michael A. Doherty, Ph.D., Chemist, RAB2

Peer Reviewer M. Sheremata Date: June 6, 2005
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Approved by Date: Jun 13/05
Henri Bietlot, Ph.D.
A/Section Head, FREAS, HED, PMRA

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 11/08/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46235708 Macpherson, D. (2003) The Distribution and Metabolism of (Carbon 14)-XDE-750 in the Lactating Goat. Project Number: 201893. Unpublished study prepared by Inveresk Research International. 111 p.

EXECUTIVE SUMMARY:

Dow AgroSciences has submitted a goat metabolism study with aminopyralid. The aminopyralid test substance used in the study was labeled at the 2- and 6-positions of the pyridine ring and was administered to one lactating goat for six consecutive days. The target dose level was 15 ppm in the total diet, while the actual achieved daily dose received was 13.96 ppm over 6 days. A control goat was similarly dosed with capsules containing no test material. Milk was collected twice daily throughout the study, and tissues (liver, kidney, muscle, and fat) were collected at sacrifice (approximately 25 hours after cessation of dosing). The in-life and analytical phases of the study were conducted by Inveresk Research (Tranent, Scotland).

Total radioactive residues (TRR) in samples of milk and tissues, collected from the treated goat, were <0.01 ppm, except in kidney which bore a TRR of 0.071 ppm. Residues in milk, kidney, and liver were extracted and partitioned with organic solvents, and the nonextractable residues were subjected to enzyme hydrolysis. However, no residues were identified in milk and liver because the extracts from these matrices contained low levels (<0.01 ppm) of radioactivity. In kidney, the parent aminopyralid was the only residue identified at 79.9% TRR (0.057 ppm) by HPLC. The identification of the parent was confirmed by LC/MS/MS.

The study reported that elimination *via* urine and feces was approximately the same, and each accounted for *ca.* 46% of the total dose administered. A steady state of daily excretion in feces was established after 48 hours, while output in urine was variable. The identity of unchanged aminopyralid was confirmed in urine and feces.

No storage stability data were generated as part of this study, and none are required since milk and tissue samples were stored frozen for <6 months from sample collection to analysis. Kidney samples were stored the longest, 4.6 months from collection to HPLC analysis and 8.1 months from collection to LC/MS/MS confirmation.

Based on the results of the submitted study, the petitioner concluded that aminopyralid is rapidly absorbed and excreted by ruminants, with only kidneys having TRR in excess of 0.01 ppm. No significant bioaccumulation of aminopyralid residues is expected in the edible tissues of ruminants as a result of the proposed uses. Any residues that are found in milk or tissue will consist almost exclusively as aminopyralid.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the goat metabolism data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D305665 and in Canada's Regulatory Decision Document.

COMPLIANCE:

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

A. BACKGROUND INFORMATION

Aminopyralid (4-amino-3,6-dichloropyridine-2-carboxylic acid) is the proposed common name of XDE-750, a new active ingredient developed by Dow AgroSciences. Aminopyralid is a systemic postemergence herbicide which belongs to the pyridine carboxylic acid class of herbicides. The petitioner is currently proposing food/feed uses on grasses grown in rangelands and permanent pastures and on wheat for the selective control of invasive and noxious broadleaf weeds. It is also proposed for weed control in sites such as parks, electric utility rights-of way, forestry, woodlands, and wildlife openings, with smaller amounts used in railroads, utility substations, pipelines, and pumping stations.

The proposed end-use product (EP) is a soluble concentrate liquid (SC/L) referred to by the trade name GF-871 (EPA Reg. No. 62719-LRI). The active ingredient in GF-871 is formulated as the triisopropanolammonium (TIPA) salt, with the product containing 40.6% of aminopyralid TIPA salt at an acid equivalent (ae) of 21.1% or 2 lb ae/gal (240 g ae/L). The petitioner stated that the

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aminopyralid TIPA salt dissociates rapidly in water to the acid (aminopyralid) at environmental pH values greater than 2.56 (the pKa).

Currently, the 2 lb ae/gal (24 g ae/L) SC/L formulation is proposed for broadcast foliar application at maximum rates of 0.11 lb ae/A (120 g ae/hectare) on rangeland and permanent pastures and 0.009 lb ae/A (10 g ae/ha) on wheat. The proposed PHIs are 0 days for wheat hay and 50 days for wheat grain and straw. No PHI is listed or proposed for grasses or wheat forage.

TABLE A.1. Test Compound Nomenclature.

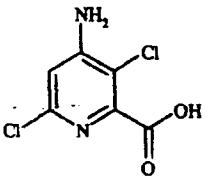
Chemical structure	
Common name	Aminopyralid
Company experimental name	XDE-750
IUPAC name	4-amino-3,6-dichloropyridine-2-carboxylic acid
CAS name	4-amino-3,6-dichloro-2-pyridinecarboxylic acid
CAS registry number	150114-71-9
End-use product (EP)	2 lb ae/gal (240 g ae/L) TIPA salt SC/L formulation (GF-871 Herbicide; EPA Reg. No. 62719-LRI; Aminopyralid Liquid Concentrate Herbicide in Canada)

TABLE A.2. Physicochemical Properties of the Aminopyralid Technical Grade Test Compound.

Parameter	Value	Reference
Melting point	163.5 °C	MRID 46235703, PMRA LS
pH	2.31 at 23.4 °C (1% solution in water)	MRID 46235703, PMRA LS
Relative density	1.72 at 20 °C	MRID 46235703, PMRA LS
Water solubility	2.48 g/L unbuffered water at 18 °C 212 g/L pH 5 buffer at 20 °C 205 g/L pH 7 buffer at 20 °C 203 g/L pH 9 Buffer at 20 °C	MRID 46235703, PMRA LS
Solvent solubility at 20 °C	methanol 52.2 g/L acetone 29.2 g/L n-octanol 3.9 g/L ethyl acetate 3.9 g/L 1,2-dichloroethane 0.2 g/L xylene 0.04 g/L heptane <10 µg/mL	MRID 46235703, PMRA LS
Vapor pressure	2.59×10^{-4} Pa at 25 °C; 9.52×10^{-9} Pa at 20 °C	MRID 46235703, PMRA LS
Dissociation constant, pK_a	2.56	MRID 46235703, PMRA LS
Octanol/water partition coefficient, $Log(K_{OW})$	0.201 unbuffered water at 19 °C; -1.76 at pH 5; -2.87 at pH 7; -2.96 at pH 9	MRID 46235703, PMRA LS

TABLE A.2. Physicochemical Properties of the Aminopyralid Technical Grade Test Compound.				
Parameter	Value			Reference
UV/visible absorption spectrum	Solution	Wavelength λ max, nm	Extinction coefficient ε, L/(mol*cm)	MRID 46235703, PMRA LS
	Neutral	217	29100	
	Basic	220	26100	
	(pH 12.6)	245	10150	
	Acidic	217	22800	
	(pH 1.4)	270	9140	

B. EXPERIMENTAL DESIGN

B.1. Livestock

TABLE B.1.1. General Test Animal Information.					
Species	Breed	Age (years)	Weight at study initiation (kg)	Health Status	Description of housing/holding area
Lactating goat (treated)	Toggenburg	2 yr 6 mo	71.0	Good	Stainless steel metabolism cage at Inveresk Research in Scotland, at 18-23 °C and 21-87% Rh, with 12 h. of light per day
Lactating goat (control)	Toggenburg	3 yr 6 mo	77.5	Good	

TABLE B.1.2. Test Animal Dietary Regime.				
Composition of Diet	Feed consumption	Water	Acclimation period	Predosing
Protein concentrate (commercial goat mix) and hay	500 g of feed offered twice daily; hay offered *ad libitum*. Actual hay consumption was 294-476 g/day, and total food consumption was 1300-1476 g/day	*ad libitum*	23 days	None

TABLE B.1.3. Test Animal Dosing Regime.			
Treatment Type	Feeding Level	Vehicle	Timing/Duration
Oral by gavage	13.31-14.79 ppm (average 13.96 ppm) in the diet; based on 19.14-19.68 mg/day and the average food consumption (1.422 kg) during the week prior to dosing.	gelatin capsule	Once a day immediately following morning milking for 6 consecutive days.

B.2. Test Materials

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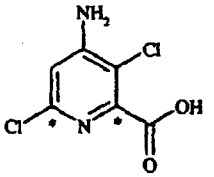
TABLE B.2.1. Test Material Characteristics.	
Chemical structure	
Radiolabel position	2- and 6-positions of the pyridinyl ring
Lot No.	Not provided
Purity	98.6%
Specific activity [1]	27.4 mCi/mmole

[1] The radiolabeled test substance was isotopically diluted with non-labeled aminopyralid for a final specific activity of 29.06 µCi/mg.

B.3. Sampling Information

TABLE B.3.1. Sample Collection Information			
Milk collected	Urine, feces and cage wash collected	Interval from last dose to sacrifice	Tissues harvested and analyzed
Twice daily: 714-1662 g/milking during dosing. Milk production during the acclimation period was not reported.	Urine and feces collected at 24-hour intervals, and cage wash collected at sacrifice.	23.5 hours	Liver, kidney, muscle (composite of triceps, semi-membranous and longissimus dorsi), and fat (composite of omental and perirenal)

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Milk was collected twice daily - in the morning immediately prior to dosing and approximately 8 hours later. Milk was also collected immediately prior to sacrifice. After samples were radioassayed, samples were stored frozen (-20 °C). Kidney and liver samples were homogenized on the day of collection; a sample was taken for radioassay, and the remaining samples were stored frozen (-20 °C) until further analysis. The different types of fat and muscle were respectively composited and frozen (-20 °C). Frozen composited muscle was homogenized in the presence of dry ice, while frozen composited fat was chopped into smaller pieces prior to being homogenized. Samples were taken for radioassay, and the remaining sample was frozen (-20 °C) pending analysis.

Only the milk (32- and 128-hour), kidney, and liver samples were extracted for metabolite characterization/identification. The extraction procedures are detailed in Figures B.4.1.1 (32-hour milk), B.4.1.2 (liver), and B.4.1.3 (kidney). Briefly, milk, kidney, and liver samples were extracted with methanol and then centrifuged. The methanol extract was then partitioned with diethyl ether/hexane and/or hexane. Only the resulting methanol phase of kidney was concentrated, and residues were redissolved in mobile phase for HPLC analysis. Milk

nonextractable residues were subjected to hydrolysis with pepsin enzyme in 0.1 M hydrochloric acid (37 °C for 24 hours); methanol was added to precipitate the pepsin hydrolysates.

Figure B.4.1.1. Extraction Flowchart for 32-hour Milk.

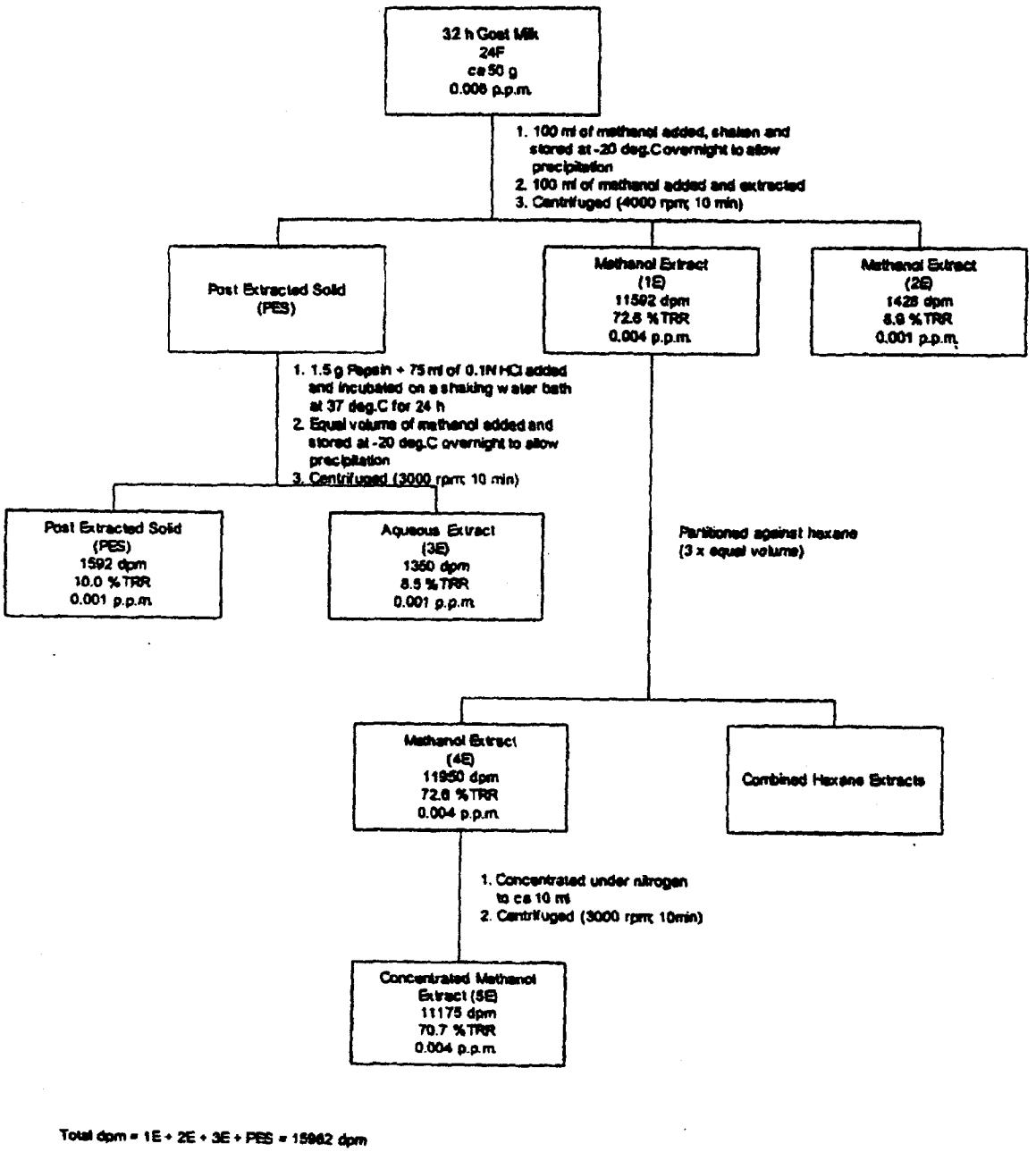


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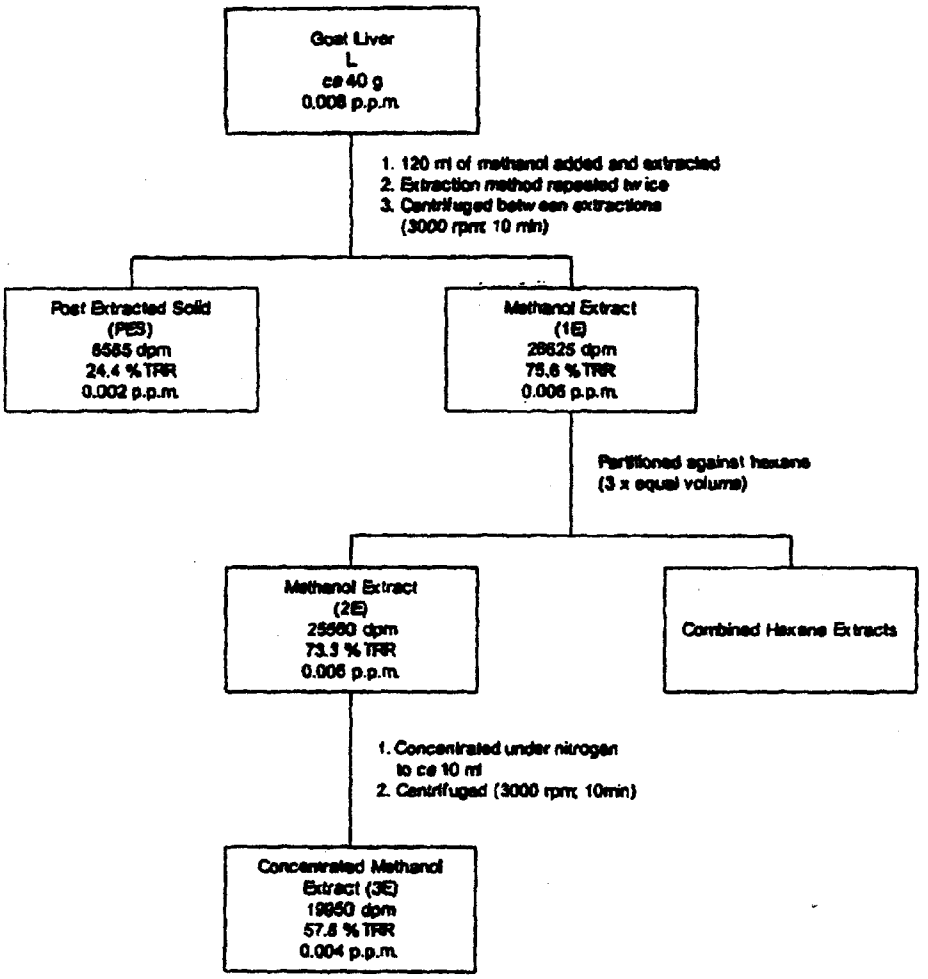
Figure B.4.1.2. Extraction Flowchart for Liver.



Total dpm = 1E + PES = 35210 dpm

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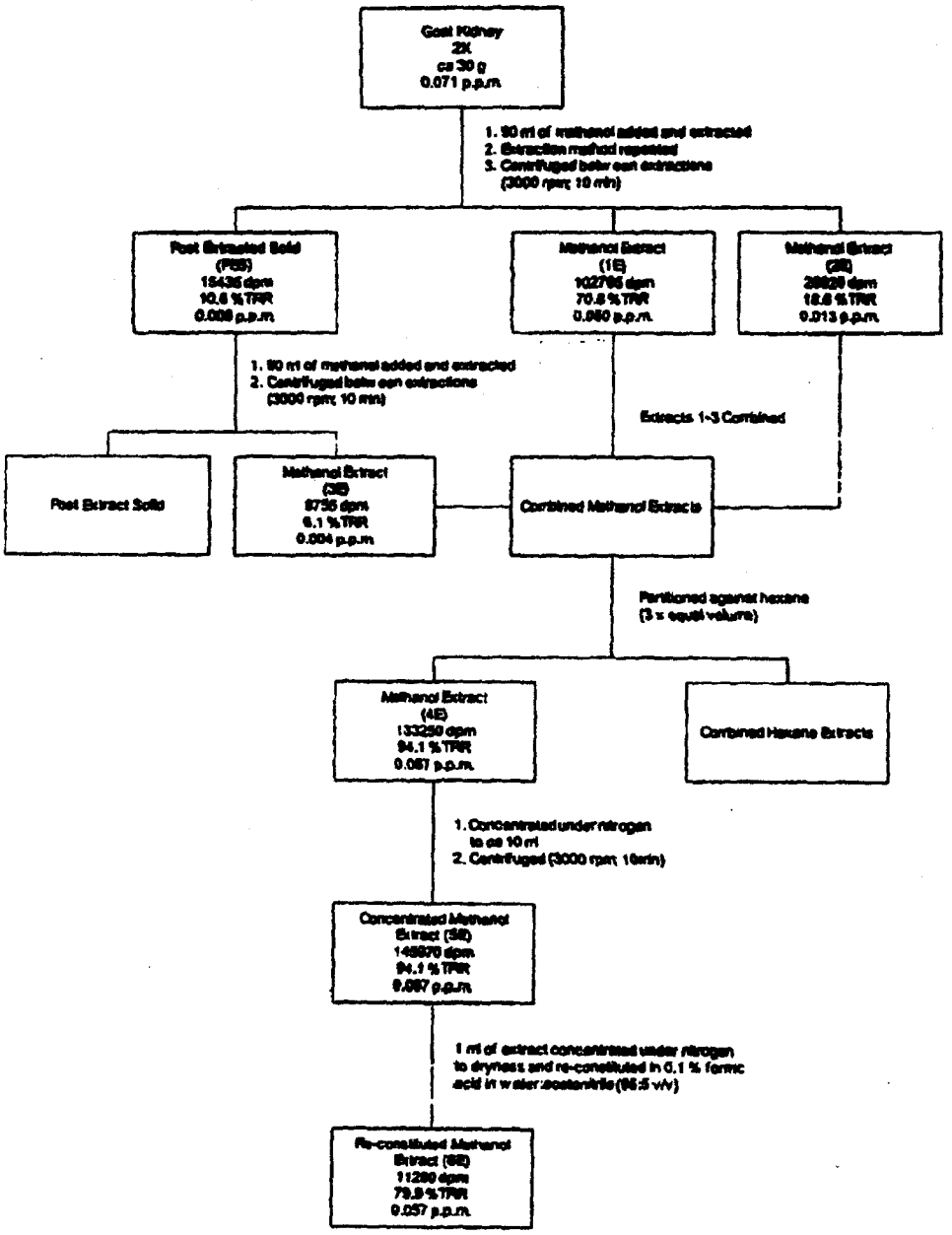
Figure B.4.1.3. Extraction Flowchart for Kidney.



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B.4.2. Analytical Methodology

Total radioactive residues in milk were determined by LSC, and TRR in tissues were determined by combustion/LSC. Extracts were radioassayed by LSC, and nonextractable residues were radioassayed by combustion/LSC. The reported limits of detection (LODs) were 0.00007 ppm for milk, 0.00088 ppm for kidney, liver, and muscle, and 0.00040 ppm for fat.

The kidney extract was analyzed by HPLC using a system equipped with an Inertsil ODS-2 (C8) column, a UV detector (254 nm), and fraction collection/LSC for radiodetection; a gradient mobile phase of water and acetonitrile, each containing 1% formic acid was used. Because of the low radioactivity in the kidney extract, multiple aliquots were injected and 20 fractions were collected for analysis by LSC. The single metabolite detected in the kidney extract was co-chromatographed with aminopyralid standard for identification.

Kidney extract Fractions 11-18 were combined and concentrated for LC/MS/MS analysis using positive ion electrospray ionization, to confirm residues as the parent, aminopyralid. The spectrum was compared to that of the aminopyralid standard.

C. RESULTS AND DISCUSSION

The storage intervals and conditions for the goat metabolism study are presented in Table C.1. The petitioner provided the dates of dose administration, initial and final sample extraction, and HPLC and LC/MS analyses of kidney extracts; actual LSC analysis dates were not provided for the individual matrices. Based on these dates, kidney samples were stored the longest, for up to 4.6 months from collection to HPLC analysis; confirmation of residues by LC/MS/MS was conducted 8.1 months after collection of kidney samples. Because milk and tissue samples were stored frozen for <6 months, no storage stability data are required to support the storage conditions and intervals of the samples from the goat metabolism study.

Total radioactive residues (TRR) in goat milk and tissues are reported in Table C.2.1. TRR were 0.003-0.008 ppm in milk, 0.001 ppm in fat, 0.071 ppm in kidney, 0.008 ppm in liver, and nondetectable (<0.00088 ppm) in muscle from a goat dosed orally with [2,6-^{14}C]aminopyralid at ~13.96 ppm in the diet for 6 consecutive days. Radioactivity was highest in kidney and very low (<0.01 ppm) in milk, fat, liver, and muscle. Residues in milk were generally highest in samples collected 8 hours after dosing, and appeared to plateau within 24-48 hours after initiation of dosing. Graphical depictions of residue levels in milk over the course of the study are presented in Figure C.2.1. A large portion of the administered dose was excreted; radioactivity in urine and feces accounted for a total of ~96% of the administered dose.

The distribution of the radioactivity in goat matrices is presented in Table C.2.2. Fat and muscle samples were not extracted due to extremely low radioactivity (≤0.001 ppm). The majority of the radioactivity (~72-96%) in milk, liver, and kidney was initially extracted using methanol. An additional methanol extraction of 32- and 128-hour milk samples released ≤10% TRR (≤0.001 ppm), and further radioactivity (4-9% TRR, ≤0.001 ppm) was released from milk using pepsin

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digestion. Only residues in kidney were identified by HPLC analysis. LC/MS/MS analysis was used for residue confirmation. Nonextractable residues accounted for ≤0.003 ppm in milk, liver, and kidney.

The characterization and identification of residues in goat matrices is summarized in Table C.2.3. The parent aminopyralid was the only residue identified in kidney, at 79.9% TRR (0.057 ppm). Aminopyralid was also identified as the major residue (>94% TRR) present in goat urine and feces.

C.1. Storage Stability

TABLE C.1. Summary of Storage Conditions

Matrix	Storage Temp. (°C)	Actual Storage Duration	Interval of Demonstrated Storage Stability
Goat, milk, fat, liver, and muscle	-20	83 days (2.7 months) [1]	None required.
Goat, kidney		140 days (4.6 months); 245 days (8.1 months) LC/MS confirmation	

[1] Maximum storage interval based on the first dose and final extraction dates, actual LSC analysis dates were not provided.

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C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRR) in Milk, Tissue and Excreta

Matrix	Collection Timing (hours after first dose)	[2,6-^{14}C]Aminopyralid	
		% of administered dose	ppm
Urine	24	3.10	Not reported
	48	5.91	Not reported
	72	1.29	Not reported
	96	12.90	Not reported
	120	14.47	Not reported
	144 (sacrifice)	8.36	Not reported
	Total	46.03	--
Feces	24	1.31	Not reported
	48	10.67	Not reported
	72	7.65	Not reported
	96	8.69	Not reported
	120	9.70	Not reported
	144	8.60	Not reported
	Total	46.62	--
Cage Wash	144 (sacrifice)	2.88	Not reported
Total excreta	over study period	95.53	--
Milk	8	0.0	0.005
	24	0.0	0.003
	32	0.01	0.006
	48	0.0	0.003
	56	0.01	0.007
	72	0.0	0.003
	80	0.01	0.008
	96	0.0	0.003
	102	0.01	0.007
	120	0.0	0.003
	128	0.01	0.008
	144 (sacrifice)	0.01	0.004
Muscle	sacrifice	--	ND (<0.00088)
Fat	sacrifice	--	0.001
Kidney	sacrifice	0.01	0.071
Liver	sacrifice	0.01	0.008
% of Administered Dose	over study period	95.60	--

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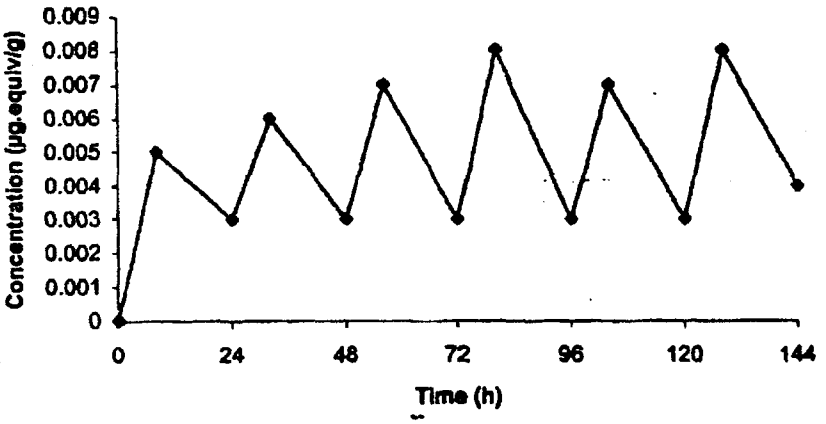
FIGURE C.2.1. Pharmacokinetics of Aminopyralid in Milk of Lactating Goat
(Copied without revision from MRID 46235708)

Results for individual timepoints



Results for 24 h intervals

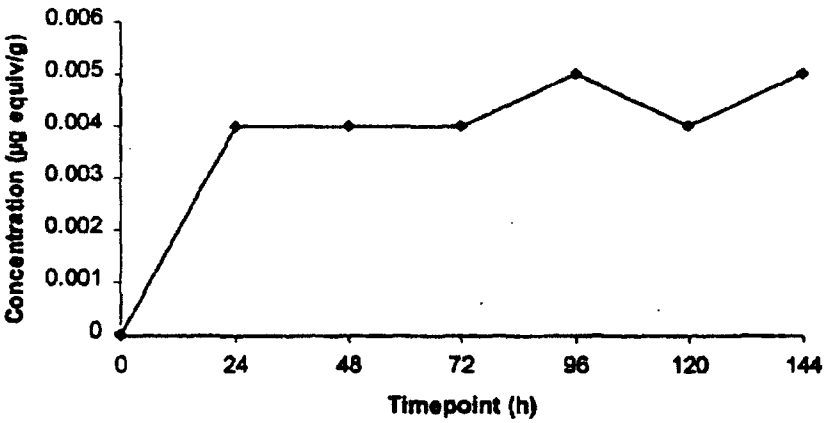


TABLE C.2.2. Distribution of the Parent and the Metabolites in Livestock Matrices Following Dosing with ^{14}C-labeled Aminopyralid. [1]

Metabolite Fraction [2]	Milk, 32-hour		Milk, 128-hour		Liver		Kidney	
	TRR = 0.006 ppm		TRR = 0.008 ppm		TRR = 0.008 ppm		TRR = 0.071 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Methanol extract	72.6	0.004	72.4	0.006	75.6	0.006	95.5	0.067
Methanol phase	70.7	0.004	70.9	0.006	57.8	0.004	79.9	0.057
Aminopyralid							79.9	0.057
Hexane phase	NDR	NDR	NDR	NDR	NDR	NDR	NDR	NDR
Methanol	8.9	0.0005	10.3	0.001				
Pepsin hydrolysate	8.5	0.001	4.4	<0.001				
Solids	10.0	0.001	12.9	0.001	24.4	0.002	4.5	0.003

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] Refer to the extraction flowcharts for a description of the extract fractions.
NDR = No Detectable Residue

TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Livestock Matrices Following Dosing with Radiolabeled Aminopyralid at 13.96 ppm in the Diet.

Compound	Milk, 32-hour		Milk, 128-hour		Liver		Kidney	
	TRR = 0.006 ppm		TRR = 0.008 ppm		TRR = 0.008 ppm		TRR = 0.071 ppm	
	% TRR	ppm	%TRR	ppm	% TRR	ppm	%TRR	ppm
Aminopyralid	--	--	--	--	--	--	79.9	0.057
Methanol extractable	79.6	0.0045	81.2	0.007	57.8	0.004	--	--
Pepsin hydrolysate	8.5	0.001	4.4	<0.001	--	--	--	--
Total identified	0.0	0.0	0.0	0.0	0.0	0.0	79.9	0.057
Total characterized	88.1	0.0055	85.6	<0.008	57.8	0.004	0.0	0.0
Total extractable	90.0	0.0055	87.1	<0.008	75.6	0.006	95.5	0.067
Unextractable (PES)[1]	10.0	0.001	12.9	0.001	24.4	0.002	4.5	0.003
Accountability[2]	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.

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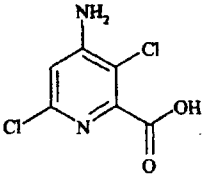
C.3. Proposed Metabolic Profile

Aminopyralid does not appear to be extensively metabolized or significantly conjugated in lactating goat.

TABLE C.3.1. Identification of Compounds from Goat Metabolism Study

Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
Aminopyralid/XDE-750	4-amino-3,6-dichloro-2-pyridinecarboxylic acid	

D. CONCLUSION

Results from this study indicate that aminopyralid is rapidly absorbed and excreted by ruminants, with only kidneys having residues in excess of 0.01 ppm; urine and feces accounted for a total of ~96% of the administered dose. Any residues that might be present in edible tissues will consist almost exclusively of aminopyralid itself, since the compound does not appear to be either extensively metabolized or significantly conjugated.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: MADoherty, 06/28/05; TSheramata, 06/13/05; HBietlot, 6/13/05
Petition Number(s): PP#4F6827
DP Barcode(s): D305665
PC Code: 005100/005209

Template Version September 2003

Primary Evaluator _____ Date: 6/28/05
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Evaluation Officer, FREAS, HED, PMRA

Approved by _____ Date: June 13/05
Henri Bietlot, Ph.D.
A/Section Head, FREAS, HED, PMRA

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 11/08/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46235711 Magnussen, J. (2004) 14C XDE-750 Poultry Nature of the Residue Study. Project Number: 030009, 379/131. Unpublished study prepared by Dow AgroSciences, LLC and Wildlife International, Ltd. 117 p.

EXECUTIVE SUMMARY:

Dow AgroSciences has submitted a hen metabolism study with aminopyralid. The aminopyralid test substance used in the study was labeled at the 2- and 6-positions of the pyridine ring and was administered to ten single-comb, white laying hens at a dose level of 1.024 mg/kg bw/day. Based on feed consumption during the acclimation period, this dose level was equivalent to ~12 ppm (or mg of test material/kg of feed consumed). Hens were dosed for seven consecutive days by oral administration each day of a single gelatin capsule containing the test material. During the dosing phase, excreta was collected at 24-hour intervals, while eggs were collected twice a day (morning and evening) and pooled to give a single sample for each day. Within approximately 25 hours of the final dose, the hens were sacrificed, and samples of muscle, fat, liver and skin with subcutaneous fat were collected for analysis. The in-life phase (including sample preparation and total radioactivity determinations) of the study was conducted by Wildlife International, Ltd. (Easton, MD), and the analytical phase was conducted by Dow AgroSciences, Regulatory Laboratories (Indianapolis, Indiana).

Following preparation, all samples were assayed for total radioactive residues (TRR) either by solubilization (fat only) or by combustion analysis. TRR in/on all collected samples of eggs and tissues were <0.01 ppm. Due to the low TRR levels in all egg and tissue samples, none of these samples was subjected to residue characterization/identification.

The study reported that a large portion (~79%) of the administered dose was excreted. To provide information concerning the nature of the residue in hen excreta, samples of Day-7 excreta were subjected to residue characterization. Approximately 97% of the TRR in excreta was extracted with acetonitrile/water, and an additional 3.1% TRR was released by acid reflux. The nonextractable residues were <0.1% TRR. The parent aminopyralid was identified as the major residue in excreta, at 92.9% TRR. Two poorly resolved fractions (3.3% TRR) were characterized as conjugates of aminopyralid following acid and base hydrolysis.

Based on the results of this study, the petitioner concluded that aminopyralid is rapidly excreted in laying hens with minimal transference of residues to eggs and tissues. Furthermore, any metabolism which might take place would result in the formation of conjugated residues of aminopyralid.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the hen metabolism data are classified as scientifically acceptable. Because the dosing level used in the study resulted in low radioactivity levels in tissues and eggs, preventing identification of metabolites in any matrix, the study may only be used to support aminopyralid uses yielding a poultry dietary burden that is not significantly greater than 12 ppm.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D305665 and in Canada's Regulatory Decision Document.

COMPLIANCE:

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

A. BACKGROUND INFORMATION

Aminopyralid (4-amino-3,6-dichloropyridine-2-carboxylic acid) is the proposed common name of XDE-750, a new active ingredient developed by Dow AgroSciences. Aminopyralid is a systemic postemergence herbicide which belongs to the pyridine carboxylic acid class of herbicides. The petitioner is currently proposing food/feed uses on grasses grown in rangelands and permanent pastures and on wheat for the selective control of invasive and noxious broadleaf weeds. It is also proposed for weed control in sites such as parks, electric utility rights-of way, forestry, woodlands, and wildlife openings, with smaller amounts used in railroads, utility substations, pipelines, and pumping stations.

The proposed end-use product (EP) is a soluble concentrate liquid (SC/L) referred to by the trade name GF-871 (EPA Reg. No. 62719-LRI). The active ingredient in GF-871 is formulated as the

triisopropanolammonium (TIPA) salt, with the product containing 40.6% of aminopyralid TIPA salt at an acid equivalent (ae) of 21.1% or 2 lb ae/gal (240 g ae/L). The petitioner stated that the aminopyralid TIPA salt dissociates rapidly in water to the acid (aminopyralid) at environmental pH values greater than 2.56 (the pKa).

Currently, the 2 lb ae/gal (240 g ae/L) SC/L formulation is proposed for broadcast foliar application at maximum rates of 0.11 lb ae/A (120 g ae/hectare) on rangeland and permanent pastures and 0.009 lb ae/A (10 g ae/ha) on wheat. The proposed PHIs are 0 days for wheat hay and 50 days for wheat grain and straw. No PHI is listed or proposed for grasses or wheat forage.

TABLE A.1. Test Compound Nomenclature.

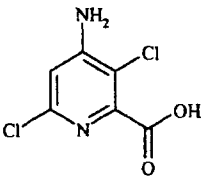
Chemical structure	
Common name	Aminopyralid
Company experimental name	XDE-750
IUPAC name	4-amino-3,6-dichloropyridine-2-carboxylic acid
CAS name	4-amino-3,6-dichloro-2-pyridinecarboxylic acid
CAS registry number	150114-71-9
End-use product (EP)	2 lb ae/gal (240 g ae/L) TIPA salt SC/L formulation (GF-871 Herbicide; EPA Reg. No. 62719-LRI; Aminopyralid Liquid Concentrate Herbicide in Canada)

TABLE A.2. Physicochemical Properties of the Aminopyralid Technical Grade Test Compound.

Parameter	Value	Reference
Melting point	163.5 °C	MRID 46235703, PMRA LS
pH	2.31 at 23.4 °C (1% solution in water)	MRID 46235703, PMRA LS
Relative density	1.72 at 20 °C	MRID 46235703, PMRA LS
Water solubility	2.48 g/L unbuffered water at 18 °C 212 g/L pH 5 buffer at 20 °C 205 g/L pH 7 buffer at 20 °C 203 g/L pH 9 Buffer at 20 °C	MRID 46235703, PMRA LS
Solvent solubility at 20 °C	methanol 52.2 g/L acetone 29.2 g/L n-octanol 3.9 g/L ethyl acetate 3.9 g/L 1,2-dichloroethane 0.2 g/L xylene 0.04 g/L heptane <10 µg/mL	MRID 46235703, PMRA LS
Vapor pressure	2.59 x 10^{-8} Pa at 25 °C; 9.52 x 10^{-9} Pa at 20 °C	MRID 46235703, PMRA LS
Dissociation constant, pK_a	2.56	MRID 46235703, PMRA LS

TABLE A.2. Physicochemical Properties of the Aminopyralid Technical Grade Test Compound.				
Parameter	Value			Reference
Octanol/water partition coefficient, Log(K_{OW})	0.201 unbuffered water at 19 °C; -1.76 at pH 5; -2.87 at pH 7; -2.96 at pH 9			MRID 46235703, PMRA LS
UV/visible absorption spectrum	Solution	Wavelength λ max, nm	Extinction coefficient ε, L/(mol*cm)	MRID 46235703, PMRA LS
	Neutral	217	29100	
	Basic	220	26100	
	(pH 12.6)	245	10150	
	Acidic	217	22800	
	(pH 1.4)	270	9140	

B. EXPERIMENTAL DESIGN

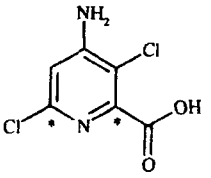
B.1. Livestock

TABLE B.1.1. General Test Animal Information.					
Species	Breed	Age (weeks)	Weight at study initiation (g)	Health Status	Description of housing/holding area
Laying hen (*Gallus domesticus*)	single-comb, white laying hens	45	1.288-2.012	Good	Individual pens at Wildlife International in MD, at 22.24 ± 0.15 °C and 60 ± 4% Rh, with 12 h. of light per day

TABLE B.1.2. Test Animal Dietary Regime.				
Composition of Diet	Feed consumption	Water	Acclimation period	Predosing
Game bird ration that contained a minimum of 27% protein, 2.5% crude fat and 5% crude fiber and that was supplemented by 5% limestone	102-181 g/bird/day (daily average of treated hens) 117-183 g/bird/day (daily average of control hens)	*ad libitum*	8 weeks	Animals were not predosed.

TABLE B.1.3. Test Animal Dosing Regime			
Treatment Type	Feeding Level	Vehicle	Timing/Duration
Oral	11.56 ppm in the diet; based on the average daily feed consumption (147 g feed/bird/day) of the treated hens during dosing.	A single capsule for each hen administered once each day.	7 days (21 May 2003 - 28 May 2003). All doses administered at approximately the same time each morning.

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics.	
Chemical structure	
Radiolabel position	2- and 6-positions of the pyridinyl ring
Lot No.	SPS Reference No. DE3-E1004-77; Inventory No. INV1893
Purity	98.2%
Specific activity [1]	28.6 mCi/mmol; 138.2 µCi/mg

[1] The radiolabeled test substance was isotopically diluted with non-labeled aminopyralid for a final specific activity of 30 µCi/mg (66,600 dpm/µg or 1,110 Bq/µg at the time of application).

B.3. Sampling Information

TABLE B.3.1. Sample Collection Information			
Eggs collected	Excreta and cage wash collected	Interval from last dose to sacrifice	Tissues harvested and analyzed
Eggs were collected twice daily: 0.6-1.1 eggs/bird/day during dosing; egg production during the acclimation period was not reported.	Excreta collected at 24-hour intervals, and cage wash collected at sacrifice.	24.5-25.5 hours	Liver, muscle (composite of light and dark meat), abdominal fat, and skin with subcutaneous fat

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Eggs were collected twice daily: the evening samples were stored at ~4 °C until they were combined with the following morning samples for daily composite samples for each group (treated or untreated). Daily egg samples were then stored frozen. Group tissue samples were respectively combined, cut into small pieces, and stored frozen. Liver, fat, and skin samples were homogenized with dry ice, while muscle samples were homogenized in a food cutter. Homogenized samples were frozen pending radioassay.

B.4.2. Analytical Methodology

Total radioactive residues (TRR) in eggs and tissues, except fat, were determined by combustion/LSC. Fat samples were first solubilized and then analyzed by LSC. Radioassay was conducted at the in-life laboratory; the reported limits of detection (LODs) were 0.0012 ppm for eggs, 0.0017 ppm for liver and muscle, 0.0019 ppm for fat, and 0.0014-0.0016 ppm for skin with subcutaneous fat.

The excreta extract was analyzed by HPLC using a system equipped with an Inertsil ODS-2 (C8) column, a UV detector (270 nm), and fraction collection/LSC for radiodetection; a gradient

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mobile phase of water and acetonitrile (90:10 to 50:50, water/acetonitrile), each containing 0.5% trifluoroacetic acid (TFAA) was used. The single metabolite detected in the excreta was compared to the HPLC chromatogram of aminopyralid standard for identification (UV detector set at 270 nm).

C. RESULTS AND DISCUSSION

The storage intervals and conditions for the hen metabolism study are presented in Table C.1. All hen egg and tissue samples were radioassayed within 0.5 months of collection, therefore, no storage stability data are required to support the storage conditions and intervals of the samples from the hen metabolism study.

Total radioactive residues in hen eggs and tissues are reported in Table C.2.1. TRR were nondetectable (<0.0012-0.0040 ppm) in eggs, nondetectable (<0.0017 and 0.0019 ppm) in muscle and fat, 0.0024 ppm in liver, and 0.0029 ppm in skin with subcutaneous fat from ten laying hens dosed orally with [2,6-^{14}C]aminpyralid at ~12 ppm in the diet for 7 consecutive days. Residues in eggs gradually increased over the 7 day dosing period, but remained low; a graph of the residue levels in eggs over the course of the study is presented in Figure C.2.1. A large portion of the administered dose was excreted; excreta accounted for a total of ~79% of the administered dose.

Because of low radioactivity (<0.005 ppm) in hen eggs and tissues, none of these samples were extracted for metabolite characterization/identification. Therefore, no distribution or summary of characterized metabolite tables are included in this DER.

To provide information concerning the metabolism of aminopyralid in hens, the residues in Day-7 excreta were investigated. Approximately 97% of the TRR in excreta was extracted with acetonitrile/water and an additional 3.1% TRR was released by acid reflux. Nonextractable residues were <0.1% TRR. The parent aminopyralid was identified as the major residue in excreta, at 92.9% TRR. Two poorly resolved fractions (3.3% TRR) were characterized as conjugates of aminopyralid following acid and base hydrolysis.

C.1. Storage Stability

TABLE C.1. Summary of Storage Conditions

Matrix	Storage Temp. (°C)	Actual Storage Duration	Interval of Demonstrated Storage Stability
Hen, eggs and tissues	-20	13-15 days (<0.5 months) [1]	None required.

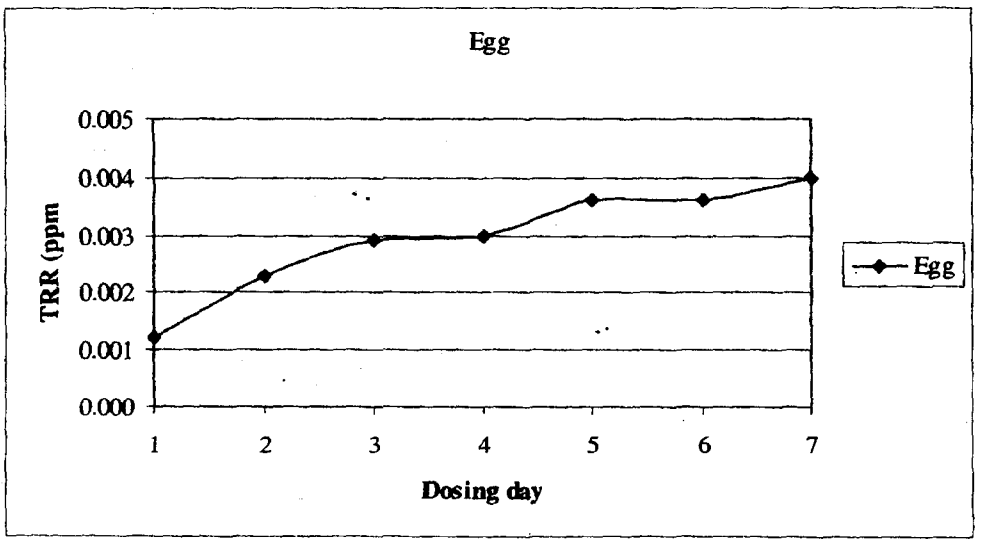
[1] Storage interval from collection to radioassay.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRR) in Eggs, Tissue and Excreta

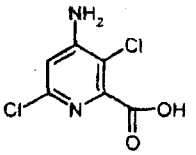
Matrix	Collection Timing (days)	[2,6-^{14}C]Aminopyralid	
		% of daily administered dose	ppm
Excreta	1	55.8	7.81
	2	55.1	8.45
	3	80.2	9.94
	4	84.3	9.63
	5	70.3	7.90
	6	87.2	10.75
	7	117.2	12.37
	Total of administered dose	78.6	--
Egg	1	<0.01	ND (<0.0012)
	2		0.0023
	3		0.0029
	4		0.0030
	5		0.0036
	6		0.0036
	7		0.0040
Muscle	sacrifice	<0.01	ND (<0.0017)
Fat	sacrifice		ND (<0.0019)
Liver	sacrifice		0.0024
Skin with subcutaneous fat	sacrifice		0.0029
% of Administered Dose	over study period	78.6	-

FIGURE C.2.1. Pharmacokinetics of Aminopyralid in Eggs of Laying Hens



C.3. Proposed Metabolic Profile

FIGURE C.3.1. Proposed Metabolic Profile of Aminopyralid in Laying Hen (Copied without alteration from MRID 46235711)



XDE-750

Less than 10% converted to aqueous soluble residues that in part can be converted back to XDE-750 using base or acid hydrolysis.

D. CONCLUSION

Based on the results of this study, aminopyralid is rapidly excreted in laying hens, with minimal transference of residues to eggs and tissues. The TRR accounted for in the treated egg and tissue samples represented < 0.01 % of the administered dose. The residue levels in all egg and edible tissue samples were < 0.01 ppm. Furthermore, any metabolism which might take place would result in the formation of conjugated residues of aminopyralid.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: MADoherty, 06/28/05; TSheramata, 06/13/05; HBietlot, 6/13/05
Petition Number(s): PP#4F6827
DP Barcode(s): D305665
PC Code: 005100/005209

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Aminopyralid/XDE-750/PC Code 005100/Dow AgroSciences/62719
DACO 7.2.1, 7.2.2, and 7.2.3/OPPTS 860.1340/OECD IIA 4.2.5, 4.2.6 and 4.3
Residue Analytical Method: Ruminant

Primary Evaluator _____ Date: 6/25/05
Michael A. Doherty, Ph.D.; Chemist, RAB2

Peer Reviewer _____ Date: June 6/05
Tamara Sheremata, Ph.D.,
Evaluation, FREAS, HED, PMRA

Approved by _____ Date: 7/- 13/05
Henri Bietlot, Ph.D.,
A/Section Head, FREAS, HED, PMRA

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 11/08/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

46235714 Reed, R. (2004) Independent Laboratory Validation of Dow AgroSciences LLC Method GRM 03.18 - Determination of Residues of Aminopyralid in Bovine Tissues by Liquid Chromatography with Tandem Mass Spectrometry. Project Number: 030098, ML03/1122/DOW. Unpublished study prepared by Morse Laboratories. 130 p.

46235716 Rutherford, L.; Hastings, M. (2003) Method Validation Report for the Determination of Aminopyralid in Bovine Tissues by Liquid Chromatography with Tandem Mass Spectrometry Using Dow AgroSciences LLC Method GRM 03.18. Project Number: 021327, GRM/03/18. Unpublished study prepared by Dow AgroSciences LLC. 53 p.

EXECUTIVE SUMMARY:

Dow AgroSciences has proposed LC/MS/MS Method GRM 03.18, entitled "Determination of Residues of Aminopyralid in Bovine Tissues by Liquid Chromatography with Tandem Mass Spectrometry" for the enforcement of tolerances for residues of aminopyralid in ruminant milk and tissues. The proposed LC/MS/MS method was used to determine residues of aminopyralid in/on samples from the cattle feeding study associated with DP Barcode D305665.

Briefly, milk or ground tissue samples are extracted with methanol/sodium bicarbonate. The extract is cleaned up through an anion-exchange solid-phase extraction (SPE) plate. The internal standard, $^{13}C_2{}^{15}N$-aminopyralid, is added to the eluate, and residues are derivatized with butyl chloroformate to form the 1-butyl esters of aminopyralid for LC/MS/MS analysis. The validated limit of quantitation (LOQ) is 0.01 ppm for all matrices, and the calculated limit of detection (LOD) is 0.003 ppm.

Method validation data for LC/MS/MS Method GRM 03.18 demonstrated adequate method recoveries of aminopyralid from bovine whole milk, cream, skimmed milk, fat, kidney, liver, and muscle fortified at the LOQ (0.01 ppm) and up to 100x LOQ (1.0 ppm) for milk, fat, liver, and muscle, or 250x LOQ (2.5 ppm) for kidney. The fortification levels and samples used in method validation adequately bracket expected residue levels in ruminant milk and tissues. Two recoveries were below the acceptable 70-120% range: 67% in one kidney sample fortified at the LOQ and 64% in one milk cream sample fortified at the higher level (1.0 ppm). The petitioner stated that the low recoveries were due to random error and not systematic error. Acceptable concurrent method recovery data were included with the feeding study submitted in conjunction with DP Barcode D305665.

Adequate independent laboratory validation data have been submitted using bovine milk and kidney. The petitioner concluded that confirmatory analysis procedures are not required for the proposed enforcement method due to the high specificity of the LC/MS/MS method. Because the method only monitors one transition ion, HED defers to ACB to determine whether confirmatory analysis procedures are needed for the method.

A radiovalidation study was not conducted for the LC/MS/MS enforcement method because residues in samples from a goat metabolism study were very low; only kidney samples had TRR >0.01 ppm. However, the extraction solvent used in the proposed enforcement method is similar to that used in the goat metabolism study. In the goat metabolism study (refer to the DER for MRID 46235708), 76-96% TRR was extracted from milk, liver, and kidney samples using methanol (fat and muscle samples were not subjected to extraction procedures due to low residue levels). In the proposed enforcement method, methanol containing 5% sodium bicarbonate is used as the extraction solvent.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the analytical method residue data are classified as scientifically acceptable. The petitioner is required to show that the proposed enforcement method (GRM 03.18) can differentiate between aminopyralid, clopyralid, and picloram, as they are all similar in structure. The proposed enforcement method will be forwarded to ACB for petition method validation.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D305665 and in Canada's Regulatory Decision Document.

COMPLIANCE:

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

A. BACKGROUND INFORMATION

Aminopyralid (4-amino-3,6-dichloropyridine-2-carboxylic acid) is the proposed common name of XDE-750, a new active ingredient developed by Dow AgroSciences. Aminopyralid is a systemic postemergence herbicide which belongs to the pyridine carboxylic acid class of herbicides. The petitioner is currently proposing food/feed uses on grasses grown in rangelands and permanent pastures and on wheat for the selective control of invasive and noxious broadleaf weeds. It is also proposed for weed control in sites such as parks, electric utility rights-of way, forestry, woodlands, and wildlife openings, with smaller amounts used in railroads, utility substations, pipelines, and pumping stations.

The proposed end-use product (EP) is a soluble concentrate liquid (SC/L) referred to by the trade name GF-871 (EPA Reg. No. 62719-LRI). The active ingredient in GF-871 is formulated as the triisopropanolammonium (TIPA) salt, with the product containing 40.6% of aminopyralid TIPA salt at an acid equivalent (ae) of 21.1% or 2 lb ae/gal (240 g ae/L). The petitioner stated that the aminopyralid TIPA salt dissociates rapidly in water to the acid (aminopyralid) at environmental pH values greater than 2.56 (the pKa).

Currently, the 2 lb ae/gal (240 g ae/L) SC/L formulation is proposed for broadcast foliar application at maximum rates of 0.11 lb ae/A (120 g ae/hectare) on rangeland and permanent pastures and 0.009 lb ae/A (10 g ae/ha) on wheat. The proposed PHIs are 0 days for wheat hay and 50 days for wheat grain and straw. No PHI is listed or proposed for grasses or wheat forage.

TABLE A.1. Test Compound Nomenclature.

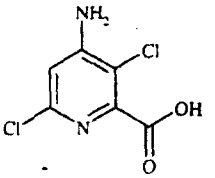
Chemical structure	
Common name	Aminopyralid
Company experimental name	XDE-750
IUPAC name	4-amino-3,6-dichloropyridine-2-carboxylic acid
CAS name	4-amino-3,6-dichloro-2-pyridinecarboxylic acid
CAS registry number	150114-71-9
End-use product (EP)	2 lb ae/gal (240 g ae/L) TIPA salt SC/L formulation (GF-871 Herbicide; EPA Reg. No. 62719-LRI; Aminopyralid Liquid Concentrate Herbicide)

TABLE A.2. Physicochemical Properties of the Aminopyralid Technical Grade Test Compound.

Parameter	Value	Reference
Melting point	163.5 °C	MRID 46235703, PMRA LS
pH	2.31 at 23.4 °C (1% solution in water)	MRID 46235703, PMRA LS
Relative density	1.72 at 20 °C	MRID 46235703, PMRA LS
Water solubility	2.48 g/L unbuffered water at 18 °C 212 g/L pH 5 buffer at 20 °C 205 g/L pH 7 buffer at 20 °C 203 g/L pH 9 Buffer at 20 °C	MRID 46235703, PMRA LS
Solvent solubility at 20 °C	methanol 52.2 g/L acetone 29.2 g/L n-octanol 3.9 g/L ethyl acetate 3.9 g/L 1,2-dichloroethane 0.2 g/L xylene 0.04 g/L heptane <10 µg/mL	MRID 46235703, PMRA LS
Vapor pressure	2.59×10^{-8} Pa at 25 °C; 9.52×10^{-9} Pa at 20 °C	MRID 46235703, PMRA LS
Dissociation constant, pK_a	2.56	MRID 46235703, PMRA LS
Octanol/water partition coefficient, $Log(K_{OW})$	0.201 unbuffered water at 19 °C; -1.76 at pH 5; -2.87 at pH 7; -2.96 at pH 9	MRID 46235703, PMRA LS
UV/visible absorption spectrum	Solution: Neutral; Wavelength λ max, nm: 217; Extinction coefficient ε, L/(mol*cm): 29100 Basic: 220; 26100 (pH 12.6): 245; 10150 Acidic: 217; 22800 (pH 1.4): 270; 9140	MRID 46235703, PMRA LS

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

Samples from the cattle feeding studies, associated with DP Barcode D305665, were analyzed for residues of aminopyralid using LC/MS/MS Method GRM 03.18, entitled "Determination of Residues of Aminopyralid in Bovine Tissues by Liquid Chromatography with Tandem Mass Spectrometry."

B.1.1. Principle of the Method:

Briefly, milk or ground tissue samples are extracted with methanol/sodium bicarbonate. The extract was cleaned up through an anion-exchange SPE plate. The internal standard, $^{13}C_2{}^{15}N$-aminopyralid, was added to the eluate and residues derivatized with butyl chloroformate to form the 1-butyl esters of aminopyralid for LC/MS/MS analysis.

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TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Aminopyralid Residues in Ruminant Milk and Tissues.	
Method ID	GRM 03.18
Analyte(s)	Aminopyralid
Extraction solvent/technique	Samples are extracted with methanol:sodium bicarbonate (20:1, v:w) using an homogenizer and the extract is isolated by centrifugation.
Cleanup strategies	An aliquot of the supernatant is diluted with water and cleaned up through a mixed-mode polymeric anion-exchange SPE plate with a vacuum manifold; residues are eluted with ethyl acetate:trifluoroacetic acid (99:1, v:v). The internal standard, $^{13}C_2{}^{15}N$-aminopyralid, is added to the SPE eluate. The eluate is then evaporated to dryness, residues are reconstituted in acetonitrile (ACN):pyridine:1-butanol (22:2:1, v:v:v) and derivatized with butyl chloroformate to form the 1-butyl esters of aminopyralid and the internal standard. The derivatized solution is diluted with methanol:water:acetic acid (50:49.9:0.1, v:v:v) for analysis.
Instrument/Detector	HPLC utilizing a reverse-phase column and a gradient mobile phase of methanol and water, each containing 0.1% acetic acid, with tandem mass spectrometry (MS/MS) detection using electrospray ionization operating in the positive ion mode with multiple reaction monitoring. The ions monitored for aminopyralid butyl ester are 263 amu (precursor ion) and 134 amu (product ion); ions monitored for the internal standard $^{13}C_2{}^{15}N$-aminopyralid butyl ester are 268 amu (precursor ion) and 139 amu (product ion).
Standardization method	Stable-isotope labeled internal standard, $^{13}C_2{}^{15}N$-aminopyralid, and external bracketing calibration standards of aminopyralid, each derivatized to their 1-butyl esters. Derivatized cross-over standards of $^{13}C_2{}^{15}N$-aminopyralid and aminopyralid are analyzed to determine the isotopic crossover factor of unlabeled and labeled aminopyralid (see text below).
Stability of std solutions	The petitioner indicated that based on previous environmental fate studies, aminopyralid has been proven to be stable in a multitude of solvents under varying temperatures.
Retention times	4.7-4.9 mins. (based on submitted liver chromatograms)

The petitioner noted that when using stable-isotope labeled internal standards, there is a possibility that isotopic contributions will occur between the MS/MS transitions used for quantitation of unlabeled and labeled compounds; therefore, the method includes instructions for the determination of isotopic crossover factors. The only isotopic overlap of consequence was the crossover of the $^{13}C_2{}^{15}N$-aminopyralid stable isotope internal standard to aminopyralid (ISTD → analyte). The transitions measured were: m/z 263 → 134 for aminopyralid and m/z 268 → 139 for $^{13}C_2{}^{15}N$-aminopyralid. The crossover factor was calculated as: peak area at m/z 263 → 134 divided by peak area at m/z 268 → 139. The average crossover factor determined for analyte to internal standard (ISTD) was 0.00387, while the average crossover factor determined for ISTD to analyte was 0.00666. These factors demonstrated a higher percentage of crossover from analyte to ISTD. The petitioner stated that the amount of the internal standard used in the method was chosen to minimize the crossover of analyte to ISTD over the calibration range. Therefore, the measured quantitation ratio only needs to be corrected for the ISTD to analyte crossover contribution. Correction is made by subtracting the determined ISTD-to-analyte crossover factor from the analyte/ISTD peak area ratio.

B.2. Enforcement Method

The proposed enforcement method is the same as the data-gathering method, LC/MS/MS Method GRM 03.18.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

TABLE C.1.1. Recovery Results from Method Validation of Ruminant Milk and Tissues using the Data-Gathering Analytical Method.[1]

Matrix	Spiking Level (ppm)	Recoveries (%) Obtained [2]	Mean Recovery ± SD [CV]
Bovine, whole milk	0.003	(0.0020 ppm)	--
	0.01	78, 78, 79, 85, 87	80 ± 8 [10]
	0.10	86, 91	
	1.0	76, 77, 80, 80, 83	
Bovine, milk cream	0.01	71, 91	
	0.10	88	
	1.0	64, 71	
Bovine, skim milk	0.01	77, 82	
	0.10	99	
	1.0	71, 75	
Bovine, fat	0.003	(0.0028 ppm)	--
	0.01	85, 90, 91, 95, 99	93 ± 4 [4]
	0.10	94, 97	
	1.0	88, 92, 93, 95, 97	
Bovine, kidney	0.003	(0.0016 ppm)	--
	0.01	67, 78, 81, 84, 86	81 ± 8 [9]
	0.50	83, 89	
	2.5	72, 76, 83, 84, 95	
Bovine, liver	0.003	(0.0016 ppm)	--
	0.01	77, 80, 83, 86, 88	83 ± 5 [6]
	0.10	89, 93	
	1.0	77, 79, 79, 79, 82	
Bovine, muscle	0.003	(0.0019 ppm)	--
	0.01	81, 81, 85, 89, 96	84 ± 7 [8]
	0.10	91, 92	
	1.0	75, 76, 79, 80, 85	

[1] Fortification standards were prepared in acetonitrile.
[2] Recoveries were not reported or calculated for fortification levels below the LOQ (0.01 ppm); however ppm values are reported in parentheses.

The fortification levels and samples used in method validation adequately bracket expected residue levels in ruminant milk and tissues. Two recoveries were below the acceptable 70-120% range: 67% in one kidney sample fortified at the LOQ and 64% in one milk cream sample fortified at the higher level (1.0 ppm). The petitioner stated that the low recoveries were due to

random error and not systematic error, and that because the low recoveries were not significantly <70%, the results were included in the statistical calculations for total recoveries and LOD/LOQ determination. We note additionally that acceptable concurrent method recovery data were included with the feeding study submitted in conjunction with the currently requested uses.

The petitioner concluded that Method GRM 03.18 is specific for the quantitation of aminopyralid residues because MS/MS selective detection is used; confirmation of the presence of the analyte is achieved by observation of a precursor ion plus one structurally significant product ion at the same retention time. Because the method only monitors one transition ion, HED defers to ACB to determine whether confirmatory analysis procedures are needed for the method.

TABLE C.1.2. **Characteristics for the Analytical Method Used for the Quantitation of Aminopyralid Residues in Ruminant Milk and Tissues.**	
Analyte	Aminopyralid
Equipment ID	Agilent Model 1100 autosampler, binary pump, and degasser, MDS/Sciex API 3000 LC/MS/MS system with a Diazem 3000 (C18) column, and MS/MS electrospray detection (TurboIonSpray) in the positive mode.
Limit of quantitation (LOQ)	0.01 ppm; calculated LOQs based on 10x the standard deviation from the 0.01 ppm recovery results were 0.0044 ppm for liver, 0.0053 ppm for fat, 0.0060 ppm for milk, 0.0063 ppm for muscle, and 0.0075 ppm for kidney.
Limit of detection (LOD)	0.003 ppm; calculated LODs based on 3x the standard deviation from the 0.01 ppm recovery results were 0.0013 ppm for liver, 0.0016 ppm for fat, 0.0018 ppm for milk, 0.0019 ppm for muscle, and 0.0022 ppm for kidney.
Accuracy/Precision	Adequate recoveries, demonstrating acceptable accuracy/precision of the LC/MS/MS method were obtained with bovine milk and tissues fortified at the LOQ (0.01 ppm) and up to 100x LOQ (1.0 ppm) for milk, fat, liver, and muscle, and 250x LOQ (2.5 ppm) for kidney. The range of percent recoveries [± coefficient of variation] were 64-99% [10] for milk including cream and skimmed, 85-99% [4] for fat, 67-95% [9] for kidney, 77-93% [6] for liver, and 75-96% [8] for muscle; see Table C.1.1.
Reliability of the Method/ [ILV]	An independent laboratory method validation [ILV] was conducted to verify the reliability of LC/MS/MS method GRM 03.18 for the determination of aminopyralid residues in ruminant milk and tissues. Adequate recoveries were obtained with milk fortified at the method LOQ (0.01 ppm) and 0.10 ppm, and with kidney fortified at the method LOQ (0.01 ppm) and 0.50 ppm, indicating that method GRM 03.18 is reliable; see Table C.3.1.
Linearity	The detector response was linear (coefficient of determination, r^2= >0.999) within the range of 0.004-2.0 ppm aminopyralid; a representative calibration curve (r^2 = 0.99919) was provided.
Specificity	The method is specific for aminopyralid due to the use of tandem MS detection. Monitored MS/MS ion transitions were 263/134 m/z for aminopyralid butyl ester and 268/139 m/z for $^{13}C_2{}^{15}N$-aminopyralid butyl ester (internal standard).

No extraction efficiency data were generated for the LC/MS/MS enforcement method (GRM 03.18) because residues in samples from a goat metabolism study were very low; only kidney samples had TRR >0.01 ppm. However, the extraction solvent used in the proposed enforcement

method is similar to that used in the goat metabolism study. In the goat metabolism study (refer to the DER for MRID 46235708), 76-96% TRR was extracted from milk, liver, and kidney samples using methanol (fat and muscle samples were not subjected to extraction procedures due to low residue levels). In the proposed enforcement method, methanol containing 5% sodium bicarbonate is used as the extraction solvent. Furthermore, in the goat metabolism study, although residues in the milk and liver samples were too low for identification, 96 % of the TRR was extracted from kidney using methanol and ~80 % of the TRR was identified as aminopyralid. HED concludes that based on the current goat metabolism study, no radiovalidation study is needed for the proposed enforcement method. However, if a new goat metabolism study is conducted, radiovalidation data will be required for the proposed enforcement method.

C.2. Enforcement Method

The proposed enforcement method is the same as the data-gathering method, LC/MS/MS Method GRM 03.18. As such, the petitioner is required to show that the proposed enforcement method (GRM 03.18) can differentiate between aminopyralid, clopyralid, and picloram, as they are all similar in structure.

C.3. Independent Laboratory Validation

An independent laboratory validation (ILV) study was conducted by Morse Laboratories (Sacramento, CA). Bovine kidney and milk were chosen as the representative matrices to be tested because milk has a high aqueous content and higher residues are expected in kidney. Untreated samples of milk and kidney, obtained from a local grocery, were fortified with aminopyralid at 0.01 ppm (LOQ) and 0.10 ppm (10x LOQ; milk) or 0.50 ppm (50x LOQ; kidney). Fortified and unfortified samples were extracted and analyzed using LC/MS/MS Method GRM 03.18. We note that the method as written uses a power regression curve for calculations, however, the ILV laboratory used a quadratic non-linear regression equation to generate the calibration curve for wheat grain and a linear regression equation was used for grass forage because of the wider range of analyte concentrations. This change was communicated by the laboratory to the petitioner, which noted that the method does allow for the use of other regression analyses.

Adequate recoveries were achieved for both bovine milk and kidney with the first trial; all recoveries were within the 70-120% acceptable recovery range. Residues in unfortified milk and kidney samples were nondetectable.

The laboratory stated that no changes or modifications were made to the method, except for substitution of some of the equipment used. A mistake in the draft method was noted by the laboratory; this typographical error has been corrected in the final version of the method dated 12/19/03. One set of 13 samples required approximately 8 person-hours for sample preparation and automated HPLC analyses were run overnight unattended.

TABLE C.3.1. Recovery Results Obtained by an Independent Laboratory Validation of the Enforcement Method for the Determination of Aminopyralid in Bovine Milk and Kidney.

Matrix	Spiking Level (ppm)	Recoveries Obtained	Mean Recovery ± SD [CV]
Bovine, milk	0.01	72, 75, 75, 80, 97	83 ± 7.9 [9.5]
	0.10	84, 84, 88, 89, 90	
Bovine, kidney	0.01	72, 74, 75, 77, 97	87 ± 11 [13]
	0.50	93, 95, 96, 97, 97	

D. CONCLUSION

Adequate method validation data have been submitted for LC/MS/MS Method GRM 03.18 for the determination of residues of aminopyralid in ruminant milk and tissues.

The petitioner is proposing the LC/MS/MS method for enforcement. Adequate independent laboratory validation data have been submitted using bovine milk and kidney. HED concludes that based on the current goat metabolism study, no radiovalidation study is needed for the proposed enforcement method. However, if a new goat metabolism study is conducted, radiovalidation data will be required for the proposed enforcement method. Furthermore, the petitioner is required to show that the proposed enforcement method (GRM 03.18) can differentiate between aminopyralid, clopyralid, and picloram, as they are all similar in structure. The proposed enforcement method will be forwarded to ACB for petition method validation.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: MADoherty, 06/28/05; TSheramata, 06/13/05; HBietlot, 6/13/05
Petition Number(s): PP#4F6827
DP Barcode(s): D305665
PC Code: 005100/005209

Template Version September 2003

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Aminopyralid/XDE-750/PC Code 005100/Dow AgroSciences/62719
DACO 6.3/OPPTS 860.1300/OECD II 6.2.2, 6.2.3 & IIIA 8.2, 8.4.1, 8.4.2
Nature of the Residues in Plants - Grass

Primary Evaluator _____ Date: 6/28/05
Michael A. Doherty, Ph.D., Chemist, RAB2

Peer Reviewer _____ Date: June 6/05
Tamara Sheremata, Ph.D.,
Evaluation Officer, FREAS, HED, PMRA

Approved by _____ Date: Jun 13/05
Henri Bietlot, Ph.D.,
A/Section Head, FREAS, HED, PMRA

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 11/08/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46235710 Magnussen, J.; Balcer, J. (2004) 14C XDE-750 Grass Nature of the Residue Study. Project Number: 010071. Unpublished study prepared by Dow AgroSciences LLC. 139 p.

EXECUTIVE SUMMARY:

Dow AgroSciences has submitted a grass metabolism study with aminopyralid. The aminopyralid test substance used in the study was labeled at the 2- and 6-positions of the pyridine ring and formulated as the potassium salt. The test substance was foliarly applied once to each of three types of pasture grasses (Big bluestem, Perennial rye grass, and *Panicum maximum*) approximately 8-10 weeks after planting at a rate of 0.321 lb ai/A (360 g ai/ha). Grasses were grown in individual plastic tubs that were maintained outdoors on a concrete patio that was adjacent to a greenhouse. The in-life and analytical phases of the study were conducted by Dow AgroSciences (Indianapolis, Indiana).

Samples were collected at 0, 14, 21, and 42 days after treatment (DAT). Portions of the 42-DAT samples for each grass species were allowed to air dry in order to produce hay samples. Following sample preparation, the total radioactive residues (TRR) were determined by combustion/LSC. The TRR in grass matrices are tabulated below.

Matrix	Days After Treatment	[^{14}C]Aminopyralid Equivalents, ppm		
		Ryegrass	Big Bluestem	*Panicum maximum*
Forage	0 DAT	48.84	25.84	17.96
	7 DAT	36.91	21.03	18.87
	14 DAT	22.90	8.29	10.08
	21 DAT	20.85	9.03	10.03
	42 DAT	6.57	5.61	4.81
Hay	42 DAT	23.34	12.64	19.13

The 0-DAT samples were rinsed sequentially with water followed by methanol, and residues in rinsed grasses were subsequently extracted by homogenizing in acetonitrile/water (70:30, v:v). All other samples were extracted by first homogenizing in acetonitrile/water (70:30, v:v) followed by homogenizing and refluxing in acetonitrile/2 N HCl (50:50, v:v). Potential conjugate fractions from the 21-DAT samples were further analyzed using a base hydrolysis step followed by organic solvent partitioning. All extracts and rinses were analyzed for aminopyralid and metabolites by reverse phase HPLC. Selected fractions following isolation and cleanup were also analyzed by LC/MS.

Radioactive residues in/on grass forage and hay were readily extractable at all harvest intervals as evidenced by the fact that only 2.8-4.4% of the TRR remained as nonextractable residues in the 42-DAT samples after solvent extraction. Chromatographic analyses of the extractable residues identified the parent aminopyralid as the major residue component, and indicated that the residue level of the parent declined at subsequent intervals, suggesting rapid metabolism of the applied test substance. Aminopyralid accounted for approximately 92-97% of TRR in 0-DAT grass forage, 48-68% of TRR in 7-DAT forage, 33-45% of TRR in 14-DAT forage, 25-38% of TRR in 21-DAT forage, 22-31% TRR in 42-DAT forage, and 24-35% of TRR in 42-DAT hay.

In all three grasses, metabolism resulted in the formation of three metabolite complexes (Fractions C-1, C-2, and C-3). By 42 DAT, the least polar of these fractions represented 50-60% of TRR, while each of the other two fractions represented about 5-10% of the TRR. Subsequent characterization work confirmed that the two largest of these fractions were multi-component in nature and that most of all three fractions could be converted back to aminopyralid following base or acid hydrolysis. Based on these findings, the petitioner concluded that the metabolite fractions observed in the study consisted primarily of isomeric mixtures of acid and base-labile-N-glucosides and glucose conjugate esters of aminopyralid. Other than the formation of these conjugates, the only other metabolic alteration observed as part of this study involved the addition of a hydroxyl group to the pyridine ring to form a minor, conjugated metabolite that was observed at levels estimated to be <1% of the TRR.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the grass metabolism data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D305665 and in Canada's Regulatory Decision Document.

COMPLIANCE:

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

A. BACKGROUND INFORMATION

Aminopyralid (4-amino-3,6-dichloropyridine-2-carboxylic acid) is the proposed common name of XDE-750, a new active ingredient developed by Dow AgroSciences. Aminopyralid is a systemic postemergence herbicide which belongs to the pyridine carboxylic acid class of herbicides. The petitioner is currently proposing food/feed uses on grasses grown in rangelands and permanent pastures and on wheat for the selective control of invasive and noxious broadleaf weeds. It is also proposed for weed control in sites such as parks, electric utility rights-of way, forestry, woodlands, and wildlife openings, with smaller amounts used in railroads, utility substations, pipelines, and pumping stations.

The proposed end-use product (EP) is a soluble concentrate liquid (SC/L) referred to by the trade name GF-871 (EPA Reg. No. 62719-LRI). The active ingredient in GF-871 is formulated as the triisopropanolammonium (TIPA) salt, with the product containing 40.6% of aminopyralid TIPA salt at an acid equivalent (ae) of 21.1% or 2 lb ae/gal (240 g ae/L). The petitioner stated that the aminopyralid TIPA salt dissociates rapidly in water to the acid (aminopyralid) at environmental pH values greater than 2.56 (the pKa).

Currently, the 2 lb ae/gal (240 g ae/L) SC/L formulation is proposed for broadcast foliar application at maximum rates of 0.11 lb ae/A (120 g ae/hectare) on rangeland and permanent pastures and 0.009 lb ae/A (10 g ae/ha) on wheat. The proposed PHIs are 0 days for wheat hay and 50 days for wheat grain and straw. No PHI is listed or proposed for grasses or wheat forage.

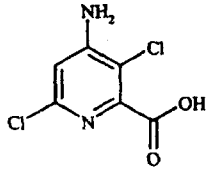
TABLE A.1. Test Compound Nomenclature.	
Chemical structure	

TABLE A.1. Test Compound Nomenclature.	
Common name	Aminopyralid
Company experimental name	XDE-750
IUPAC name	4-amino-3,6-dichloropyridine-2-carboxylic acid
CAS name	4-amino-3,6-dichloro-2-pyridinecarboxylic acid
CAS registry number	150114-71-9
End-use product (EP)	2 lb ae/gal (240 g ae/L) TIPA salt SC/L formulation (GF-871 Herbicide; EPA Reg. No. 62719-LRI, Aminopyralid Liquid Concentrate Herbicide in Canada)

TABLE A.2. Physicochemical Properties of the Aminopyralid Technical Grade Test Compound.

Parameter	Value	Reference
Melting point	163.5 °C	MRID 46235703, PMRA LS
pH	2.31 at 23.4 °C (1% solution in water)	MRID 46235703, PMRA LS
Relative density	1.72 at 20 °C	MRID 46235703, PMRA LS
Water solubility	2.48 g/L unbuffered water at 18 °C 212 g/L pH 5 buffer at 20 °C 205 g/L pH 7 buffer at 20 °C 203 g/L pH 9 Buffer at 20 °C	MRID 46235703, PMRA LS
Solvent solubility at 20 °C	methanol 52.2 g/L acetone 29.2 g/L n-octanol 3.9 g/L ethyl acetate 3.9 g/L 1,2-dichloroethane 0.2 g/L xylene 0.04 g/L heptane <10 µg/mL	MRID 46235703, PMRA LS
Vapor pressure	2.59×10^{-8} Pa at 25 °C; 9.52×10^{-9} Pa at 20 °C	MRID 46235703, PMRA LS
Dissociation constant, pK_a	2.56	MRID 46235703, PMRA LS
Octanol/water partition coefficient, $Log(K_{ow})$	0.201 unbuffered water at 19 °C; -1.76 at pH 5; -2.87 at pH 7; -2.96 at pH 9	MRID 46235703, PMRA LS
UV/visible absorption spectrum	Solution: Neutral; Basic; (pH 12.6); Acidic; (pH 1.4) Wavelength λ max, nm: 217; 220; 245; 217; 270 Extinction coefficient ε, L/(mol*cm): 29100; 26100; 10150; 22800; 9140	MRID 46235703, PMRA LS

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B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

TABLE B.1.1. Test Site Information

Testing Environment	Soil characteristics			
	Type	%OM	pH	CEC
Plastic tubs (26 cm x 36 cm x 20 cm) maintained outdoors at Dow AgroSciences (Indianapolis, IN). Plants were germinated in a greenhouse and moved to an outdoor patio area adjacent to a greenhouse.	Silt loam (58% silt, 18% clay, and 24% sand)	2.1	7.1	10.5 meq/100 g

No weather data were provided, but the petitioner stated that no unusual weather conditions were noted during the in-life phase of the study. Plants were irrigated (bottom watering) as needed. Prior to treatment, grasses were cut to approximately 12-14 cm to provide a uniform stand.

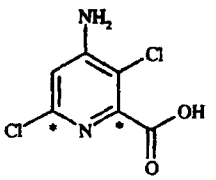
TABLE B.1.2. Crop Information.

Crop; crop group	Variety	Growth stage at application [1]	Growth stage at harvest	Harvested RAC	Harvesting procedure
Grass; grass, forage, fodder and hay, group 17	Big bluestem (*Andropogon gerardii var. Bison)*	12-14 cm tall (68 DAP)	68, 75, 82, 89, and 110 DAP	Forage and hay	Plant were cut with scissors 2-5 cm above soil surface.
	Perennial rye grass (*Lolium perenne var. Manhatten 3)*	12-14 cm tall (56 DAP)	56, 63, 70, 77, and 98 DAP		
	Panicum maximum (common pasture grass in South America)	12-14 cm tall (69 DAP)	69, 76, 83, 90, and 111 DAP		

[1] DAP = Days after planting; grasses were cut to 12-14 cm prior to application.

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics.

Chemical structure	
Radiolabel position	2- and 6-positions of the pyridine ring
Lot No.	Inventory No. INV1590 (radiolabeled lot no.); TSN 102298 (non-radiolabeled lot no.)
Purity	98.6%
Specific activity	39,960 dpm/µg or 666 Bq/µg at time of application

B.3. Study Use Pattern

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TABLE B.3.1. Use Pattern Information.	
Chemical name	Aminopyralid
Application method	Foliar spray of the potassium salt in water
Application rate	0.321 lb ai/A (360 g ai/ha)
Number of applications	1
Timing of applications	Approximately 8-10 weeks after planting.
PHI (days)	0, 7, 14, 21, and 42 for forage, and 42 for hay

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Following collection, all samples were returned to the laboratory on ice. The 0-DAT samples were sequentially rinsed with water followed by methanol, and the rinsed grasses were frozen in liquid nitrogen and then ground. Half of the 7-, 14-, and 21-DAT samples for rye grass and bluestem grass and half of the 7- and 14-DAT samples for the *Panicum* grass were handled in a similar fashion. The remainder of the 7- to 21-DAT samples were weighed and ground in the presence of liquid nitrogen without any rinse steps. These were designated as the unrinsed grass samples and were used for the bulk of the extraction and characterization work done in the study. For the 42-DAT samples, half were ground without any prior rinse step. The other half were allowed to air dry over a 1-2 day period to provide hay samples. After drying, the hay samples were ground in the same manner as the fresh grass samples. All prepared samples were stored frozen at approximately –20 °C pending analysis.

TRR levels in all prepared samples were determined by combustion analysis followed by LSC. Aliquots of the rinsed 0-DAT samples were extracted by homogenizing for approximately 5 minutes in nominal portions of acetonitrile/water (70:30) using a Polytron tissue homogenizer. Solvent was removed by vacuum filtration, and portions of the filtrate and extracted grass were assayed for total radioactivity. Portions of the unrinsed 7-, 14-, 21- and 42-DAT grasses as well as the 42-DAT hay samples were initially extracted by homogenizing in acetonitrile/water (70:30) using a Polytron tissue homogenizer. Spent grass was removed by vacuum filtration and homogenized a second time using portions of acetonitrile/2 N HCl (50:50). Homogenized samples from this latter step were then transferred to a reflux flask using additional extraction solution, and the sample was refluxed with stirring for approximately one hour. Following the reflux period, remaining solids were removed by vacuum filtration and portions of the two extracts and of the extracted grass were assayed for total radioactivity. The extracts from each sample was reserved for HPLC analysis. Use of the acetonitrile/acid reflux step in the extraction scheme was necessary due to the steady increase over time in the level of residues that could not be extracted by simply homogenizing with acetonitrile/water.

Extracts of the 21-DAT samples were further analyzed to confirm the presence of labile conjugates. Initially, both the acetonitrile/water and acetonitrile/acid extracts were partitioned

with acetonitrile/dichloromethane (50:50) in order to remove any nonconjugated residues. Portions of the remaining aqueous fractions were then prepared in 1.0 N in sodium hydroxide and refluxed for approximately two hours. After cooling, samples were acidified (<pH 2) and then partitioned up to three times with acetonitrile/dichloromethane (50:50). Acid hydrolysis of these fractions with 1-3 N in hydrochloric acid was also attempted but was not found to be as effective when compared with base hydrolysis at releasing the conjugated residues.

The extraction procedures for grass matrices are summarized in the flow charts (Figures B.4.1.1 and B.4.1.2.) below, which were copied without alteration from MRID 46235710. The 0- and 21-DAT ryegrass flowcharts are representative for the other grasses.

Figure B.4.1.1. Extraction Flowchart for 0 Time Ryegrass.

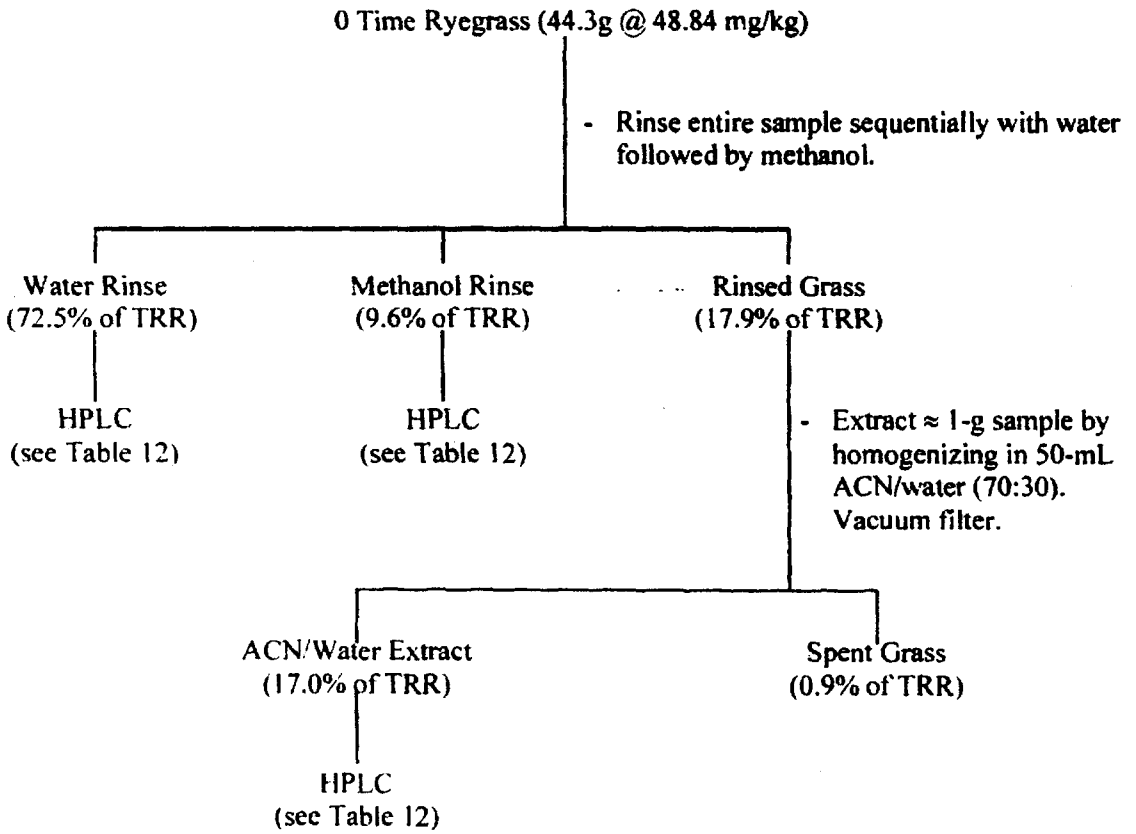
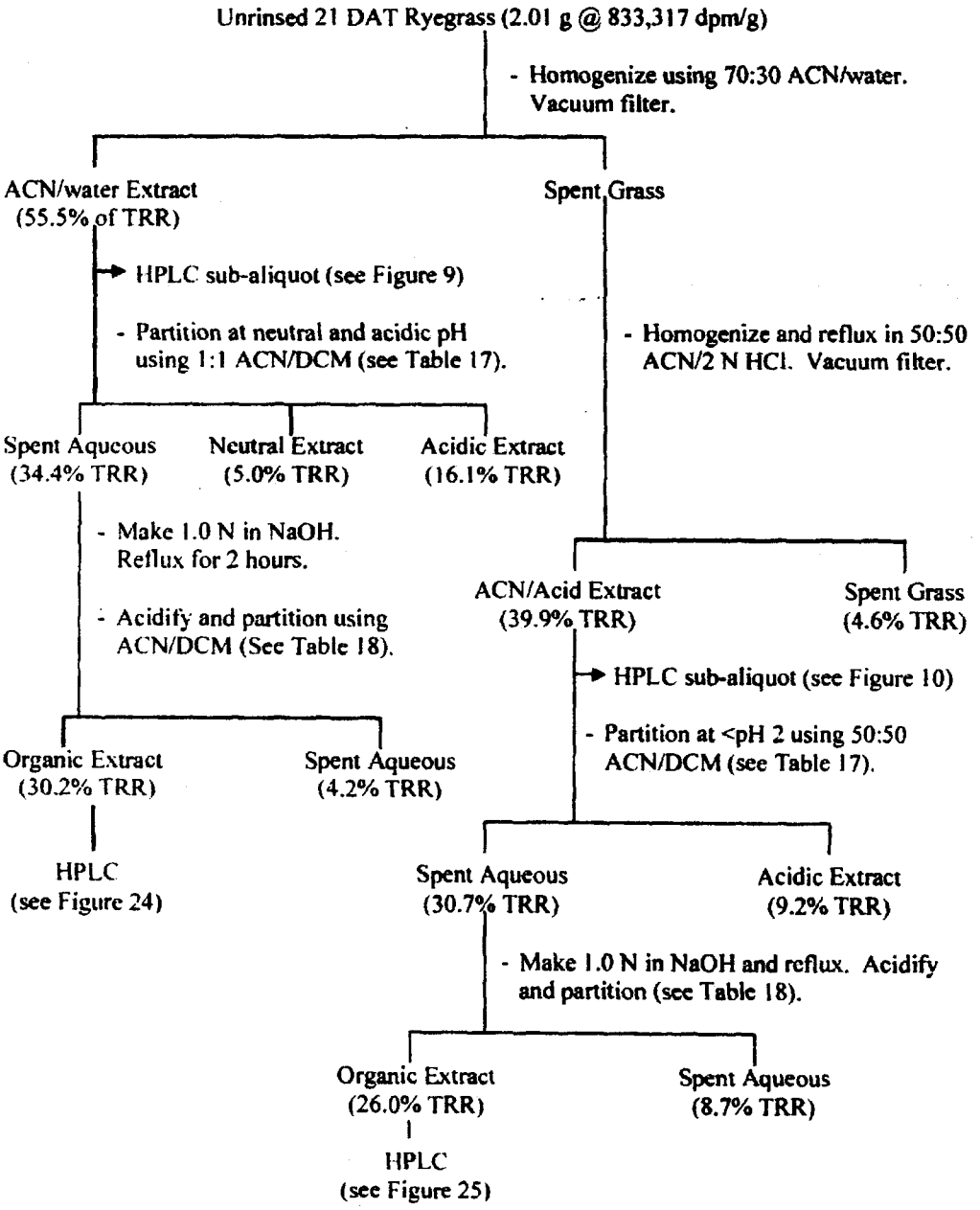


Figure B.4.1.2. Extraction Flowchart for Unrinsed 21 DAT Ryegrass.



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B.4.2. Analytical Methodology

Total radioactive residues (TRR) in grass forage and hay, and nonextractable residue fractions were determined by combustion/LSC. Radioactivity in the rinses, extracts, and hydrolysates were determined by direct LSC. The 0-DAT samples were rinsed prior to extraction, and the TRR was determined by the summation of radioactivity in the rinses and the rinsed forage. The limit of quantitation (LOQ) ranged 0.02 to 0.09 ppm depending on the grass matrix.

Concentrated extracts of grass matrices were analyzed by reverse phase HPLC equipped with an Inertsil ODS-2 (C18) column, a UV detector (270 nm), and fraction collection/LSC for radiodetection; an isocratic or gradient mobile phase of water and acetonitrile, each containing 0.5% trifluoroacetic acid (TFAA), was used. Residues of aminopyralid were identified by co-chromatography with unlabeled standard.

The identification of unconjugated aminopyralid was confirmed in the ACN/water and acidic ACN extracts of 14-DAT ryegrass and 21-DAT *Panicum* using LC/MS. Aminopyralid released from a conjugated fraction following base hydrolysis was also confirmed by LC/MS. LC/MS analyses were conducted using an Intersil ODS-2 or Synergi Hydro-RP column with a gradient solvent system of ACN and water each containing 1.0% glacial acetic acid or 0.5% formic acid as acidic modifiers instead of 0.5% TFAA. Quantitation was by MS electrospray ionization in the negative mode.

C. RESULTS AND DISCUSSION

Storage Stability

The storage intervals for grass samples are presented in Table C.1. Samples were initially extracted within 30 days of collection; however, some of the earlier samples had to be reanalyzed due to an improvement made in the extraction scheme. These re-analyses generally occurred within one to two months of collection. As part of subsequent metabolite isolation and characterization work, additional portions of several samples were re-extracted after more than 650 days of frozen storage. For those samples, extraction results were nearly the same as when originally analyzed using the improved extraction scheme. Results from the HPLC analyses of the extracts of these samples were also nearly identical to the original analyses. Based on these findings, there appeared to be no instability of aminopyralid or its metabolites in grass samples during long term frozen storage.

TRR Levels

Table C.2.1 shows the TRR levels, expressed as aminopyralid equivalents in ppm, for all the unrinsed grass forage and hay samples. For all three grasses, residues were highest in the 0-DAT and 7-DAT samples and were reduced by 50% or more thereafter. Based on sample weight data, it appeared that most of the reduction in residue levels over the first 14 days after application was

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due to weathering (i.e., washed off due to rainfall events), while reduction over the latter portion of the study could be attributed primarily to growth dilution.
Residues in grass hay produced at the time of the final sampling were consistently 3-4 times higher than those in the corresponding fresh forage samples collected at the same time. These levels were consistent with the levels that would be predicted based on the amounts of moisture that were lost from the samples during the drying process.

Extraction and characterization of residues

As summarized in Tables C.2.2, C.2.3, C.2.4 and C.2.5, residues in all grass and hay samples were readily extractable as there was minimal build over time in the levels of nonextractable residues. With the exception of rye grass hay, nonextractable residue levels never exceeded 5% of the TRR. Over the course of the study, however, there was a significant change in the amounts of the TRR that could be readily extracted by simply homogenizing in an aqueous acetonitrile solution. While this step recovered approximately 80-90% of the TRR in samples collected within the first 7-14 days after application, it only recovered about 50% of the TRR by 42 DAT. These more difficult-to-extract residues were readily recovered using the acidified acetonitrile reflux step. Air drying of the grass to produce hay appeared to have no effect on the overall extractability of the residues.

Results from the HPLC analyses showed that aminopyralid was rapidly metabolized as less than 50% of the TRR in any of the grasses could still be accounted for as the unchanged test material within 7-14 days after application. By 42 DAT, only about 20-30% of the TRR remained as aminopyralid. The metabolism of aminopyralid was the same in all three grasses and lead to the formation of three metabolite complexes that were subsequently shown to consist almost exclusively of labile conjugates of aminopyralid itself. One of these fractions (complex C-1) was more polar than aminopyralid as it eluted just after the column void volume. A second (complex C-2) was slightly less polar than the parent, while the third (complex C-3) was even less polar. At all time points other than 0-DAT, the C-3 complex was the primary metabolite component as it generally represented about 40% or more of the TRR within 14 days after application. By 42 DAT, this fraction represented 50-60% of the TRR. Each of the other two complexes reached levels representing approximately 5-10% of the TRR within 7 DAT and did not change significantly thereafter.

Analyses of the hay samples showed that the air drying process used to produce these samples had no effect on the residue profile as the distribution of the residues in the hay samples was virtually the same as that observed in the 42-DAT forage from which they were generated. For all samples, no significant losses of radioactivity were observed during either extraction or HPLC analysis. High recoveries (115-175%) were observed, however, during extraction of several of the rye and bluestem samples. It was thought that these high recoveries might be due to the partial loss of moisture from the finely ground plant material during frozen storage since the highest recoveries were encountered in samples that were being analyzed a second time using an improved extraction procedure and as such had been stored for 6-8 weeks after collection and preparation.

Characterization and Hydrolysis of Conjugate Fractions

Table C.2.6 shows the distribution of the residues in the 21-DAT samples among the fractions generated. For each of the acetonitrile/water extracts, approximately 40-60% of the radioactivity in the samples (approximately 27-34% of TRR) was accounted for in the spent aqueous fractions. HPLC analyses of these fractions confirmed that they consisted almost exclusively of conjugate fractions. Following base hydrolysis, approximately 80-90% of the radioactivity in these samples could be partitioned into organic solvent. HPLC analysis of these fractions showed over 90% of each to elute as aminopyralid.

For the acetonitrile/acid extracts, approximately 60-75% of the radioactivity in each (approximately 14-31% of the TRR) was accounted for in the spent aqueous fractions. HPLC analyses of these fractions again confirmed that they consisted primarily of the suspected conjugate fractions. Following base hydrolysis, approximately 75% of each could be partitioned into organic solvent. HPLC analyses confirmed that approximately 85-95% of these organosoluble residues was aminopyralid.

Acid hydrolysis of these aqueous soluble fractions also resulted in the liberation of aminopyralid; however, the efficiency of this release was significantly lower than that seen for base hydrolysis. No significant losses of radioactivity were observed during either the acid or base hydrolysis steps.

Isolation and Identification of Metabolites

LC/MS analyses of residues eluting in the region of aminopyralid in the HPLC chromatograms of both the nonconjugated fractions and of the organosoluble extracts of the spent aqueous (conjugated) fractions following base hydrolysis confirmed the identity of these residues as aminopyralid.

Cleanup of the C-1 and C-3 metabolite fractions using extended gradient systems confirmed that both were multi-component in nature. Analysis of a minor fraction (estimated to be less than 1% of the TRR) from the C-1 complex that eluted just after aminopyralid in the extended gradient used for cleanup showed it to be a glucose conjugate of aminopyralid that had been hydroxlyated at the 5-position of the pyridine ring. Analyses of other isolated fractions from both the C-1 and C-3 complexes showed the presence of high molecular weight components that contained a two chlorine pattern consistent with that seen for aminopyralid. While exact structures could not be assigned to these metabolites, it was thought that they might represent aminopyralid that had been conjugated with multiple sugars and/or other components such as malonic acid.

Table C.2.7 shows the summary of characterization and identification of residues in 21-DAT grass forage. A similar profile was seen in all three grass species at all sampling time points with the only differences over time being the levels at which each component was observed. Thus, only the 21-DAT data are summarized in this table since 21 days represented the midpoint of the

study and the residue in these samples were considered to be representative of the other samples analyzed during the study. Table C.2.8 shows the summary of characterization and identification of residues in 42-DAT grass hay. The proposed metabolic pathway of aminopyralid in grass is depicted in Figure C.3.1.

C.1. Storage Stability

TABLE C.1. Summary of Storage Conditions.			
Matrix	Storage Temp. (°C)	Actual Study Duration [1]	Interval of Demonstrated Storage Stability [2]
Grass, forage	-20	0-13 days (<0.5 months); 12-69 days (≤2.3 months)	604-638 days (≤21 months)
Grass hay		13-29 days (<1 month)	None provided

[1] Storage interval from harvest to initial extraction/HPLC analysis, the storage interval from harvest to re-analysis of samples using an improved extraction procedure are listed secondary.
[2] Based on the storage of samples of forage used for metabolite characterization work.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. Total Radioactive Residues (TRR) in Grass Matrices Following a Single Foliar Spray Application of [2,6-^{14}C]Aminopyralid at 0.321 lb ai/A (360 g ai/ha).				
Matrix	Days After Treatment	[2,6-^{14}C]Aminopyralid Equivalents, ppm		
		Ryegrass	Big Bluestem	*Panicum maximum*
Forage	0 DAT	48.84	25.84	17.96
	7 DAT	36.91	21.03	18.87
	14 DAT	22.90	8.29	10.08
	21 DAT	20.85	9.03	10.03
	42 DAT	6.57	5.61	4.81
Hay	42 DAT	23.34	12.64	19.13

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TABLE C.2.2. Distribution of the Parent and Metabolites in Ryegrass Forage Following a Single Foliar Spray Application of [2,6-^{14}C]Aminopyralid at 0.321 lb ai/A (360 g ai/ha).

Metabolite Fraction	0-Time Forage		7-DAT Forage		14-DAT Forage		21-DAT Forage		42-DAT Forage	
	TRR=48.84 ppm		TRR=36.91 ppm		TRR=22.90 ppm		TRR=20.85 ppm		TRR=6.57 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Surface Rinses [1]	82.1	40.10	NA [4]	--	NA	--	NA	--	NA	--
Aminopyralid	80.3	39.22	--	--	--	--	--	--	--	--
Fraction C-1 [2]	1.4	0.68	--	--	--	--	--	--	--	--
Fraction C-2 [2]	0.3	0.15	--	--	--	--	--	--	--	--
Fraction C-3 [2]	--	--	--	--	--	--	--	--	--	--
ACN/Water Extract	17.0	8.30	81.9	30.23	57.9	13.26	55.5	11.57	53.6	3.52
Aminopyralid	16.7	8.16	42.0	15.50	23.8	5.45	17.1	3.57	13.8	0.91
Fraction C-1	<MQL*	<0.10	4.7	1.73	3.6	0.82	3.0	0.63	3.4	0.22
Fraction C-2	0.2	0.10	2.3	0.85	0.9	0.21	0.6	0.13	1.2	0.08
Fraction C-3	--	--	32.9	12.14	29.5	6.76	34.7	7.20	35.2	2.31
ACN/Acid Reflux [3]	NA	--	17.0	6.27	38.8	8.89	39.9	8.32	42.0	2.76
Aminopyralid	--	--	5.8	2.14	10.9	2.06	8.2	1.71	8.1	0.53
Fraction C-1	--	--	3.8	1.40	4.6	1.05	3.8	0.79	4.1	0.27
Fraction C-2	--	--	1.0	0.37	4.0	0.92	4.0	0.83	5.5	0.36
Fraction C-3	--	--	6.4	2.36	19.3	4.42	23.3	4.86	24.3	1.60
Nonextractable	0.9	0.44	1.1	0.41	3.3	0.76	4.6	0.96	4.4	0.29

[1] Complete solvent rinse analyses were done only with the 0-Time samples.
[2] Metabolite Fraction C-1 eluted just after the column void volume; fraction C-2 eluted just after aminopyralid; and fraction C-3 eluted significantly later in the gradient run than aminopyralid.
[3] 0 Time samples were not extracted using the reflux step.
[4] Not analyzed using this procedure.
*MQL is the same as LOQ

TABLE C.2.3. Distribution of the Parent and Metabolites in Big Bluestem Forage Following a Single Foliar Spray Application of [2,6-^{14}C]Aminopyralid at 0.321 lb ai/A (360 g ai/ha).

Metabolite Fraction	0-Time Forage		7-DAT Forage		14-DAT Forage		21-DAT Forage		42-DAT Forage	
	TRR=25.84 ppm		TRR=21.03 ppm		TRR=8.29 ppm		TRR=9.03 ppm		TRR=5.61 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Surface Rinses [1]	87.4	22.58	NA [4]	--	NA	--	NA	--	NA	--
Aminopyralid	80.3	22.07	--	--	--	--	--	--	--	--
Fraction C-1 [2]	1.8	0.47	--	--	--	--	--	--	--	--
Fraction C-2 [2]	0.1	0.03	--	--	--	--	--	--	--	--
Fraction C-3 [2]	--	--	--	--	--	--	--	--	--	--
ACN/Water Extract	12.0	3.10	85.7	18.03	82.4	6.83	74.7	6.75	67.7	3.80
Aminopyralid	11.7	3.02	61.3	12.89	38.1	3.16	28.2	2.55	18.3	1.03
Fraction C-1	--	--	6.2	1.30	7.4	0.61	8.5	0.77	8.9	0.50
Fraction C-2	0.3	0.08	3.5	0.74	5.2	0.43	3.3	0.30	2.3	0.13
Fraction C-3	--	--	14.6	3.07	31.5	2.61	34.8	3.14	37.9	2.13
ACN/Acid Reflux [3]	NA	--	12.9	2.71	15.7	1.30	22.8	2.06	29.5	1.65
Aminopyralid	--	--	5.3	1.11	6.8	0.56	9.8	0.88	10.3	0.58
Fraction C-1	--	--	3.2	0.67	2.8	0.23	2.5	0.23	2.3	0.13
Fraction C-2	--	--	<MQL	<0.08	--	--	1.7	0.15	3.7	0.21
Fraction C-3	--	--	4.2	0.88	6.1	0.51	8.8	0.79	12.2	0.68
Nonextractable	0.6	0.16	1.3	0.27	1.9	0.16	2.5	0.23	2.8	0.16

[1] Complete solvent rinse analyses were done only with the 0-Time samples.
[2] Metabolite Fraction C-1 eluted just after the column void volume; fraction C-2 eluted just after aminopyralid; and fraction C-3 eluted significantly later in the gradient run than aminopyralid.
[3] 0-Time samples were not extracted using the reflux step.
[4] Not analyzed using this procedure.

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TABLE C.2.4. Distribution of the Parent and Metabolites in *Panicum maximum* Forage Following a Single Foliar Spray Application of [2,6-14C]Aminopyralid at 0.321 lb ai/A (360 g ai/ha).

Metabolite Fraction	0-Time Forage		7-DAT Forage		14-DAT Forage		21-DAT Forage		42-DAT Forage	
	TRR=17.96 ppm		TRR=18.87 ppm		TRR=10.08 ppm		TRR=10.03 ppm		TRR=4.81 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Surface Rinses [1]	89.5	16.07	NA [4]	--	NA	--	NA	--	NA	--
Aminopyralid	86.2	15.48	--	--	--	--	--	--	--	--
Fraction C-1 [2]	0.1	0.02	--	--	--	--	--	--	--	--
Fraction C-2 [2]	<MQL	<0.02	--	--	--	--	--	--	--	--
Fraction C-3 [2]	2.4	0.43	--	--	--	--	--	--	--	--
ACN/Water Extract	10.3	1.85	90.5	17.08	80.4	8.10	72.7	7.29	56.0	2.69
Aminopyralid	9.9	1.78	64.3	12.13	27.0	2.72	25.9	2.60	19.9	0.96
Fraction C-1	--	--	5.5	1.04	3.6	0.36	4.0	0.40	3.9	0.19
Fraction C-2	<MQL	<0.02	1.6	0.30	2.2	0.22	1.4	0.14	2.1	0.10
Fraction C-3	0.2	0.04	19.0	3.59	47.6	4.80	41.4	4.15	30.0	1.44
ACN/Acid Reflux [3]	NA	--	8.4	1.59	17.1	1.72	23.6	2.37	39.9	1.92
Aminopyralid	--	--	3.4	0.64	5.9	0.59	8.0	0.80	10.7	0.51
Fraction C-1	--	--	1.9	0.36	1.8	0.18	2.0	0.20	2.5	0.12
Fraction C-2	--	--	<MQL	<0.07	1.8	0.18	2.8	0.28	5.6	0.27
Fraction C-3	--	--	2.7	0.51	7.6	0.77	10.8	1.09	21.1	1.01
Nonextractable	0.2	0.04	1.1	0.21	2.4	0.24	3.7	0.37	4.1	0.20

[1] Complete solvent rinse analyses were done only with the 0-Time samples.
[2] Metabolite Fraction C-1 eluted just after the column void volume; fraction C-2 eluted just after aminopyralid; and fraction C-3 eluted significantly later in the gradient run than aminopyralid.
[3] 0-Time samples were not extracted using the reflux step.
[4] Not analyzed using this procedure.

TABLE C.2.5. Distribution of the Parent and Metabolites in Grass Hay Following a Single Foliar Spray Application of [2,6-^{14}C]Aminopyralid at 0.321 lb ai/A (360 g ai/ha).

Metabolite Fraction	42-DAT Ryegrass Hay		42-DAT Big Bluestem Hay		42-DAT *Panicum* Hay	
	TRR = 23.34 ppm		TRR = 12.64 ppm		TRR = 19.13 ppm	
	%TRR	ppm	%TRR	ppm	% TRR	ppm
ACN/Water Extract	41.5	9.69	59.0	7.46	54.5	10.43
Aminopyralid	12.6	2.94	18.8	2.38	24.0	4.59
Fraction C-1 [1]	2.0	0.47	6.3	0.80	3.7	0.71
Fraction C-2 [1]	ND [2]	--	1.2	0.15	0.8	0.15
Fraction C-3 [1]	26.9	6.28	32.8	4.15	26.0	4.97
ACN/Acid Reflux	48.3	11.27	36.5	4.61	41.1	7.86
Aminopyralid	11.2	2.61	13.0	1.64	10.9	2.09
Fraction C-1	4.2	0.98	2.6	0.33	2.6	0.50
Fraction C-2	3.9	0.91	2.1	0.27	5.2	0.99
Fraction C-3	29.1	6.79	18.8	2.38	22.4	4.29
Nonextractable	10.2	2.38	4.5	0.57	4.4	0.84

[1] Metabolite Fraction C-1 eluted just after the column void volume; fraction C-2 eluted just after aminopyralid; and fraction C-3 eluted significantly later in the gradient run than aminopyralid.
[2] Not detected.

TABLE C.2.6. Distribution of the Parent and Metabolites in Grass Forage Following Base Hydrolysis of Conjugates in the Spent Aqueous Fractions from 21-DAT Grass Samples Treated with a Single Foliar Spray Application of [2,6-^{14}C]Aminopyralid at 0.321 lb ai/A (360 g ai/ha).

Fraction ID	Ryegrass		Big Bluestem		*Panicum*	
	%TRR	ppm	%TRR	ppm	% TRR	ppm
ACN/Water Extract [1]	55.5	11.57	74.7	6.75	72.7	7.29
Neutral Extract	5.0	1.04	6.2	0.56	13.8	1.38
Acidic Extract	16.1	3.36	35.6	3.21	31.5	3.16
Spent Aqueous	34.4	7.17	32.9	2.97	27.4	2.75
Organic Extract After Base Hydrolysis	30.2	6.30	26.2	2.37	23.8	2.39
Spent Aqueous After Base Hydrolysis	4.2	0.87	6.7	0.60	3.6	0.36
HPLC of Spent Aqueous Before Base Hydrolysis						
Aminopyralid	2.9	0.60	1.4	0.13	1.6	0.16
Fraction C-1	3.1	0.65	12.2	1.10	8.0	0.80
Fraction C-2	ND [3]	--	1.0	0.09	<MQL	<MQL
Fraction C-3	28.4	5.92	17.4	1.57	17.5	1.76
HPLC of Organic Extract After Base Hydrolysis						
Aminopyralid	28.7	5.98	25.2	2.28	22.8	2.29
Fraction C-1	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Fraction C-2	ND	-	ND	-	ND	--
Fraction C-3	0.6	0.13	0.7	0.23	0.5	0.05
ACN/Acid Reflux [2]	39.9	8.32	22.8	2.06	23.6	2.37
Acidic Extract	9.2	1.92	8.3	0.75	8.7	0.87
Spent Aqueous	30.7	6.40	14.5	1.31	14.9	1.49
Organic Extract After Base Hydrolysis	23.0	4.80	11.0	0.99	11.4	1.14
Spent Aqueous After Base Hydrolysis	7.7	1.60	3.5	0.32	3.5	0.35
HPLC of Spent Aqueous After Base Hydrolysis						
Aminopyralid	~4.3 [4]	~0.90	ND	--	1.2	0.12
Fraction C-1	4.6	0.96	9.3	0.84	7.4	0.74
Fraction C-2	~3.1 [4]	~0.65	<MQL	<MQL	<MQL	<MQL
Fraction C-3	18.7	3.90	5.0	0.45	6.2	0.62
HPLC of Organic Extract After Base Hydrolysis						
Aminopyralid	20.0	4.17	10.6	0.96	10.4	1.04
Fraction C-1	0.9	0.19	<MQL	<MQL	ND	--
Fraction C-2	ND	--	ND	--	ND	--
Fraction C-3	1.9	0.40	<MQL	<MQL	1.0	0.10

[1] The ACN/acid extracts were partitioned with organic solvent at a neutral and acidic pH and a portion of the resulting spent aqueous fractions then subjected to base hydrolysis step followed again by organic solvent partitioning at an acidic pH.

[2] The ACN/acid extracts were partitioned with organic solvent at an acidic pH and a portion of the resulting spent aqueous fractions then subjected to base hydrolysis step followed again by organic solvent partitioning at an acidic pH.

[3] Not detected.

[4] Aminopyralid and C-2 fractions were poorly resolved in this HPLC run and thus the values shown for each fraction represent

an estimation of the amount of each component that was actually present.

TABLE C.2.7. Summary of Characterization and Identification of Radioactive Residues in 21-DAT Grass Forage Samples [1] Following Application of Radiolabeled Aminopyralid at 0.321 lb ai/A (360 g ai/ha).

Compound	Ryegrass, forage		Bluestem, forage		*Panicum*, forage	
	TRR = 20.85 ppm		TRR = 9.03 ppm		TRR = 10.03 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Aminopyralid	25.3	5.28	38.0	3.43	33.9	3.40
Fraction C-1 [2]	6.8	1.42	11.0	1.00	6.0	0.60
Fraction C-2 [2]	4.6	0.96	5.0	0.45	4.2	0.42
Fraction C-3 [2]	58.0	12.06	43.6	3.93	52.2	5.24
Glucose Conjugate of Aminopyralid [3]	<1.0	<0.21	– [3]	--	– [3]	--
Total identified [4]	69.5	14.49	72.2	6.52	63.9	6.41
Total characterized	95.4	19.89	97.5	8.80	96.3	9.64
Total extractable	95.4	19.89	97.5	8.81	96.3	9.66
Unextractable (PES)[5]	4.6	0.96	2.5	0.23	3.7	0.37
Accountability [6]	100		100		100	

[1] A similar profile was seen in all three grass species at all sampling time points with the only differences over time being the levels at which each component was observed. Only the 21-DAT data are summarized in this table since 21 days represented the midpoint of the study and the residue in these samples were considered to be representative of the other samples analyzed during the study.

[2] All three of these fractions were multi-component in nature and consisted primarily of acid- and base-labile conjugates of aminopyralid.

[3] This metabolite was observed only during the isolation and cleanup of the C-1 fraction from an extract of rye grass in preparation for LC/MS analysis. This work was done using a 75-minute, extended gradient elution HPLC system that was developed especially for the cleanup step. Since this system was not used for the routine analyses of all samples, it was not possible to determine if this metabolite might also be present at low levels in the other two grass species.

[4] The total identified represents the total residue accounted for as aminopyralid following HPLC analyses of the residues that could be partitioned into organic solvent before and after base hydrolysis (see Table C.2.6). This is a conservative value since significant amounts of the C-3 metabolite fraction were accounted for in the solvent extracts before hydrolysis, and thus were never subjected to base hydrolysis which would have freed additional aminopyralid.

[5] Nonextractable residues that remained after exhaustive extractions.

[6] No losses of radioactivity were observed during extraction, base hydrolysis or HPLC analysis of the sample fractions. Overall recoveries for many samples following initial extraction steps were generally on the high side. This was thought to be due to partial drying of the finely ground plant material during frozen storage between the time of the combustion analyses and the time of extraction for analyses. Due to the higher recoveries, all other percentage values in the table were normalized by the petitioner.

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TABLE C.2.8. Summary of Characterization and Identification of Radioactive Residues in 42-DAT Grass Hay Following Application of Radiolabeled Aminopyralid at 0.321 lb ai/A (360 g ai/ha).

Compound	Ryegrass, hay		Bluestem, hay		*Panicum*, hay	
	TRR = 23.34 ppm		TRR = 12.64 ppm		TRR = 19.13 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Aminopyralid	23.8	5.55	31.8	4.02	34.9	6.68
Fraction C-1	6.2	1.45	8.9	1.13	6.3	1.21
Fraction C-2	3.9	0.91	3.3	0.42	6.0	1.14
Fraction C-3	56.0	13.07	51.6	6.68	48.4	9.26
Total identified	23.8	5.55	31.8	4.02	34.9	6.68
Total characterized	66.10	15.43	63.8	8.23	60.70	11.61
Total extractable	89.8	20.94	95.5	12.07	95.6	18.29
Unextractable (PES)[1]	10.2	2.38	4.5	0.57	4.4	0.84
Accountability[2]	100		100		100	

[1] Residues remaining after exhaustive extractions.
[2] Values were normalized by the petitioner, therefore, accountabilities were 100%.

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C.3. Proposed Metabolic Profile

FIGURE C.3.1. Proposed Metabolic Profile of Aminopyralid in Grass

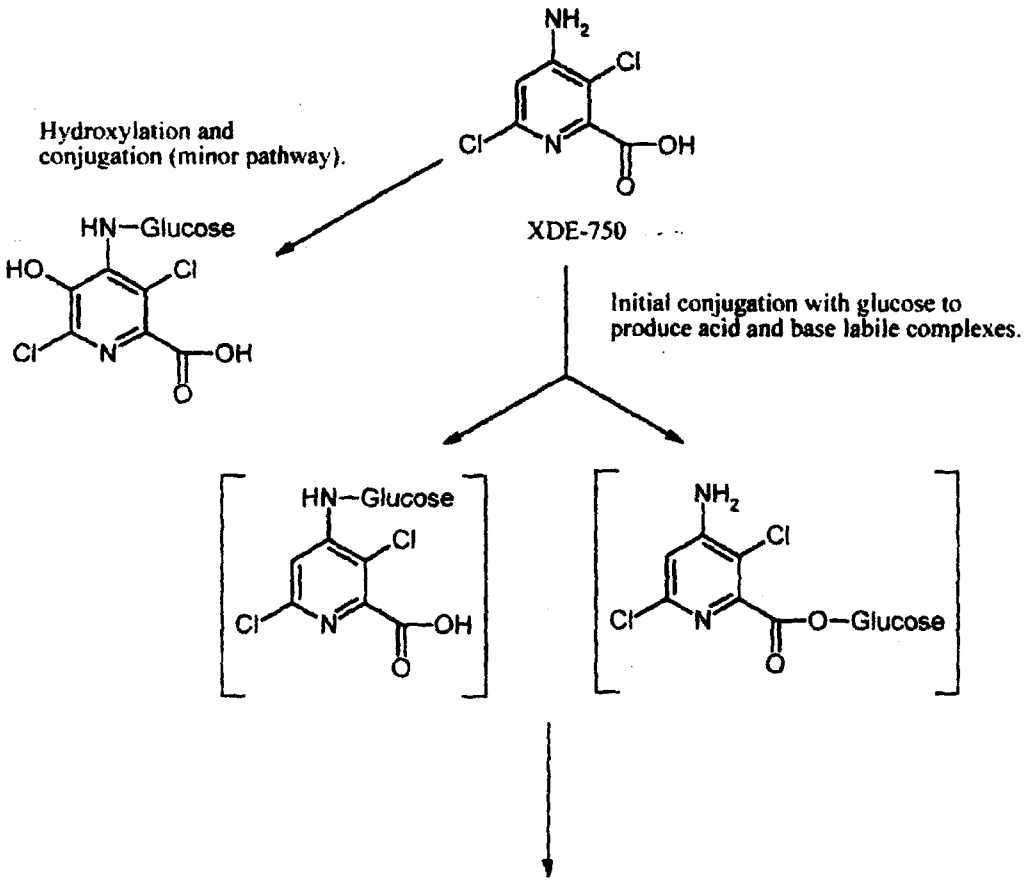

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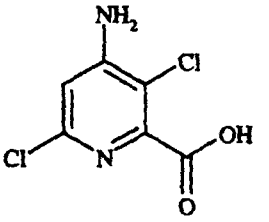
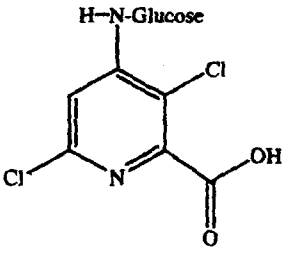
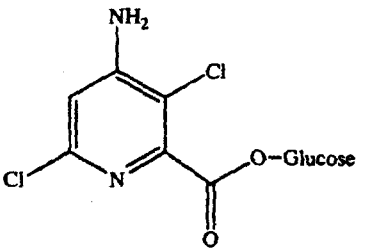
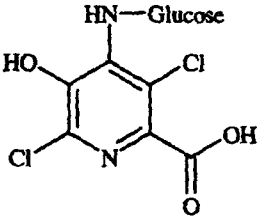
TABLE C.3.1. Identification of Compounds from Grass Metabolism Study.

Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
Aminopyralid/XDE-750	4-amino-3,6-dichloro-2-pyridinecarboxylic acid	
Glucose conjugates of aminopyralid	glucose conjugates of 4-amino-3,6-dichloro-2-pyridinecarboxylic acid	
		
Glucose conjugate of hydroxylated aminopyralid	glucose conjugate of 4-amino-3,6-dichloro-5-hydroxypyridine -2-carboxylic acid	

D. CONCLUSION

Total radioactive residues (TRR) were 48.84, 25.84, and 17.96 ppm, respectively, in ryegrass, bluestem, and *Panicum* forage harvested 0 days following a single foliar application of [2,6-^{14}C]aminopyralid, formulated as a potassium salt, made to pasture grasses at 0.321 lb ai/A (360 g ai/ha). TRR generally declined with each subsequent sampling interval, with a TRR of 6.57, 5.61, and 4.81 ppm, respectively, in ryegrass, bluestem, and *Panicum* forage harvested 42 days after treatment (DAT). The 42-DAT forage samples were dried for hay, and the respective TRR were 23.34, 12.64, and 19.13 ppm in ryegrass, bluestem, and *Panicum* hay.

The study adequately characterized and identified radioactive residues in grass forage and hay. Unconjugated aminopyralid was the primary residue component identified through the first 7-14 days after application and still represent about 20% or more of the TRR at 42 DAT. Most of the remaining residue in samples collected at later intervals could be accounted for in a single, water-soluble complex that was less polar than aminopyralid, while each of the other two complexes were observed at much lower levels (generally 10% or less of the TRR)

Hydrolysis of the water-soluble residues using either acid or base reflux conditions resulted in the liberation of additional aminopyralid; however, base hydrolysis proved to be the most effective means for achieving this release. The petitioner concluded that based on these findings and on published work with the related herbicide picloram, it was assumed that the water-soluble complexes observed in this study represented isomeric mixtures of acid and alkali-labile N-glucosides and glucose ester conjugates of aminopyralid. Other than conjugation, the only metabolic change to the basic structure of the parent molecule itself was the addition of a hydroxyl group to produce a conjugated metabolite that was observed only at <1% of the TRR.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: MADoherty, 06/28/05; TSheramata, 06/13/05; HBietlot, 6/13/05
Petition Number(s): PP#4F6827
DP Barcode(s): D305665
PC Code: 005100/005209

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Aminopyralid/XDE-750/PC Code 005100/Dow AgroSciences/62719
DACO 7.3/OPPTS 860.1380/OECD IIA 6.1.1 and IIIA 8.1.1
Storage Stability - Grasses and Wheat

Primary Evaluator _____ Date: 6/28/05
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Peer Reviewer _____ Date: June 6/05
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Evaluation Officer, FREAS, HED, PMRA

Approved by _____ Date: 13/05
Henri Bietlot, Ph.D.
A/Section Head, FREAS, HED, PMRA

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 11/08/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46235719 Lindsay, D. (2004) Frozen Storage Stability of XDE-750 in Range Land and Pasture Grass and Hay and Wheat Straw and Wheat Grain - Interim Report. Project Number: 030004. Unpublished study prepared by Dow AgroSciences LLC. 50 p.

EXECUTIVE SUMMARY:

Dow AgroSciences has submitted interim data from a study investigating the stability of aminopyralid in/on grass hay and forage, and wheat grain and straw under frozen storage conditions. Samples of untreated homogenized grass hay and forage and wheat grain and straw were fortified with aminopyralid at 0.1 ppm and stored frozen (~-20°C) for up to 187 days for grass hay and forage, 168 days for wheat grain, and 175 days for wheat straw. Samples of grass hay and forage were analyzed, along with fresh fortification samples, after 0, 28, 130, and 187 days of storage. Samples of wheat grain and straw were analyzed, along with fresh fortification samples, after 0, 113, and 168/175 days of storage. The final analysis data, targeted for 18 months of storage, will be submitted when the study has been completed.

The results indicate that under these conditions residues of aminopyralid are stable for up to 187 days in/on grass hay and forage, 168 days in/on wheat grain, and 175 days in/on wheat straw.

Samples from the storage stability study were analyzed for residues of aminopyralid using LC/MS/MS Method GRM 02.31. The method is adequate for data collection for the purposes of this storage stability study based on acceptable concurrent method recovery and radiovalidation data.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the storage stability data are classified as provisionally scientifically acceptable (see Compliance, below). The final report is expected to have fully QA-audited report data.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D305665, and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated GLP and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study. A Quality Assurance statement was provided, however, it was not signed and dated; the petitioner stated that the interim report data were not audited by the QA unit.

A. BACKGROUND INFORMATION

Aminopyralid (4-amino-3,6-dichloropyridine-2-carboxylic acid) is the proposed common name of XDE-750, a new active ingredient developed by Dow AgroSciences. Aminopyralid is a systemic postemergence herbicide which belongs to the pyridine carboxylic acid class of herbicides. The petitioner is currently proposing food/feed uses on grasses grown in rangelands and permanent pastures and on wheat for the selective control of invasive and noxious broadleaf weeds. It is also proposed for weed control in sites such as parks, electric utility rights-of way, forestry, woodlands, and wildlife openings, with smaller amounts used in railroads, utility substations, pipelines, and pumping stations.

The proposed end-use product (EP) is a soluble concentrate liquid (SC/L) referred to by the trade name GF-871 (EPA Reg. No. 62719-LRI). The active ingredient in GF-871 is formulated as the triisopropanolammonium (TIPA) salt, with the product containing 40.6% of aminopyralid TIPA salt at an acid equivalent (ae) of 21.1% or 2 lb ae/gal (240 g ae/L). The petitioner stated that the aminopyralid TIPA salt dissociates rapidly in water to the acid (aminopyralid) at environmental pH values greater than 2.56 (the pKa).

Currently, the 2 lb ae/gal (240 g ae/L) SC/L formulation is proposed for broadcast foliar application at maximum rates of 0.11 lb ae/A (120 g ae/hectare) on rangeland and permanent pastures and 0.009 lb ae/A (10 g ae/ha) on wheat. The proposed PHIs are 0 days for wheat hay and 50 days for wheat grain and straw. No PHI is listed or proposed for grasses or wheat forage.

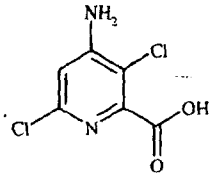
TABLE A.1. Test Compound Nomenclature.	
Chemical structure	
Common name	Aminopyralid
Company experimental name	XDE-750
IUPAC name	4-amino-3,6-dichloropyridine-2-carboxylic acid
CAS name	4-amino-3,6-dichloro-2-pyridinecarboxylic acid
CAS registry number	150114-71-9
End-use product (EP)	2 lb ae/gal (240 g ae/L) TIPA salt SC/L formulation (GF-871 Herbicide; EPA Reg. No. 62719-LRI; Aminopyralid Liquid Concentrate Herbicide in Canada)

TABLE A.2. Physicochemical Properties of the Aminopyralid Technical Grade Test Compound.			
Parameter	Value		Reference
Melting point	163.5 °C		MRID 46235703, PMRA LS
pH	2.31 at 23.4 °C (1% solution in water)		MRID 46235703, PMRA LS
Relative density	1.72 at 20 °C		MRID 46235703, PMRA LS
Water solubility	2.48 g/L unbuffered water at 18 °C 212 g/L pH 5 buffer at 20 °C 205 g/L pH 7 buffer at 20 °C 203 g/L pH 9 Buffer at 20 °C		MRID 46235703, PMRA LS
Solvent solubility at 20 °C	methanol acetone n-octanol ethyl acetate 1,2-dichloroethane xylene heptane	52.2 g/L 29.2 g/L 3.9 g/L 3.9 g/L 0.2 g/L 0.04 g/L <10 µg/mL	MRID 46235703, PMRA LS
Vapor pressure	2.59 x 10^{-8} Pa at 25 °C; 9.52 x 10^{-9} Pa at 20 °C		MRID 46235703, PMRA LS
Dissociation constant, pK_a	2.56		MRID 46235703, PMRA LS
Octanol/water partition coefficient, Log(K_{ow})	0.201 unbuffered water at 19 °C; -1.76 at pH 5; -2.87 at pH 7; -2.96 at pH 9		MRID 46235703, PMRA LS
UV/visible absorption spectrum	Solution Neutral Basic (pH 12.6) Acidic (pH 1.4)	Wavelength λ max, nm 217 220 245 217 270	Extinction coefficient ε, L/(mol*cm) 29100 26100 10150 22800 9140	MRID 46235703, PMRA LS

B. EXPERIMENTAL DESIGN

B.1. Sample Handling and Preparation

Samples of untreated grass hay and forage, and wheat grain and straw were obtained from the Dow AgroSciences Regulatory Laboratories. All samples were frozen with liquid nitrogen, ground, and fortified with aminopyralid at 0.1 ppm. The aminopyralid fortification standard was prepared in methanol. Fortified and unfortified samples were then stored frozen (ca. -20 ° C) in polyethylene containers. Stored samples and fresh fortification samples of grass hay and forage were analyzed after 0, 28, 130, and 187 days of frozen storage. Stored samples and fresh fortification samples of wheat grain and straw were analyzed after 0, 113, and 168/175 days of frozen storage. Additional data for samples stored for up to 18 months under frozen conditions will be submitted in the final report upon conclusion of the study. We note that the 0-day grass hay and forage samples were actually fortified and analyzed concurrently with the 28-day stored samples and the 0-day wheat grain and straw samples were fortified and analyzed a day before 113-day stored samples.

B.2. Analytical Methodology

Samples of grass hay and forage and wheat grain and straw were analyzed for residues of aminopyralid using Dow AgroSciences Method GRM 02.31. The method LOQ was 0.01 ppm for aminopyralid. A complete description of the method is provided in the Residue Analytical Method DER for plants (MRID 46235712).

Briefly, homogenized samples of grass hay and forage and wheat grain and straw were extracted using 0.1 N sodium hydroxide and centrifuged. Residues were concentrated and purified using solid phase extraction (SPE) cartridges. The internal standard, $^{13}C_2{}^{15}N$-aminopyralid, was added to the eluate. The eluate was evaporated to incipient dryness and residues were redissolved in coupling reagent, derivatized with butyl chloroformate, and diluted with mobile phase for analysis by LC/MS/MS. The limit of quantitation was 0.01 ppm.

C. RESULTS AND DISCUSSION

The concurrent method validation data included in the study indicate that the LC/MS/MS method GRM 02.31 is adequate for the determination of residues of aminopyralid in/on grass hay and forage and wheat grain and straw. Apparent residues were nondetectable (<0.01 ppm) in all unfortified samples (one control sample each of grass hay and forage, and wheat grain and straw). We note that raw data for the 6-month storage interval for wheat straw were not provided; in the raw data section (Figure 14 of the MRID), the results for the 6-month analysis indicate DNU (data not used). Presumably the raw data for wheat straw for this interval will be included in the final report of this storage stability study.

Residues of aminopyralid appear to be stable in/on grass hay and forage stored frozen for up to 187 days (6.2 months) and in/on wheat grain and straw stored frozen for up to 168 days (5.5

months) and 175 days (5.8 months), respectively. A graph of the stability of aminopyralid in grass hay and forage, and wheat grain and straw is presented in Figure C.1.

TABLE C.1. Summary of Concurrent Recoveries of Aminopyralid from Grass Forage and Hay and Wheat Grain and Straw.

Matrix	Spike level (ppm)	Storage Interval (days)	Sample size (n)	Recoveries (%)	Mean
Grass hay	0.1	0	2	93, 94	94
		28	2	91, 93	92
		130	2	73, 79	76
		187	2	81, 83	82
Grass forage	0.1	0	2	91, 94	93
		28	2	95, 95	95
		130	2	71, 78	75
		187	2	82, 85	84
Wheat grain	0.1	0	2	85, 87	86
		113	2	89, 90	90
		168	2	93, 97	95
Wheat straw	0.1	0	2	82, 84	83
		113	2	81, 88	85
		175	2	87, 96	92

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FIGURE C.1. Graph of Storage Stability of Aminopyralid in Grass Hay and Forage and Wheat Grain and Straw.

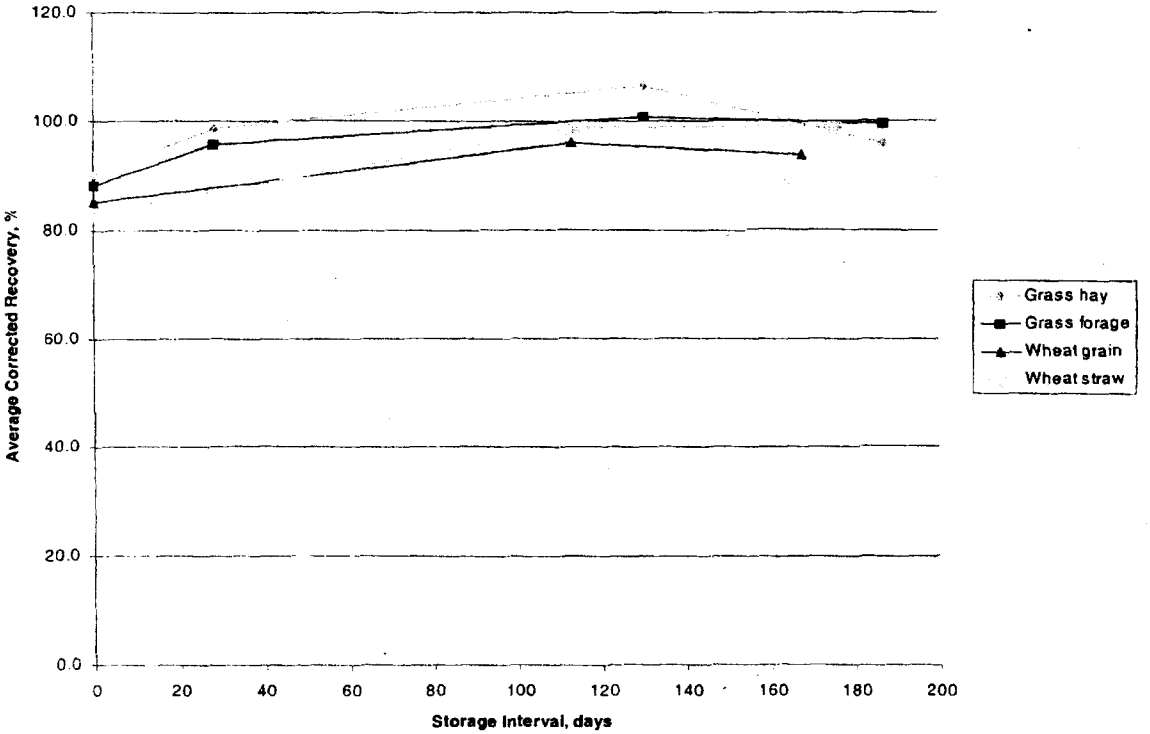

TABLE C.2. Stability of Aminopyralid Residues in Grass Hay and Forage and Wheat Grain and Straw Following Storage at -20 °C.

Commodity	Spike level (ppm)	Storage interval (days)	Recovered residues (ppm)	Corrected % recovery[1]
Grass hay	0.1	0	0.0862, 0.0885, 0.0898	--
		28	0.0880, 0.0909, 0.0944	95.7, 98.8, 103
		130	0.0689, 0.0763, 0.0984	90.7, 100, 129
		187	0.0769, 0.0784, 0.0807	93.8, 95.6, 98.4
Grass forage	0.1	0	0.0765, 0.0898, 0.0908	--
		28	0.0904, 0.0907, 0.0922	95.2, 95.5, 97.1
		130	0.0678, 0.0790, 0.0803	90.4, 105, 107
		187	0.0771, 0.0863, 0.0870	91.8, 103, 104
Wheat grain	0.1	0	0.0824, 0.0853, 0.0867	--
		113	0.0800, 0.0898, 0.0900	88.9, 99.8, 100
		168	0.0882, 0.0883, 0.0911	92.8, 92.9, 95.9

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TABLE C.2. Stability of Aminopyralid Residues in Grass Hay and Forage and Wheat Grain and Straw Following Storage at -20 °C.				
Commodity	Spike level (ppm)	Storage interval (days)	Recovered residues (ppm)	Corrected % recovery[1]
Wheat straw	0.1	0	0.0816, 0.0847, 0.0854	--
		113	0.0821, 0.0821, 0.0878	96.6, 96.6, 103
		175	0.0897, 0.0918, 0.0920	97.5, 99.8, 100

[1] Corrected for average concurrent method recoveries.

D. CONCLUSION

The submitted interim storage stability study adequately demonstrates the stability of aminopyralid residues in grass hay and forage, and wheat grain and straw stored frozen for ~6 months. The data indicate that residues of aminopyralid are stable under frozen storage conditions for up to 187 days for grass hay and forage, 168 days for wheat grain, and 175 days for wheat straw. The petitioner stated that the full study will include storage intervals of up to 18 months for grass hay and forage, and wheat grain and straw.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: MADoherty, 06/28/05; TSheramata, 06/13/05; HBietlot, 6/13/05
Petition Number(s): PP#4F6827
DP Barcode(s): D305665
PC Code: 005100/005209

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Aminopyralid/XDE-750/PC Code 005100/Dow AgroSciences/62719
DACO 7.2.4/OPPTS 860.1360/OECD IIIA 5.3.1
Multiresidue Analytical Methods

Primary Evaluator _____ Date: 6/2/05
Michael A. Doherty, Ph.D.; Chemist, RAB2

Peer Reviewer _____ Date: June 6/05
Tamara Sheremata, Ph.D.,
Evaluation Officer, FREAS, HED, PMRA

Approved by _____ Date: 6/13/05
Henri Bietlot, Ph.D.,
A/Section Head, FREAS, HED, PMRA

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 11/08/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

46235718 Lala, M.; Mollica, J.; West, S. (2002) PAM I Multiresidue Protocol Testing for XDE-750: Final Report. Project Number: 021197, DOW/1413. Unpublished study prepared by Pyxant Labs Inc. 267 p.

EXECUTIVE SUMMARY:

Dow AgroSciences has submitted multiresidue method data for aminopyralid. Aminopyralid was analyzed according to the FDA Multi-Residue Method Test guidelines in PAM Vol. I, 3rd Edition (dated 1/94). Aminopyralid was tested through Protocols A and C, and as a result of Protocol C testing, was also tested through Protocols D and E. Based on the results using Protocol E, testing under Protocol F was not required. Since methylated aminopyralid provided good response on the column/detector combinations outlined in Protocol C, additional testing was performed with aminopyralid methyl ester under Protocol B.

We note that the testing laboratory (Pyxant Labs Inc.) did not address the testing of aminopyralid using Protocol G; however, testing of this compound using Protocol G is not required because the compound is not a substituted urea. The petitioner and the testing laboratory should note that the most recent version of PAM Vol. I is dated 10/99.

Aminopyralid is not an N-methylcarbamate and was not found to be naturally fluorescent; therefore, further testing under Protocol A was not required. Methylation efficiency was low for aminopyralid using Protocol B. Aminopyralid was not recovered using Protocol D (with no cleanup), or using Florisil cleanup under Protocols E and F. The results of the study indicate that the FDA MRM guidelines in PAM Vol. I are not applicable to aminopyralid.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the multiresidue method residue data are classified as scientifically acceptable. These data will be forwarded to the U.S. FDA for further evaluation.

The petitioner and the testing laboratory should note that the most recent version of PAM Vol. I is dated 10/99 and has additional protocols not provided in the 1/94 version.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D305665 and in Canada's Regulatory Decision Document.

COMPLIANCE:

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

A. BACKGROUND INFORMATION

Aminopyralid (4-amino-3,6-dichloropyridine-2-carboxylic acid) is the proposed common name of XDE-750, a new active ingredient developed by Dow AgroSciences. Aminopyralid is a systemic postemergence herbicide which belongs to the pyridine carboxylic acid class of herbicides. The petitioner is currently proposing food/feed uses on grasses grown in rangelands and permanent pastures and on wheat for the selective control of invasive and noxious broadleaf weeds. It is also proposed for weed control in sites such as parks, electric utility rights-of way, forestry, woodlands, and wildlife openings, with smaller amounts used in railroads, utility substations, pipelines, and pumping stations.

The proposed end-use product (EP) is a soluble concentrate liquid (SC/L) referred to by the trade name GF-871 (EPA Reg. No. 62719-LRI). The active ingredient in GF-871 is formulated as the triisopropanolammonium (TIPA) salt, with the product containing 40.6% of aminopyralid TIPA salt at an acid equivalent (ae) of 21.1% or 2 lb ae/gal (240 g ae/L). The petitioner stated that the aminopyralid TIPA salt dissociates rapidly in water to the acid (aminopyralid) at environmental pH values greater than 2.56 (the pKa).

Currently, the 2 lb ae/gal (240 g ae/L) SC/L formulation is proposed for broadcast foliar application at maximum rates of 0.11 lb ae/A (120 g ae/hectare) on rangeland and permanent pastures and 0.009 lb ae/A (10 g ae/ha) on wheat. The proposed PHIs are 0 days for wheat hay and 50 days for wheat grain and straw. No PHI is listed or proposed for grasses or wheat forage.

TABLE A.1. Test Compound Nomenclature.

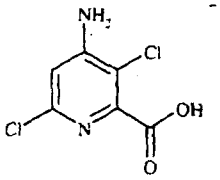
Chemical structure	
Common name	Aminopyralid
Company experimental name	XDE-750
IUPAC name	4-amino-3,6-dichloropyridine-2-carboxylic acid
CAS name	4-amino-3,6-dichloro-2-pyridinecarboxylic acid
CAS registry number	150114-71-9
End-use product (EP)	2 lb ae/gal (240 g ae/L) TIPA salt SC/L formulation (GF-871 Herbicide; EPA Reg. No. 62719-LRI; Aminopyralid Liquid Concentrate in Canada)

TABLE A.2. Physicochemical Properties of the Aminopyralid Technical Grade Test Compound.

Parameter	Value	Reference
Melting point	163.5 °C	MRID 46235703, PMRA LS
pH	2.31 at 23.4 °C (1% solution in water)	MRID 46235703, PMRA LS
Relative density	1.72 at 20 °C	MRID 46235703, PMRA LS
Water solubility	2.48 g/L unbuffered water at 18 °C 212 g/L pH 5 buffer at 20 °C 205 g/L pH 7 buffer at 20 °C 203 g/L pH 9 Buffer at 20 °C	MRID 46235703, PMRA LS
Solvent solubility at 20 °C	methanol 52.2 g/L acetone 29.2 g/L n-octanol 3.9 g/L ethyl acetate 3.9 g/L 1,2-dichloroethane 0.2 g/L xylene 0.04 g/L heptane <10 µg/mL	MRID 46235703, PMRA LS
Vapor pressure	2.59×10^{-8} Pa at 25 °C; 9.52×10^{-9} Pa at 20 °C	MRID 46235703, PMRA LS
Dissociation constant, pK_a	2.56	MRID 46235703, PMRA LS
Octanol/water partition coefficient, $Log(K_{OW})$	0.201 unbuffered water at 19 °C; -1.76 at pH 5; -2.87 at pH 7; -2.96 at pH 9	MRID 46235703, PMRA LS
UV/visible absorption spectrum	Solution / Wavelength λ max, nm / Extinction coefficient ε, L/(mol*cm) Neutral 217 29100 Basic 220 26100 (pH 12.6) 245 10150 Acidic 217 22800 (pH 1.4) 270 9140	MRID 46235703, PMRA LS

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B. MATERIALS AND METHODS

Aminopyralid was analyzed according to the FDA Multi-Residue Method Test guidelines in PAM Vol. I, Appendix II (1/94). Aminopyralid was tested through Protocols A and C, and as a result of Protocol C testing, was also tested through Protocols D and E. Based on the results of Protocol E testing, testing under Protocol F was not required. Since methylated aminopyralid provided good response on the column/detector combinations outlined in Protocol C, additional testing was performed with aminopyralid-Me under Protocol B. Testing using Protocol G was not conducted; this testing is not required because aminopyralid is not a substituted urea.

C. RESULTS AND DISCUSSION

TABLE C.1. Results of Multiresidue Methods Testing with Aminopyralid.

PAM I Protocol	Results	Comments
A	Aminopyralid was not found to be naturally fluorescent.	Aminopyralid is not an *N*-methylcarbamate. No further testing needed.
B	Aminopyralid was methylated with diazomethane and tested. Aminopyralid-Me was recovered (83.6%) from the Florisil column, but the methylation efficiency, by comparison to diazomethane derived methyl ester standard, was low (8.2%).	No further testing conducted because of low methylation efficiency.
C	Aminopyralid demonstrated reasonable relative retention times using modules DG1, DG13, and DG18 (electron capture and nitrogen/phosphorus detection), but had a relative retention time <0.3 using modules DG5 and DG17. Level II testing was not conducted. All modules had deflections greater than 50%. Two peaks were observed for aminopyralid with modules DG13, DG17, and DG18. Because aminopyralid is an acid, the methyl ester from Protocol B was also tested. Aminopyralid-Me demonstrated reasonable relative retention times using modules DG1, DG5, DG13, DG17, and DG18 (electron capture and nitrogen/phosphorus detection) with greater than 50% deflection.	Further work conducted using Protocols D, E, and F
D	Aminopyralid was tested in a non-fatty matrix without Florisil cleanup; recoveries from wheat grain were ≤16.5% at 0.05 ppm and ≤9.0% at 0.25 ppm, using Method 302 E1. A strong matrix effect was observed.	
E	Aminopyralid was not recovered using Florisil cleanup methods C1 and C2 for Protocol E.	Because aminopyralid could not be recovered during Florisil column cleanup, no further testing was conducted.
F	Not tested, because aminopyralid could not be recovered from the Florisil column cleanup.	
G	Not tested.	Not required because aminopyralid is not a substituted urea.

D. CONCLUSION

Aminopyralid was adequately evaluated for recovery through FDA multiresidue methods. The results of the study indicate that the FDA MRM guidelines in PAM Vol. I are not applicable to aminopyralid. Aminopyralid was not found to be naturally fluorescent; therefore, further testing under Protocol A was not required. Methylation efficiency was low for aminopyralid using Protocol B. Aminopyralid was not recovered using Protocol D (with no cleanup), or using Florisil cleanup under Protocols E and F. The submitted data will be forwarded to the U.S. FDA for further evaluation.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: MADoherty, 06/28/05; TSheramata, 06/13/05; HBietlot, 6/13/05
Petition Number(s): PP#4F6827
DP Barcode(s): D305665
PC Code: 005100/005209

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Aminopyralid/XDE-750/PC Code 005100/Dow AgroSciences/62719
DACO 7.2.1, 7.2.2, and 7.2.3/OPPTS 860.1340/OECD IIA 4.2.5, 4.2.6 and 4.3
Residue Analytical Method: Plant

Primary Evaluator _____ Date: 6/28/05
Michael A. Doherty, Ph.D., Chemist, RAB2

Peer Reviewer _____ Date: June 6/05
Tamara Sheremata, Ph.D.,
Evaluation Officer, FREAS, HED, PMRA

Approved by _____ Date: Jun 13/05
Henri Bietlot, Ph.D.,
A/Section Head, FREAS, HED, PMRA

In the absence of signatures, this document is considered to be a draft with deliberative material for internal use only.

This DER was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 11/08/2004). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

46235712 Reed, R. (2004) Independent Laboratory Validation of Dow AgroSciences LLC Method GRM 02.31 - Determination of Residues of Aminopyralid in Agricultural Commodities by Liquid Chromatography with Tandem Mass Spectrometry Detection. Project Number: 020157, ML03/1110/DOW. Unpublished study prepared by Morse Laboratories. 147 p.

46235717 Olberding, E.; Hastings, M. (2004) Validation Report for Method GRM 02.31 - Determination of Residues of Aminopyralid in Agricultural Commodities by Liquid Chromatography with Tandem Mass Spectrometry Detection. Project Number: 021310, GRM/02/31. Unpublished study prepared by Dow AgroSciences LLC. 61 p.

EXECUTIVE SUMMARY:

Dow AgroSciences has proposed an LC/MS/MS Method GRM 02.31, entitled "Determination of Residues of Aminopyralid in Agricultural Commodities by Liquid Chromatography with Tandem Mass Spectrometry Detection" for the enforcement of tolerances for residues of aminopyralid in plant commodities. The proposed LC/MS/MS method was used to determine residues of free and conjugated aminopyralid in/on grass and wheat samples from the storage stability, field trial, and processing studies associated with the currently requested uses.

Briefly, ground samples are extracted with 0.1 N sodium hydroxide, releasing bound residues and hydrolyzing base-labile conjugates to free aminopyralid. The extract is then acidified with hydrochloric acid and heated to release acid-labile conjugates. Following hydrolysis, the extract is cleaned up through an anion-exchange solid-phase extraction (SPE) column. The internal standard, $^{13}C_2{}^{15}N$-aminopyralid, is added to the eluate, and residues are derivatized with butyl

chloroformate to form the 1-butyl esters of aminopyralid for LC/MS/MS analysis. The validated limit of quantitation (LOQ) is 0.01 ppm for all matrices, and the calculated limit of detection (LOD) is 0.002 ppm.

Method validation data for LC/MS/MS Method GRM 02.31 demonstrated adequate method recoveries of aminopyralid from barley grain, forage, and straw; grass forage and hay; sorghum grain, forage, and stover; and wheat grain, forage, and straw fortified at the LOQ (0.01 ppm) and at up to 0.50 ppm for cereal grain, 5.00 ppm for cereal forage and straw, and 20.0 ppm for grasses.

Adequate radiovalidation data have been submitted for the extraction procedures of Method GRM 02.31 using samples of grass and wheat commodities bearing weathered residues, from the respective metabolism studies, in which crops were treated with [2,6-^{14}C]aminopyralid. Adequate independent laboratory validation data have been submitted using grass forage and wheat grain. The petitioner concluded that confirmatory analysis procedures are not required for the proposed enforcement method due to the high specificity of the LC/MS/MS method. Because the method only monitors one transition ion, HED defers to ACB to determine whether confirmatory analysis procedures are needed for the method.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the analytical method residue data are classified as scientifically acceptable. The petitioner is required to show that the proposed enforcement method (GRM 02.31) can differentiate between aminopyralid, clopyralid, and picloram, as they are all similar in structure. The proposed enforcement method will be forwarded to ACB for petition method validation.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D305665 and in Canada's Regulatory Decision Document.

COMPLIANCE:

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

A. BACKGROUND INFORMATION

Aminopyralid (4-amino-3,6-dichloropyridine-2-carboxylic acid) is the proposed common name of XDE-750, a new active ingredient developed by Dow AgroSciences. Aminopyralid is a systemic postemergence herbicide which belongs to the pyridine carboxylic acid class of herbicides. The petitioner is currently proposing food/feed uses on grasses grown in rangelands and permanent pastures and on wheat for the selective control of invasive and noxious broadleaf

weeds. It is also proposed for weed control in sites such as parks, electric utility rights-of way, forestry, woodlands, and wildlife openings, with smaller amounts used in railroads, utility substations, pipelines, and pumping stations.

The proposed end-use product (EP) is a soluble concentrate liquid (SC/L) referred to by the trade name GF-871 (EPA Reg. No. 62719-LRI). The active ingredient in GF-871 is formulated as the triisopropanolammonium (TIPA) salt, with the product containing 40.6% of aminopyralid TIPA salt at an acid equivalent (ae) of 21.1% or 2 lb ae/gal (240 g ae/L). The petitioner stated that the aminopyralid TIPA salt dissociates rapidly in water to the acid (aminopyralid) at environmental pH values greater than 2.56 (the pKa).

Currently, the 2 lb ae/gal (240 g ae/L) SC/L formulation is proposed for broadcast foliar application at maximum rates of 0.11 lb ae/A (120 g ae/hectare) on rangeland and permanent pastures and 0.009 lb ae/A (10 g ae/ha) on wheat. The proposed PHIs are 0 days for wheat hay and 50 days for wheat grain and straw. No PHI is listed or proposed for grasses or wheat forage.

TABLE A.1. Test Compound Nomenclature.

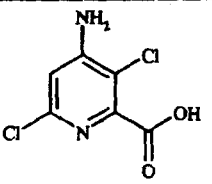
Chemical structure	
Common name	Aminopyralid
Company experimental name	XDE-750
IUPAC name	4-amino-3,6-dichloropyridine-2-carboxylic acid
CAS name	4-amino-3,6-dichloro-2-pyridinecarboxylic acid
CAS registry number	150114-71-9
End-use product (EP)	2 lb ae/gal (240 g ae/L) TIPA salt SC/L formulation (GF-871 Herbicide; EPA Reg. No. 62719-LRI; Aminopyralid Liquid Concentrate in Canada)

TABLE A.2. Physicochemical Properties of the Aminopyralid Technical Grade Test Compound.

Parameter	Value	Reference
Melting point	163.5 °C	MRID 46235703, PMRA LS
pH	2.31 at 23.4 °C (1% solution in water)	MRID 46235703, PMRA LS
Relative density	1.72 at 20 °C	MRID 46235703, PMRA LS
Water solubility	2.48 g/L unbuffered water at 18 °C 212 g/L pH 5 buffer at 20 °C 205 g/L pH 7 buffer at 20 °C 203 g/L pH 9 Buffer at 20 °C	MRID 46235703, PMRA LS

TABLE A.2. Physicochemical Properties of the Aminopyralid Technical Grade Test Compound.

Parameter	Value	Reference
Solvent solubility at 20 °C	methanol 52.2 g/L acetone 29.2 g/L n-octanol 3.9 g/L ethyl acetate 3.9 g/L 1,2-dichloroethane 0.2 g/L xylene 0.04 g/L heptane <10 µg/mL	MRID 46235703, PMRA LS
Vapor pressure	2.59×10^{-4} Pa at 25 °C; 9.52×10^{-9} Pa at 20 °C	MRID 46235703, PMRA LS
Dissociation constant, pK_a	2.56	MRID 46235703, PMRA LS
Octanol/water partition coefficient, $Log(K_{ow})$	0.201 unbuffered water at 19 °C; -1.76 at pH 5; -2.87 at pH 7; -2.96 at pH 9	MRID 46235703, PMRA LS
UV/visible absorption spectrum	Solution: Neutral; Basic; (pH 12.6); Acidic; (pH 1.4) Wavelength λ max, nm: 217; 220; 245; 217; 270 Extinction coefficient ε, L/(mol*cm): 29100; 26100; 10150; 22800; 9140	MRID 46235703, PMRA LS

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

Samples from the storage stability, grass and wheat field trial, and wheat processing studies associated with the currently requested uses were analyzed for residues of free and conjugated aminopyralid using LC/MS/MS Method GRM 02.31, entitled "Determination of Residues of Aminopyralid in Agricultural Commodities by Liquid Chromatography with Tandem Mass Spectrometry Detection."

B.1.1. Principle of the Method:

Briefly, ground samples were extracted with 0.1 N sodium hydroxide, releasing bound residues and hydrolyzing base-labile conjugates to free aminopyralid. The extract is then acidified with hydrochloric acid and heated to release acid-labile conjugates. Note that samples of grass hay and forage and wheat grain and straw from the storage stability study were subjected only to base hydrolysis. Following hydrolysis, the extract was cleaned up through an anion-exchange SPE column. The internal standard, $^{13}C_2{}^{15}N$-aminopyralid, was added to the eluate and residues derivatized with butyl chloroformate to form the 1-butyl esters of aminopyralid for LC/MS/MS analysis.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Aminopyralid Residues in Barley, Grass, Sorghum, and Wheat.	
Method ID	GRM 02.31
Analyte(s)	Aminopyralid
Extraction solvent/technique	Ground samples are extracted with 0.1 N sodium hydroxide, releasing bound residues and hydrolyzing base-labile conjugates to free aminopyralid; the extract is isolated by centrifugation. An aliquot of the supernatant is acidified with 2.0 N hydrochloric acid and heated at 80 °C for 90 minutes, to hydrolyze acid-labile conjugates. After returning to ambient temperatures, the sample is sonicated and centrifuged.
Cleanup strategies	Following hydrolysis, the extract is cleaned up through a mixed-mode polymeric anion-exchange SPE column; residues are eluted with ethyl acetate:trifluoroacetic acid (99:1, v:v). The internal standard, $^{13}C_2{}^{15}N$-aminopyralid, is added to the SPE eluate. The eluate is then evaporated to dryness, residues are reconstituted in acetonitrile:pyridine:1-butanol (22:2:1, v:v:v) and derivatized with butyl chloroformate to form the 1-butyl esters of aminopyralid and the internal standard. The derivatized solution is diluted with methanol:water:acetic acid (50:49.9:0.1, v:v:v) for analysis.
Instrument/Detector	HPLC utilizing a reverse-phase column and a gradient mobile phase of methanol and water, each containing 0.1% acetic acid, with tandem mass spectrometry (MS/MS) detection using electrospray ionization operating in the positive ion mode with multiple reaction monitoring. The ions monitored for aminopyralid butyl ester are 263 amu (precursor ion) and 134 amu (product ion); ions monitored for the internal standard $^{13}C_2{}^{15}N$-aminopyralid butyl ester are 268 amu (precursor ion) and 139 amu (product ion).
Standardization method	Stable-isotope labeled internal standard, $^{13}C_2{}^{15}N$-aminopyralid, and external bracketing calibration standards of aminopyralid, each derivatized to their 1-butyl esters. The relative response of analyte peak to internal standard peak is calculated for each standard and a calibration curve is generated using power regression. Derivatized cross-over standards of $^{13}C_2{}^{15}N$-aminopyralid and aminopyralid are analyzed to determine the isotopic crossover factor of unlabeled and labeled aminopyralid (see text below).
Stability of std solutions	The petitioner indicated that based on previous environmental fate studies, aminopyralid has been proven to be stable in a multitude of solvents under varying temperatures.
Retention times	4.4-4.6 mins (based on submitted wheat and grass chromatograms)

The petitioner noted that when using stable-isotope labeled internal standards, there is a possibility that isotopic contributions will occur between the MS/MS transitions used for quantitation of unlabeled and labeled compounds; therefore, the method includes instructions for the determination of isotopic crossover factors. The average crossover factor determined for analyte to internal standard (ISTD) was 0.00543, while the average crossover factor determined for ISTD to analyte was 0.00287. These factors demonstrated a higher percentage of crossover from analyte to ISTD. The petitioner stated that the amount of the internal standard used in the method was chosen to minimize the crossover of analyte to ISTD over the calibration range. Therefore, the measured quantitation ratio only needs to be corrected for the ISTD to analyte crossover contribution. Correction is made by subtracting the determined ISTD-to-analyte crossover factor from the analyte/ISTD peak area ratio.

B.2. Enforcement Method

The proposed enforcement method is the same as the data-gathering method, LC/MS/MS Method GRM 02.31.

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C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

TABLE C.1.1. Recovery Results from Method Validation of Barley, Grass, Sorghum, and Wheat using the Data-Gathering Analytical Method.[1]

Matrix	Spiking Level (ppm)	Recoveries (%) Obtained [2]	Mean Recovery ± SD [CV]
Barley, grain	0.003	(0.0024 ppm), (0.0029 ppm)	–
	0.010	92, 99, 106, 107	102 ± 6.6 [6.4]
	0.025	107	
	0.050	96, 111	
	0.100	110	
	0.250	95	
	0.500	97, 103	
Barley, forage	0.003	(0.0023 ppm)	–
	0.010	88, 96, 100, 100	96 ± 6.3 [6.5]
	0.050	86, 100	
	0.250	93	
	1.000	101	
	5.000	93, 106	
Barley, straw	0.003	(0.0028 ppm)	–
	0.010	91, 92, 95, 98	96 ± 4.0 [4.2]
	0.050	98, 104	
	0.250	93	
	5.000	94, 97	
Grass, forage	0.003	(0.0023 ppm), (0.0030 ppm)	–
	0.010	87, 88, 89, 90, 91, 92, 95, 101	97 ± 4.7 [4.9]
	0.050	94, 97, 97, 98	
	0.250	99, 101, 103, 103	
	1.000	95, 96, 99, 101	
	5.00	95, 95, 97	
	20.0	93, 97, 100, 102, 102, 103	
Grass, hay	0.003	(0.0029 ppm), (0.0042 ppm)	–
	0.010	89, 94, 97, 98, 101, 101, 101, 105	99 ± 4.1 [4.1]
	0.050	95, 100, 101, 105	
	0.250	92, 95, 99, 101	
	1.000	93, 99, 100, 100	
	5.00	92, 99, 99, 108	
	20.0	98, 98, 99, 100, 101, 102	

TABLE C.1.1. Recovery Results from Method Validation of Barley, Grass, Sorghum, and Wheat using the Data-Gathering Analytical Method.[1]

Matrix	Spiking Level (ppm)	Recoveries (%) Obtained [2]	Mean Recovery ± SD [CV]
Sorghum, grain	0.003	(0.0029 ppm)	–
	0.010	98, 103	103 ± 2.9 [2.8]
	0.050	104	
	0.250	103	
	0.500	103, 107	
Sorghum, forage	0.003	(0.0028 ppm)	–
	0.010	95, 100, 103, 105	100 ± 4.6 [4.6]
	0.050	95, 102	
	0.250	98	
	1.000	106	
	5.000	94, 105	
Sorghum, stover	0.003	(0.0024 ppm)	–
	0.010	89, 94, 95, 105	96 ± 4.1 [4.3]
	0.050	95, 98	
	0.250	94	
	1.000	97	
	5.000	93, 95	
Wheat, grain	0.003	(0.0025 ppm), (0.0037 ppm)	–
	0.010	97, 98, 110, 112	105 ± 5.3 [5.0]
	0.025	109	
	0.050	101, 106	
	0.100	109	
	0.250	101	
	0.500	105	
Wheat, forage	0.003	(0.0026 ppm)	–
	0.010	89, 92, 94, 100	97 ± 6.4 [6.6]
	0.050	96, 104	
	0.250	97	
	1.000	106	
	5.000	90, 106	

TABLE C.1.1. Recovery Results from Method Validation of Barley, Grass, Sorghum, and Wheat using the Data-Gathering Analytical Method.[1]

Matrix	Spiking Level (ppm)	Recoveries (%) Obtained [2]	Mean Recovery ± SD [CV]
Wheat, straw	0.003	(0.0031 ppm)	-
	0.010	97, 101, 102, 108	99 ± 5.0 [5.1]
	0.050	102, 104	
	0.250	94	
	1.000	94, 97	
	5.000	92, 95	

[1] Fortification standards were prepared in acetonitrile/pyridine/butanol (22:2:1) solution.
[2] Recoveries were not reported or calculated for fortification levels below the LOQ (0.01 ppm); however ppm values are reported in parentheses.

The fortification levels and samples used in method validation are not adequate to bracket expected residue levels, and processed commodities for which tolerances have been proposed were not included in the validation study. However, acceptable concurrent method recovery data, bracketing the reported residue levels, were included with the crop field trial and processing studies submitted in conjunction with DP Barcode D305665.

The petitioner concluded that Method GRM 02.31 is specific for the quantitation of aminopyralid residues because MS/MS selective detection is used; confirmation of the presence of the analyte is achieved by observation of a precursor ion plus one structurally significant product ion at the same retention time. Because the method only monitors one transition ion, HED defers to ACB to determine whether confirmatory analysis procedures are needed for the method.

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Aminopyralid Residues in Barley, Grass, Sorghum, and Wheat.

Analyte	Aminopyralid
Equipment ID	Agilent Model 1100 autosampler, binary pump, and degasser, MDS/Sciex API 3000 LC/MS/MS system with a Diazem 3000 (C18) column, and MS/MS electrospray detection in the positive mode.
Limit of quantitation (LOQ)	0.01 ppm; calculated LOQs based on 10x the standard deviation from the 0.01 ppm recovery results were 0.0045 ppm for grass forage, 0.0051 ppm for grass hay, 0.0055 ppm for cereal forage, 0.0057 ppm for cereal straw/stover, and 0.0062 ppm for cereal grain.
Limit of detection (LOD)	0.002 ppm; calculated LODs based on 3x the standard deviation from the 0.01 ppm recovery results were 0.0014 ppm for grass forage, 0.0015 ppm for grass hay, 0.0016 ppm for cereal forage, 0.0017 ppm for cereal straw/stover, and 0.0019 ppm for cereal grain.

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Aminopyralid Residues in Barley, Grass, Sorghum, and Wheat.	
Accuracy/Precision	Adequate recoveries, demonstrating acceptable accuracy/precision of the LC/MS/MS method were obtained with barley grain, forage, and straw, grass forage and hay, sorghum grain, forage, and stover, and wheat grain, forage, and straw fortified at the LOQ (0.01 ppm) and up to 50x LOQ (0.50 ppm) for cereal grain, 500x LOQ (5.00 ppm) for cereal forage and straw, and 2,000x LOQ (20.0 ppm) for grasses. The range of percent recoveries [± coefficient of variation] were 92-112% [2.8-6.4] for cereal grains, 86-106% [4.6-6.6] for cereal forage, 89-108% [4.2-5.1] for cereal straw/stover, and 87-108% [4.1-4.9] for grass forage and hay; see Table C.1.1.
Reliability of the Method/ [ILV]	An independent laboratory method validation [ILV] was conducted to verify the reliability of LC/MS/MS method GRM 02.31 for the determination of aminopyralid residues in grass and cereal grain matrices. Adequate recoveries were obtained with grass forage fortified at the method LOQ (0.010 ppm) and 60 ppm, and with wheat grain fortified at the method LOQ (0.010 ppm) and 0.10 ppm, indicating that method GRM 02.31 is reliable; see Table C.3.1.
Linearity	The detector response was linear (coefficient of determination, r^2= >0.9995) within the range of 0.003-2.50 ppm aminopyralid; a representative calibration curve for wheat forage (r^2 = 0.99973) was provided.
Specificity	The method is specific for aminopyralid due to the use of tandem MS detection. Monitored MS/MS ion transitions were 263/134 m/z for aminopyralid butyl ester and 268/139 m/z for $^{13}C_2{}^{15}N$-aminopyralid butyl ester (internal standard).

To demonstrate extraction efficiency of the LC/MS/MS residue method (GRM 02.31), samples of grass and wheat commodities bearing incurred residues from the respective metabolism studies (refer to the DERs for MRIDs 46235709 and 46235710; plants were treated with [2,6-^{14}C]aminopyralid formulated as a potassium salt), were extracted and hydrolyzed according to the procedures of the residue method. Following preparation, these samples were analyzed using GC with negative-ion chemical ionization MS (GC/NCI-MS). The results of the study are reported below in Table C.1.3.

TABLE C.1.3. Extraction Efficiency of the Enforcement Method with Grass and Wheat Matrices Bearing [2,6-^{14}C]Aminopyralid Incurred Residues.

Matrix	Metabolism Study		Aminopyralid (ppm)		Extraction Efficiency (%)[1]
	TRR (ppm)	Extractable (ppm)	Metabolism Method	Residue Method GRM 02.31	
42-DAT ryegrass, forage	6.57	6.28	5.84	6.65	114
42-DAT big bluestem, forage	5.61	5.45	5.04	4.45	88
42-DAT *Panicum maximum*, forage	4.81	4.61	4.24	4.85	114
14-DAT wheat forage, low rate	0.418	0.390	--	0.393	101
35-DAT wheat hay, high rate	0.691	0.611	--	0.440	72
86-DAT wheat grain, high rate	0.084	0.058	0.050	0.085	170[2]
86-DAT wheat straw, high rate	0.623	0.508	0.489	0.424	87

[1] Extraction efficiency was calculated by the petitioner by dividing the residues extracted using the residue analytical method by the residues determined in the metabolism study (or by the metabolism extractable residues, if the sample was not analyzed in the metabolism study; wheat forage and hay).

[2] For the 86 DAT grain sample, the extraction efficiency was 170% when calculated based on the extractable ^{14}C-labeled aminopyralid, but this value was corrected to 101% when calculated based on the total radioactivity. This indicates that the extraction and hydrolysis procedures of the residue method were more effective than those used in the nature of the residue study.

The submitted extraction efficiency data demonstrate that the extraction, hydrolysis, and cleanup procedures of the residue analytical method adequately extract incurred residues of aminopyralid from grass forage and wheat forage, hay, grain, and straw samples.

C.2. Enforcement Method

The proposed enforcement method is the same as the data-gathering method, LC/MS/MS Method GRM 02.31. As such, the petitioner is required to show that the proposed enforcement method (GRM 03.18) can differentiate between aminopyralid, clopyralid, and picloram, as they are all similar in structure.

C.3. Independent Laboratory Validation

An independent laboratory validation (ILV) study was conducted by Morse Laboratories (Sacramento, CA). Grass forage and wheat grain were chosen as the representative matrices to be tested because wheat grain is a dry and oily matrix while grass forage has a high water content and higher residues are expected in grasses. Untreated samples of grass forage and wheat grain, obtained from the petitioner, were fortified with aminopyralid at 0.01 ppm (LOQ) and 0.10 ppm (10x LOQ; wheat) or 60 ppm (6,000x LOQ; grass). Fortified and unfortified samples were extracted and analyzed using LC/MS/MS Method GRM 02.31. We note that the method as written uses a power regression curve for calculations, however, the ILV laboratory used a quadratic non-linear regression equation to generate the calibration curve for wheat grain and a linear regression equation was used for grass forage because of the wider range of analyte concentrations. This change was communicated by the laboratory to the petitioner, which noted that the method does allow for the use of other regression analyses.

Adequate recoveries were achieved for both grass forage and wheat grain with the first trial; recoveries of aminopyralid are reported in Table C.3.1. However, a single recovery of 69% was observed for wheat grain at the LOQ fortification level; using the Grubbs method, this value was determined to be an outlier and was not used by the petitioner for statistical evaluations. All other recoveries were within the 70-120% acceptable recovery range. Apparent residues in unfortified grass forage and wheat grain samples were nondetectable.

The laboratory stated that no changes or modifications were made to the method, except for substitution of some of the equipment used. One set of 13 samples required approximately 13 person-hours (2-day period) for sample preparation and automated HPLC analyses were run overnight unattended.

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TABLE C.3.1. Recovery Results Obtained by an Independent Laboratory Validation of the Enforcement Method for the Determination of Aminopyralid in Grass Forage and Wheat Grain.

Matrix	Spiking Level (ppm)	Recoveries Obtained	Mean Recovery ± SD [CV]
Grass, forage	0.01	94, 96, 101, 105, 105	108 ± 9.5 [8.8]
	60	111, 114, 118, 119, 120	
Wheat, grain	0.01	69 [1], 100, 110, 112, 116	110 ± 8.2 [7.5]
	0.10	93, 110, 115, 115, 118	

[1] Determined to be an outlier using the Grubbs Test and was not included by the petitioner in the statistical calculations. We note if this value is included, mean recovery would be 106 ± 15.1.

D. CONCLUSION

Adequate method validation data have been submitted for LC/MS/MS Method GRM 02.31 for the determination of residues of aminopyralid in plant commodities.

The petitioner is proposing the LC/MS/MS method for enforcement. Adequate radiovalidation data have been submitted for the extraction procedures of Method GRM 02.31 using samples of grass and wheat commodities. Adequate independent laboratory validation data have been submitted using grass forage and wheat grain. The petitioner is required to show that the proposed enforcement method (GRM 02.31). The proposed enforcement method will be forwarded to ACB for tolerance method validation.

E. REFERENCES

None.

F. DOCUMENT TRACKING

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