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OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: 10-March-2020

Subject: **Pethoxamid.** Petition for the Establishment of Permanent Tolerances and Registration for Use on Corn, Cotton, and Soybeans. Summary of Analytical Chemistry and Residue Data from the Global Joint Review.

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1.0 Executive Summary

Pethoxamid, (2-chloro-*N*-(2-ethoxyethyl)-*N*-(2-methyl-1-phenyl-1-propen-1-yl)acetamide), is a new herbicide proposed for the control of most annual grasses and certain broadleaf weeds in corn (field, sweet, and popcorn), soybean, cotton, turf (including sod farms, residential, commercial, institutional lawns and landscapes, golf courses, and sod farms), field grown ornamentals, container grown ornamentals, and non-crop areas including rights-of-ways, and others as listed on the label. Pethoxamid is a selective chloroacetamide and can be applied as a pre-plant surface-applied, pre-plant incorporated, or pre-emergence treatment. The maximum proposed annual application rate is 1.5 lb ai/A for all of the proposed uses. The label specifies a maximum of two applications per year for spring weed control of corn, soybean, and cotton. Pethoxamid is a North American Free Trade Agreement (NAFTA) joint submission for review by the EPA and Canada's Pest Management Regulatory Agency (PMRA). FMC Corporation has proposed tolerances and registrations for the use of pethoxamid on corn (field, sweet, and pop), cotton, and soybean.

The submitted plant and animal metabolism studies are acceptable. Parent pethoxamid was observed in each of the crops only at early growth stages shortly after application and was not present in mature corn grain, soybean seed, or potato tubers. Metabolic pathways are consistent in primary crops and metabolism is observed to proceed via the displacement of the chlorine by glutathione conjugation. This intermediate was then subsequently degraded to MET-30, a cysteine conjugate of pethoxamid, followed by further degradation. Pethoxamid was extensively metabolized in the goat as parent compound was not detected in any matrix. In the goat liver, the major metabolites identified were MET-30 and MET-1 glucuronide. In the poultry metabolism study, pethoxamid was extensively metabolized in hens, with only minor amounts ($\leq 10\%$ of the total toxic residue (TRR)) of parent compound detected in livestock tissues and eggs. HED has determined that the residue of concern in plant commodities (primary and rotational crops) for both tolerance enforcement and risk assessment is pethoxamid. HED has determined that the residue of concern in livestock commodities for tolerance enforcement and risk assessment is parent compound only. For ruminant liver, the residues of concern are parent compound plus MET-1 glucuronide conjugate for risk assessment. (The Residues of Concern Knowledge-based Subcommittee (ROCKS) decision memo, D450748, M. Negussie, 06/20/2019).

There are adequate number and locations of field trials are in accordance with OCSPP Guideline 860.1500 and the geographic distribution of the field trials by NAFTA growing zones. Residue field trials were conducted for corn, cotton, and soybean at preplant or preemergence application to the soil followed by a foliar broadcast application. The field trials for corn, cotton and soybean were conducted at rates greater than the proposed maximum annual application rate at 1.5 lb ai/A and included a foliar application; however, only preplant/preemergent application are proposed. Applications were made with a 5.0 lb ai/gal EC formulation of pethoxamid at maximum seasonal rates of 2.88-4.12 lb ai/A (~2-3X) in/on corn, cotton, and soybean. Residues of pethoxamid were all below the limit of quantitation (LOQ, <0.01 ppm) in/on corn grain, soybean and cottons undelinted seed samples.

Analytical standards for pethoxamid are currently not available in the National Pesticide Standards Repository. These standards should be received prior to the tolerances being issued for pethoxamid.

A livestock feeding study was not provided by the petitioner. The metabolism data on poultry and ruminant confirm that residues of pethoxamid are not expected in edible livestock tissue, milk or eggs above 0.01 ppm based on the proposed uses. However, PMRA is recommend establishing tolerances at the limit of quantitation (0.01 ppm) for residues in livestock commodities and the registrant, FMC would like to harmonize with the proposed Canadian maximum residue limits (MRLs).

No Codex MRLs have been established for pethoxamid. The confined rotational crops data support the use of pethoxamid on rotated crops planted with a 30-day plant back interval.

2.0 Regulatory Recommendations

Analytical standards for pethoxamid are currently not available in the National Pesticide Standards Repository. These standards should be received prior to the tolerances being issued for pethoxamid (Refer to Section 5.1.4). There are no residue chemistry considerations that would preclude granting the requested registration and establishing the recommended tolerances for pethoxamid residues on the proposed crops. The specific tolerance recommendations are discussed in Section 2.2.2.

A human health risk assessment is forthcoming.

2.1 Data Deficiencies/Data Needs

There are no data deficiencies or data needs at this time.

2.2 Tolerance Considerations

2.2.1 Enforcement Analytical Method

Plants

The petitioner is proposing multi-residue method QuEChERS (Method No. AGR/MOA/PTX-8) for the determination of pethoxamid in plant commodities. Samples are extracted with acetonitrile (ACN). QuEChERS extraction salts (magnesium sulfate:sodium chloride:sodium citrate tribasic dihydrate:sodium citrate dibasic sesquihydrate; 8:2:2:1, w:w:w:w) are added, and the samples are shaken and centrifuged. A portion of the supernatant is purified by the addition of cleanup salts (primary secondary amine:C18EC sorbent:magnesium sulfate; 1:1:6, w:w:w). Samples are shaken and centrifuged, and an aliquot of the supernatant is diluted with water for analysis by LC/MS/MS, conducted using a reverse-phase C18 column and a gradient mobile phase of ACN and water containing 0.2% acetic acid. The following ion transitions are monitored: m/z 296.2 $\rightarrow$ 131.2 (quantitation) and m/z 296.2 $\rightarrow$ 250.2 (confirmation). The multi-residue method QuEChERS was evaluated and found to be a valid method for determining the residue of parent pethoxamid in plant matrices corn, lettuce, rapeseed, cotton, soybean, and

orange. The LOQ of the method is 0.01 ppm.

Livestock

Method EAS Study Code S15-03519 is proposed as the enforcement method for determination of residues of pethoxamid in livestock commodities. The extraction and analysis procedures are based on the QuEChERS method and are very similar to those of the proposed enforcement method for crop commodities, EAS Method No. AGR/MOA/PTX-8.

Samples are extracted with water by shaking and vortexing with ACN. QuEChERS extraction salts (magnesium sulfate:sodium chloride:sodium citrate tribasic dihydrate:sodium citrate dibasic sesquihydrate; 8:2:2:1, w:w:w:w) are added, and the samples are shaken, vortexed, and centrifuged. A portion of the supernatant is purified by the addition of cleanup salts (primary secondary amine:C18EC sorbent:magnesium sulfate; 1:1:6, w:w:w). Samples are shaken, vortexed, and centrifuged, and an aliquot of the supernatant is diluted with water for analysis by LC/MS/MS, conducted using a reverse-phase C18 column and a gradient mobile phase of ACN and water containing 0.2% acetic acid. The following ion transitions are monitored: m/z 296.2 $\rightarrow$ 131.2 (quantitation) and m/z 296.2 $\rightarrow$ 250.2 (confirmation). The method was successfully validated by an independent laboratory. The LOQ of the method is 0.01 ppm.

2.2.2 Recommended Tolerances

HED reviewed the available residue data and determined the appropriate tolerance levels for residues of pethoxamid (Table 2.2.2). Based on the HED Interim Guidance on Tolerance Expressions (S. Knizner, 5/27/09), the following tolerance expression is recommended for primary crops and livestock commodities:

- (a) General. Tolerances are established for residues of the herbicide pethoxamid, including its metabolites and degradates, in or on the commodities in the table below. Compliance with the tolerance levels specified below is to be determined by measuring only pethoxamid, 2-chloro-*N*-(2-ethoxyethyl)-*N*-(2-methyl-1-phenyl-1-propen-1-yl)acetamide in or on the commodity.

Table 2.2.2. Tolerance Summary for Pethoxamid (40 CFR §180.xxx).			
Commodity (correct commodity definition)	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments
Corn, field, forage	0.015	0.01	
Corn, field, stover	0.02	0.01	
Corn, field, grain	0.01	0.01	
Corn, pop, stover	0.01	0.01	
Corn, pop, grain	0.01	0.01	
Corn, sweet, forage	0.50	0.01	
Corn, sweet, stover	0.60	0.01	
Corn, sweet, kernel plus cob with husk removed	0.01	0.01	
Cotton, undelinted seed	0.01	0.01	
Cotton, gin byproducts	0.09	0.01	
Soybean, forage	3.0	0.01	

Table 2.2.2. Tolerance Summary for Pethoxamid (40 CFR §180.xxx).			
Commodity (correct commodity definition)	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments
Soybean, hay	4.5	0.01	
Soybean, seed	0.01	0.01	
Milk	--	0.01	HED is recommending tolerances following PMRA's recommendation to establish tolerances at the limit of quantitation (0.01 ppm) for residues in livestock
Eggs	--	0.01	
Cattle, fat	--	0.01	
Cattle, meat	--	0.01	
Cattle, meat byproducts	--	0.01	
Goat, fat	--	0.01	
Goat, meat	--	0.01	
Goat, meat byproducts	--	0.01	
Hog, fat	--	0.01	
Hog, meat	--	0.01	
Hog, meat byproducts	--	0.01	
Horse, fat	--	0.01	
Horse, meat	--	0.01	
Horse, meat byproducts	--	0.01	
Poultry, fat	--	0.01	
Poultry, meat	--	0.01	
Poultry, meat byproducts	--	0.01	
Sheep, fat	--	0.01	
Sheep, meat	--	0.01	
Sheep, meat byproducts	--	0.01	

2.2.3 Revisions to Petitioned-For Tolerances

HED concurs that metabolism data and dietary burden estimates indicate that residues are not expected in edible livestock tissue and feed items, milk or eggs above 0.01 ppm based on the proposed uses. However, PMRA is recommend establishing tolerances at the limit of quantitation (0.01 ppm) for residues in livestock commodities and the registrant, FMC would like to harmonize with the proposed Canadian MRLs.

2.2.4 International Harmonization

There are currently no MRLs established for residues of pethoxamid in Canada or Mexico or under Codex; therefore, there are no issues with international harmonization. HED is recommending tolerances following PMRA's recommendation to establish tolerances at the limit of quantitation (0.01 ppm) for residues in livestock commodities. Canada does not set MRLs on livestock feed items. See Appendix E for the complete list of recommended tolerances.

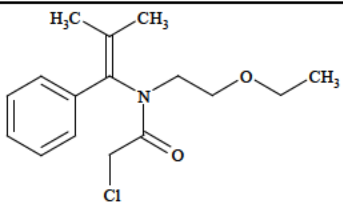
2.3 Label Recommendations

There are no label recommendations based on residue chemistry identified for this petition.

3.0 Introduction

Pethoxamid is a novel selective chloroacetamide. Classified as a Group 15 herbicide, pethoxamid works by inhibiting the germination of target weeds. Pethoxamid can be applied preemergence. In the U.S. and Canada, major crops slated for its use include corn, cotton, and soybean. Also, tolerances are established by the European Commission and the European Food Safety Authority (EFSA; 8/2017) for residues in/on plants and livestock commodities.

3.1 Chemical Identity

Table 3.1. Nomenclature for Pethoxamid.	
Common name	Pethoxamid
IUPAC name	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide
CAS registry number	106700-29-2
Company experimental name	TKC-94
	

3.2 Physical/Chemical Characteristics

Pethoxamid has high water solubility and based on its vapor pressure pethoxamid should exist in both vapor and particulate phases in the atmosphere and may volatilize from moist soil and water surfaces.

Table 3.2. Physicochemical Properties of the Technical Grade Test Compound Pethoxamid.		
Parameter	Value	Reference
Molecular weight (g/mole)	295.8	MRID 49813402
Melting point/range (°C)	37.5	
Henry's Law constant at 25°C (Pa)	7.5×10^{-11} atm-m ³ /mol at 25°C	
Bulk density (g ml ⁻¹)/Specific gravity	1.19	
Water solubility	0.400 g/L	
Vapor pressure at 25°C (Pa)	2.8×10^{-3}	
Dissociation constant (pK _a)	NA	
Octanol/water partition coefficient (log K _{ow})	2.96	

3.3 Pesticide Use Pattern/Directions for Use (860.1200)

The end-use product for agricultural applications, pethoxamid 480 g/L emulsifiable concentrate (EC; F4044-2) is a liquid product containing 46.88% of the active ingredient pethoxamid. It is a selective herbicide for use as a pre-plant surface-applied, pre-plant incorporated or pre-emergence treatment in water or liquid fertilizer for control of most annual grasses and certain broadleaf weeds in corn (field, sweet, and pop), cotton, and soybean. It may be applied preemergence for weed control in turf and ornamental sites in residential, commercial, institutional lawns and landscapes, golf courses, sod farms, utility rights-of-way, roadsides,

railways, industrial areas, container, and field grown ornamentals. Both the maximum proposed annual and single application rates are 1.5 lb ai/A for all the proposed uses. The label specifies a maximum of two applications per year for spring weed control of corn (field, sweet, and popcorn), soybean, and cotton. For ornamentals, multiple applications are proposed if needed, with a proposed annual application rate of 1.5 lb ai/A. The label supports a 30-day plant back interval for all rotated crops.

Table 3.3. Application Rates for Pethoxamid on Corn, Cotton and Soybean.					
Application Timing	Method of Application	Application Rates (lb ai/A)	Maximum Number of Applications per Year	PHI (Days)	Maximum Seasonal Use Rate (lb ai/A)
Corn (Field, Pop, and Sweet)					
Pre-plant surface; Pre-plant Incorporated	Broadcast or Banded spray to soil surface OR incorporated up to 30 days before planting	0.5-1.5	2	N/A Applied preplant	1.5
Pre-emergence Surface Application	Broadcast or Banded Spray to soil surface after planting and before crop emergence				
Cotton					
Pre-plant surface; Pre-plant Incorporated	Broadcast or Banded spray to soil surface OR incorporated up to 30 days before planting	0.5-1.5	2	N/A Applied preplant	1.5
Pre-emergence Surface Application	Broadcast or Banded Spray to soil surface after planting and before crop emergence				
Soybean					
Pre-plant surface and Pre-plant Incorporated	Broadcast or Banded spray to soil surface OR incorporated up to 30 days before planting	0.5- 1.5	2	N/A Applied preplant	1.5
Pre-emergence Surface Application	Broadcast or Banded Spray to soil surface after planting and before crop emergence				

Conclusions. The field trials reflect one treated plot reflecting a preplant or preemergence application to the soil followed by a foliar broadcast application targeting growing weeds. The submitted studies were made at exaggerated rates with a 5.0 lb ai/gal EC formulation of pethoxamid at maximum seasonal rates of 2.88-4.12 lb ai/A (~2-3x) in/on corn, cotton, and soybean. The label specifies a maximum of two applications per year for spring weed control of corn, soybean, and cotton at a maximum annual application rate of 1.5 lb ai/A. The registrant,

FMC updated the petition to remove postemergence and only include preplant or preemergent applications to the labels.

4.0 Metabolite/Degradate Residue Profile

4.1 Nature of the Residue

The metabolism of pethoxamid was investigated in commodities from three different crops (soybean, corn, and potato) following foliar application, and on rotational crops (spinach, carrots, and wheat). These studies were performed with radiolabeled ^{14}C -pethoxamid and conducted by Covance Laboratories Ltd in the United Kingdom (UK) for soybean and corn, and Excel Research Svc. in CA for potato. Livestock metabolism studies were conducted with lactating goat and laying hen. The in-life phase of the study was conducted by Wildlife International (Easton, MD) and the analytical phase of the study was conducted PTRL West (Hercules, CA) for poultry, and Huntingdon Life Science, Ltd, (Huntingdon, UK) for ruminant. Appendix A gives the structure and nomenclature of pethoxamid and its metabolites, proposed metabolic pathways in plants and livestock, and the distribution of residues in the plant and livestock metabolism studies.

4.1.1 Summary of Plant Metabolism (860.1300)

DER 49813527 Soybean

DER 49813528 Corn

DER 49813529 Potato

The metabolism of pethoxamid was investigated in soybean and corn with [phenyl- ^{14}C] pethoxamid (specific activity: 45.6 $\mu\text{Ci}/\text{mg}$) formulated as a 5.0 lb ai/gal (600 g ai/L) EC formulation and applied as a separate soil or foliar application at 1.1 lb ai/A (1.2 kg ai/ha) for each application. Applications to greenhouse-grown soybeans and corn were made as: a preemergence soil application 1 day after planting or a foliar application to plants 29 days after planting at BBCH 14-15 (4- to 5-leaf growth stage). Samples of immature whole plant were collected at preharvest intervals (PHIs) of 14 and 27 days following the postemergence soil application, 36 days following the preemergence application, and 8 days following the foliar application. Samples of immature leaves, stems, and roots were collected 61 days following preemergence application and 33 days following foliar application, and samples of mature seed or grain, pods (hulls), cobs, leaves, stems, and roots were collected 106 days for corn and 125-134 days for soybeans following preemergence application; and 106 days for corn and 97-106 days for soybean following foliar application.

The highest total radioactive residues (TRR) were observed in 8-day PHI immature whole plants collected following foliar application and were determined by high-performance liquid chromatography (HPLC) with UV detection and/or thin-layer chromatography (TLC). Identification of pethoxamid was confirmed by LC/MS in the ethyl acetate phase, and TKC-94-prethiol (MET-43) was tentatively identified based on mass spectra and molecular weight. In the aqueous phase, TKC-94- γ -glutamyl cysteine (MET-130), TKC-94-cysteine (MET-30), and TKC-94-N-malonyl cysteine (MET-47) were also tentatively identified by LC/MS. TKC-94-cysteine and TKC-94-N-malonyl cysteine eluted together, with the malonyl-cysteine conjugate eluting as a shoulder on the peak.

For soybeans and corn, pethoxamid was a minor residue component following foliar application, accounting for 1.1% TRR. TRR in corn grain and cob were <0.004 ppm. TRR in soybean seed and pod, respectively, were 0.011 and 0.028 ppm following foliar application. The major residue components in soybean were TKC-94-cysteine (MET-30) and TKC-94-N-malonyl-cysteine (MET-47), accounting for 20.3% TRR (6.403 ppm) and 17.4% TRR (5.505 ppm), respectively. No residue components were identified in mature leaves. The major residue components in corn, which accounted for 12.3% TRR, was an unknown designated as Unknown 3 that was found to be nearly entirely comprised of metabolites TKC-94-SO-lactic acid and TKC-94-S-lactic glucoside (MET-48) during analysis of 33-day PHI immature leaves. Following strong acid hydrolysis of the plant extract, benzoic acid was found at 33.7-59.4% TRR in the 2 M and 6 M HCl hydrolysates, and oxamic acid metabolite MET-31 and its geometric isomer were tentatively identified, together accounting for 16.2% TRR in the 2 M HCl hydrolysate. Unknown 3 was a major residue component in all samples, accounting for 13.6-15.1% TRR in mature leaves and 17.6% TRR in immature leaves.

The metabolism of pethoxamid was investigated in potato following one foliar spray application with [phenyl-U-¹⁴C] pethoxamid (specific activity: 1.67 MBq/mg) formulated as a 57.3% EC formulation and applied as a single foliar application at 1.21 lb ai/A (1.36 kg ai/ha). The application was made to potatoes grown outdoors at BBCH 25 (fifth basal side shoots visible, 38 days after planting). Samples of immature foliage were harvested on the day of application, samples of immature foliage and tubers were harvested 37 days after application, and samples of mature foliage and tubers were harvested 76 days after application. In potato, pethoxamid was the major residue component in 0-day foliage, at 77.1% TRR (13.960 ppm). Pethoxamid cysteine conjugate (MET-30) was also identified in 0-day foliage at 3.4% TRR (0.612 ppm) and pethoxamid-SO-cysteine was identified in 37-day foliage at 10.0% TRR (0.207 ppm). No metabolites were identified in potato tubers. See summary tables in Appendix C for details.

Conclusions. Following pre-emergence, soil-directed application, radioactive residues consisted of multiple, unidentified compounds occurring at low levels; neither pethoxamid nor the metabolites identified following foliar application were observed. Parent, pethoxamid was observed in each of the crops, only at early growth stages shortly after foliar application and was not present in mature corn grain, soybean seed, or potato tubers. Metabolic pathways are consistent in primary crops and metabolism is observed to proceed via the displacement of the chlorine atom in pethoxamid by the thiolate anion of the tripeptide glutathione. Glutamic acid and glycine are rapidly cleaved to form the cysteine conjugate (MET-30). MET-30 occurs in all immature crops. MET-30 is rapidly metabolized by a number of pathways common to cysteine conjugates, forming many products. The proposed metabolic pathways in plants are presented in Appendix B.

4.1.2 Summary of Livestock Metabolism (860.1300)

DER 49813530 Poultry

DER 49813531 Ruminant

Pethoxamid, radiolabeled in the phenyl ring, was administered orally via gelatin capsule to the test animals. This radiolabeling is considered sufficient to capture the livestock metabolism of pethoxamid. Study parameters are summarized in Table 4.1.2.1, below.

Table 4.1.2.1. Summary of Hen and Goat Metabolism Studies Experimental Parameters.		
Experimental Parameter	Lactating Goats	Laying Hens
Number of Animals	1	10
Dosing Period	5 days	7 days
Dosing Level	[¹⁴ C-Ph]-pethoxamid = 9.4 ppm	[¹⁴ C-Ph]-pethoxamid= 16.6 ppm
Egg/Milk Collection	Daily (morning and afternoon)	Daily (morning and afternoon)
Excreta Collection	Daily	Daily (morning, prior to dosing)
Time from Last Dose to Sacrifice	~23 hours	~20 hours
Tissues Harvested and Analyzed	Muscle (foreleg and rump) Liver Kidney Fat (including subcutaneous) Gastrointestinal tract and contents collected and frozen until study completion	Muscle (flank, loin) Liver Fat (including omental, subcutaneous, renal) Gastrointestinal tract and contents collected and frozen until study completion

Ruminant: The goat was dosed orally via capsule for 5 consecutive days with [¹⁴C] pethoxamid at levels equivalent to 9.4 ppm in the feed (as fed basis). The majority of the administered dose (AD) was recovered in the urine (70.15% AD) and feces (30.50% AD), with minor amounts recovered in the cage washes (0.29% AD). Another 0.53% and 0.02% of the AD were recovered in the liver and kidneys, respectively. The overall recovery of the administered dose was 101.5%. TRR levels in milk were low and remained steady throughout the study at 0.007-0.009 ppm. TRR levels in tissues were highest in liver (0.555 ppm) and kidney (0.147 ppm) and were lowest in muscle (0.003 ppm) and fat (0.002 ppm). Based on the high levels of urinary excretion and low levels of radioactivity in milk, muscle, and fat, pethoxamid was rapidly absorbed and excreted by the goat.

Analyses of liver, kidney, and urine indicated that pethoxamid was extensively metabolized in the goat. Parent compound was not detected in any matrix, and the metabolite profile was relatively complex. In liver, identified metabolites accounted for 32.5% TRR and included MET-30 (21.6% TRR, 0.120 ppm) and MET-1 glucuronide (10.9% TRR, 0.060 ppm). No metabolites were conclusively identified in kidney; metabolite MET-32 was tentatively identified at 5.3% TRR but the identification could not be confirmed using a second method.

Metabolites identified in urine included MET-30 (14.7% TRR) and MET-1 glucuronide (39.3% TRR), which together accounted for 54.0% TRR; the remainder of the TRR in urine consisted of multiple relatively polar unknowns that each accounted for ≤8.5% TRR. Treatment with β-glucuronidase converted most of these polar ¹⁴C-residues to less polar components, suggesting that a large fraction of the polar residues included glucuronide conjugates. Analysis of solvent-extractable ¹⁴C-residues from feces detected 11 unknown components, each of which accounted for ≤4.2% TRR; no further work was conducted on fecal extracts.

Poultry: The hens were dosed orally via capsules for 7 consecutive days with [¹⁴C]pethoxamid at levels equivalent to 16.6 ppm in the feed (dry weight). The majority of the AD was recovered in the excreta (77.9% AD), with minor amounts recovered in the cage wash (0.1% AD) and gastrointestinal (GI) tract (1.9% AD). Eggs accounted for 0.1% AD, and liver, muscle, and fat

together accounted for ~0.1% AD. The overall recovery of the administered dose was 80.1%. TRR levels in eggs plateaued on Day 6 at a maximum of 0.059 ppm. TRR levels in tissues were highest in liver (0.268 ppm) and were lower and similar in muscle (0.020-0.027 ppm) and fat (0.032-0.053 ppm).

Pethoxamid was extensively metabolized in hens, with only minor amounts of parent compound detected in liver (0.4% TRR), eggs (0.8% TRR), and fat (2.7-7.3% TRR), and the six metabolites identified in eggs and tissues, MET-2, MET-3, MET-30, MET-42, MET-47, and MET-103, individually accounted for $\leq 8.2\%$ TRR in any matrix. Samples of egg, liver, and muscle were extracted sequentially with aqueous ACN and ACN, and fat samples were extracted sequentially with acetone/hexane and acetone, with the extract fractions being pooled by matrix. Extraction with ACN/water released 47% TRR from liver, 67% TRR from egg, 33-40% TRR from muscle, and 79% TRR from excreta, and extraction with acetone/hexane released 38-47% TRR from fat. The overall extractability of radioactivity was 70-99% TRR from eggs and tissues, with levels of radioactivity ≤ 0.006 ppm in each of the final post-extraction solids (PES) fractions. All six metabolites were detected in liver at levels $\leq 10.0\%$ TRR. Major identified unbound residues consisted of MET-42 (2.7- 8.2% TRR), MET-3 (2.7- 3.8% TRR), MET-103(6.9 % TRR), MET-30 (0.4- 3.3% TRR), MET-47 (3.4- 3.9% TRR), and MET-2 (0.5- 1.4% TRR).

Conclusions. The submitted ruminant and poultry metabolism studies are acceptable. In the poultry metabolism study, pethoxamid was extensively metabolized in hens, with only minor amounts ($\leq 10\%$ TRR) of parent compound detected in livestock tissues and eggs. In the lactating goat, analyses of liver, kidney, and urine indicate that pethoxamid was extensively metabolized in the goat, as parent compound was not detected in any matrix. In the goat liver, the major metabolites identified were MET-30 (21.6% TRR, 0.120 ppm) and MET-1 glucuronide (10.9% TRR, 0.060 ppm). Based upon these studies, the nature of the residue of pethoxamid is considered to be understood in livestock. See summary tables in Appendix C for details. The proposed metabolic pathways in plants are presented in Appendix B.

4.1.3 Summary of Confined Rotational Crops (860.1850)

DER 49813552 Confined Rotational Crops

DER 49813553 Confined Rotational Crops

Two confined rotational crop studies were conducted on three diverse crops; the first study was on spinach, carrots, and wheat, and the second study was on turnips, spinach, and wheat. These studies were performed with radiolabeled ^{14}C -pethoxamid and conducted by PTRL West, INC in CA and Huntingdon Life Sciences in UK, respectively.

In the CA confined rotational crops study, spinach, carrots, and wheat were grown in ^{14}C -pethoxamid-treated soil. *Crops were grown outdoors.* The crops were planted 30, 120, and 365 days after a single soil application at rate of 1.16 lb ai/A (0.8x) TRR were above (>0.01 ppm) in all rotational crop matrices except 365-day PBI carrot root. Pethoxamid was detected in carrot root, at 11.3% TRR (0.008 ppm) in 30-day PBI root and 3.8% TRR (0.001 ppm) in 120-day PBI root, but the identification could not be confirmed.

In the UK confined crop rotation study, turnip, spinach, and wheat were grown in soil treated with ^{14}C -pethoxamid at a rate of 1.37 lb ai/A (0.9x) and a PBI of 30 days. Crops were *grown in*

a growth room, and samples of rotational crop commodities were harvested at three intervals: first harvest (immature); second harvest (earliest possible harvest); and third harvest (normal harvest). Samples of turnip roots and tops and spinach leaves were collected at each of the harvest intervals; at 54, 79, and 105 days after planting (DAP) for turnips and 47, 70, and 98 DAP for spinach. For wheat, the entire plant (forage) was collected at the first harvest at 70 DAP, and samples of wheat straw, grain, and chaff were collected at the remaining harvests, 126 and 146 DAP.

Pethoxamid was not detected in any rotational crop matrix. With some exceptions, the following metabolites were detected in each rotational crop matrix at one or more harvest intervals: MET-30, MET-41, MET-42, MET-48, MET-49 and MET-50. Metabolite MET-42, which was the major soil metabolite, was one of the major metabolites detected in turnip tops (14.2-26.4% TRR, 0.082- 0.132 ppm), spinach (7.7-9.1% TRR, 0.032-0.072 ppm), wheat forage (14.2% TRR, 0.068 ppm) and straw (9.9-12.9% TRR, 0.060- 0.231 ppm). Metabolite MET-50, another major metabolite, was present in turnip root (2.8-8.7% TRR, 0.002- 0.017 ppm) and tops (12.1-17.7% TRR, 0.075- 0.089 ppm), spinach (4.5-12.2% TRR, 0.018- 0.096 ppm), and wheat forage (7.5% TRR, 0.036 ppm), and was also detected at $\leq 6.0\%$ TRR in wheat straw, grain, and chaff. Metabolite MET-30 was also a major metabolite in most matrices, including turnip root ($\leq 9.8\%$ TRR, ≤ 0.019 ppm) and tops ($\leq 7.5\%$ TRR, ≤ 0.047 ppm), spinach (3.4-7.5% TRR, 0.025- 0.038 ppm), and wheat forage (11.3% TRR, 0.054 ppm) and straw (4.3-6.1% TRR, 0.026- 0.109 ppm), but was present at $\leq 2.0\%$ TRR in wheat grain and was not detected in wheat chaff.

Conclusion. The submitted confined rotational crop metabolism studies are adequate. The results of the two-confined crop rotational studies were different at the 30-day plant back interval. In the confined crop rotational study on turnip, spinach and wheat (UK indoor study), MET-42 and MET-50 were found in food items above 10% TRR: MET-42 at 14.2-26.4% TRR (0.082-0.132 ppm) in turnip tops, MET-50 at 12.1-17.7% TRR (0.075-0.089 ppm) in turnip tops, and 4.5-12.2% TRR (0.018-0.096 ppm) in spinach. In the confined crop rotational study on carrot, spinach and wheat (CA outdoor study), no major metabolites ($>10\%$ TRR) were identified. MET-42 may have resulted from direct uptake from the soil. MET-30 and MET-50 indicate that metabolism proceeds via a glutathione pathway. See summary tables in Appendix C for details. The proposed metabolic pathway for pethoxamid in rotated crops is presented in Appendix B.

4.1.4 Summary of Metabolites and Degradates

A summary of the metabolites of pethoxamid in plants, livestock, rat, and drinking water is included in Appendix A, with proposed metabolic pathways in Appendix B.

4.2 Comparison of Metabolic Pathways

The results of the plant and livestock metabolism studies (soybean, corn, cotton, rotational crops, rat, hen, and goat) are similar. Due to the predominance of sulfur-containing metabolites, the studies show that pethoxamid was metabolized primarily via glutathione conjugation, with initial displacement of the chlorine atom by the sulfhydryl group of glutathione in a reaction catalyzed by glutathione-S-transferase. In plants, the glutamic acid and glycine portions of glutathione are

then cleaved to form the cysteine conjugate MET-30, which may be metabolized by a number of pathways common to cysteine conjugates, forming many products.

The primary routes of metabolism in ruminant involve (i) cleavage of the ethoxy side chain and conjugation with glucuronic acid to yield MET-1 glucuronide and (ii) conjugation with glutathione and subsequent degradation of the glutathione moiety to yield the cysteine conjugate, MET-30. Further metabolism of the cysteine conjugate would yield MI-E, which was tentatively identified as a minor residue in liver and was previously identified in the rat metabolism study.

4.3 Residues of Concern Summary and Rationale

ROCKS/MARC Decision Memo Reference

The qualitative nature of the residue of pethoxamid in plants and animals is understood. The residue of concern in plant commodities (primary and rotational crops) for both tolerance enforcement and risk assessment is pethoxamid. The residue of concern in livestock commodities for tolerance enforcement and risk assessment is parent compound only. For ruminant liver, the residues of concern are parent compound plus MET-1 glucuronide conjugate for risk assessment. The Residues of Concern Knowledgebase Subcommittee (ROCKS) met on 12/12/2018 to discuss the residues of concern for pethoxamid (D450748, M. Negussie, 06/20/2019).

Parent pethoxamid was a minor component of the residue on all crops and livestock. Residues were generally non-detectable in all commodities at the proposed PHI with the exception of a few samples of corn forage and cotton gin-byproducts. A processing study was conducted for cotton at an exaggerated application rate; residues were non-detectable in the raw and processed commodities.

Parent pethoxamid was a minor component of the residue on all crops and livestock (tissues and eggs). In primary crops Metabolites MET-30 and MET-47 were observed at levels >10% TRR on immature soybeans foliage. Considering the minimal potential exposure in food, and they are likely to be less toxic than the parent compound as they would probably be readily excreted due to the carboxylic acid moieties, and no structural alerts were identified in the qualitative SAR analysis, they will not be included in the risk assessment. No structural alerts were identified for MET-42 and MET-50, major degradates found in the confined rotational crop study. MET-42 is a sulfonic acid and more polar than the parent so would likely be readily excreted. MET-50, weak acid at blood pH, so will be ionized and also likely excreted relatively quickly. Considering the potential exposure levels, and that these metabolites are likely to be considerably less toxic than the parent compound, they can also be excluded from the dietary risk assessment.

Pethoxamid was extensively metabolized in hens and goats with no quantifiable residues of parent detected in livestock tissues and eggs. In the goat liver, the major metabolites identified were MET-30 (22% TRR) and MET-1 glucuronide (11% TRR). As noted above, MET-30 is likely to be less toxic than the parent compound and can be excluded from the dietary risk assessment. MET-1 is a glucuronide conjugate of the de-ethylated parent compound that was also observed in the rat metabolism studies. Due to the structural similarity to the parent compound (when the glucuronide is removed), MET-1 should be included in the dietary risk

assessment. No feeding studies were provided; the registrant is encouraged to consult with the Agency on residues to monitor if the livestock dietary burden increases and a feeding study is conducted.

In drinking water, pethoxamid degrades through aerobic and anaerobic metabolism and aqueous photolysis to three major transformation products (MET-6, MET-42 and MET-102). As noted above, MET-42 does not need to be included in the dietary risk concern. Like most of the other aforementioned degradates, MET-6 and MET-102 are carboxylic acids and do not retain the chlorine moiety, so are considered to be less toxic than the parent compound. Only the parent compound will need to be considered in the dietary risk assessment for water. These degradates MET-6 (a thioether compound), MET-42 (a sulfonic acid), and MET-102 (a lactic acid, carboxylic acid) are less toxic than the parent compound.

Table 4.3. Summary of Metabolites and Degradates to be Included in the Risk Assessment and Tolerance Expression.			
Matrix		Residues Included in Risk Assessment	Residues Included in Tolerance Expression
Plants	Primary Crop	Pethoxamid	Pethoxamid
	Rotational Crop	Pethoxamid	Pethoxamid
Livestock	Ruminant (except liver)	Pethoxamid	Pethoxamid
	Ruminant liver	Pethoxamid + MET-1 glucuronide conjugate	Pethoxamid
	Poultry	Pethoxamid	Pethoxamid
Drinking Water		Pethoxamid	Not Applicable

5.0 Residue Profile

5.1 Residue Analytical Methods (860.1340)

5.1.1 Data Collection Methods

DER Reference List: 49813534- 49813538

Plant

An analytical method was developed to analyze residues of pethoxamid in corn grain, lettuce, oilseed rape seed, orange fruit, and soybean (straw). EAS Method No. AGR/MOA/PTX-8 was used for data collection and the extraction procedures are based on the QuEChERS method. Briefly, samples are extracted with ACN. QuEChERS extraction salts (magnesium sulfate:sodium chloride:sodium citrate tribasic dihydrate:sodium citrate dibasic sesquihydrate; 8:2:2:1, w:w:w:w) are added. Samples are shaken and centrifuged, and an aliquot of the supernatant is diluted with water for analysis by LC/MS/MS, conducted using a reverse-phase C18 column and a gradient mobile phase of ACN and water containing 0.2% acetic acid. The following ion transitions are monitored: m/z 296.2 $\rightarrow$ 131.2 (quantitation) and m/z 296.2 $\rightarrow$ 250.2 (confirmation). The LOQ is 0.01 ppm.

Method validation was conducted at 0.01 and 0.10 ppm using corn grain, lettuce, rape seed, and orange fruit, as well as at 0.01, 0.10, and 1.0 ppm using the following commodities: field corn forage, grain, and stover; corn meal, flour, and refined oil; sweet corn kernel plus cob with husk

removed (K+CWHR); cotton undelinted seed, gin byproducts, meal, and hulls; and soybean forage, hay, seed, meal, and hulls. Recovery was measured at both the quantitation and confirmation ion transition for all matrices and fortification levels. At the 0.01-ppm level, the mean recoveries ranged 80-119%. At the 0.10-ppm level, the mean recoveries ranged 77-108%, and at the 1.0-ppm level, mean recoveries ranged 88.4-118%. A successful ILV was conducted using samples of head lettuce and rape seed fortified at 0.01 and 0.10 ppm. Individual recoveries were within the acceptable range of 70-120%.

No radiovalidation data were submitted due to the extraction solvents used in the plant metabolism studies being very similar to those used in the proposed enforcement method.

Livestock

DER Reference List: 49813535 and 49813536

FMC Corporation has submitted an analytical method EAS Study Code S15-03519 as the enforcement method and data collection for the determination of residues of pethoxamid in livestock commodities by LC/MS/MS. The extraction and analysis procedures are based on the QuEChERS method and are very similar to those of the proposed enforcement method for crop commodities, EAS Method No. AGR/MOA/PTX-8.

Briefly, homogenized samples are extracted with water by shaking and vortexing; ACN is added and the mixture is shaken and vortexed. QuEChERS extraction salts (magnesium sulfate:sodium chloride:sodium citrate tribasic dihydrate:sodium citrate dibasic sesquihydrate; 8:2:2:1, w:w:w:w) are added, and the samples are shaken, vortexed, and centrifuged. A portion of the supernatant is purified by the addition of cleanup salts (primary secondary amine:C18EC sorbent:magnesium sulfate; 1:1:6, w:w:w). Samples are shaken, vortexed, and centrifuged, and an aliquot of the supernatant is diluted with water for analysis by LC/MS/MS, conducted using a reverse-phase C18 column and a gradient mobile phase of ACN and water containing 0.2% acetic acid. The following ion transitions are monitored: m/z 296.2 $\rightarrow$ 131.2 (quantitation) and m/z 296.2 $\rightarrow$ 250.2 (confirmation). The LOQ is 0.01 ppm.

Method validation was conducted at 0.01 and 0.1 ppm using eggs, milk, fat, kidney, liver, and muscle. At the 0.01-ppm level, the mean recoveries across all matrices ranged 97-113%, and at the 0.1-ppm level the mean recoveries ranged 98-113%. A successful ILV was conducted using samples of whole milk, liver, and muscle fortified at 0.01 and 0.10 ppm. The ILV followed method procedures as currently written. Individual recoveries were within the acceptable range of 70-120%.

No radiovalidation data were submitted since pethoxamid residues were below the LOQ of Method EAS Study Code S15-03519 in any matrix in the livestock metabolism studies.

Conclusions. EAS Method No. AGR/MOA/PTX-8 (plant) and Method EAS Study Code S15-03519 (livestock) were used for data collection. The validated LOQ is 0.01 ppm in all commodities tested. The data collection methods were adequately validated for the matrices analyzed. The methods make use of standard analytical equipment and solvents, show acceptable sensitivity and selectivity, and give adequate recoveries in a variety of matrices. A successful ILV was conducted for plants and livestock. Because the extraction solvents used in

the metabolism studies are very similar to those used in the proposed enforcement method and pethoxamid was not found at a level above the LOQ in the metabolism studies, no radiovalidation data were required.

5.1.2 Multi-Residue Methods (860.1360)

The petitioner is proposing multi-residue method QuEChERS multiresidue technique followed by detection and quantitation using LC-MS/MS. The method was successfully validated by an independent laboratory validation. No additional multiresidue method data will be required.

5.1.3 Tolerance Enforcement Methods

DER Reference List: 49813534- 49813538

The tolerance-enforcement methods are the same as the data-collection methods described above.

Conclusions. EAS Method No. AGR/MOA/PTX-8 (plant) and Method EAS Study Code S15-03519 (livestock) were used for data collection and enforcement method. The validated LOQ is 0.01 ppm in all commodities tested. The methods make use of standard analytical equipment and solvents, show acceptable sensitivity and selectivity, and give adequate recoveries in a variety of matrices. Successful ILVs were completed for both methods. Because the extraction solvents used in the metabolism studies are very similar to those used in the proposed enforcement method and pethoxamid was not found at a level above the LOQ, no radiovalidation data were required. The Agency concludes that QuEChERS (AGR/MOA/PTX-8) and (S15-03519) are acceptable for the enforcement of tolerances for residues of pethoxamid in/on plant and livestock commodities.

5.1.4 Submittal of Analytical Reference Standards (860.1650)

No analytical reference standards have been submitted to the EPA National Pesticide Standards Repository (personal communication from G. Verdin of US EPA National Pesticide Standard Repository to S. Piper on February 4, 2019). Analytical standards must be available before a tolerance can be published in the 40 CFR.

The address to submit standards is:

USEPA

National Pesticide Standards Repository/Analytical Chemistry Branch/OPP

701 Mapes Road

Fort George G. Meade, MD 20755-5350

Note: The full 9-digit zip code is mandatory, or the mail will be returned to you.

5.2 Storage Stability (860.1380)

DER Reference List: 49813534, 49813540- 49813541; 49813542- 49813543; 49813544- 49813545

A freezer storage stability study (MRID 49813540) with pethoxamid in maize (corn) grain, oilseed rape seed and soybean matrices at 0.1 ppm was submitted and samples were placed in

frozen storage at ≤ -20 °C. Stored samples and freshly fortified samples were analyzed for residues of pethoxamid at nominal intervals of 0, 3, 6, 9, and 12 months. The study was conducted by Eurofins Agrosience Service Chem SAS (Vergeze, France). A supplemental report (MRID 49813541) indicates the dates of sample fortification, extraction, and analysis in the storage stability study of pethoxamid residues in corn grain, oilseed rape seed and soybean. After 12 months of storage at -20°C , corrected recoveries were soybean 89- 109%, 70- 117% for oilseed rape seed and 100- 108% for corn grain. Corrected recoveries were calculated due to not all samples were analyzed within 24 hours after extraction. The maximum extract storage time was 5 days from extraction to analysis.

Pethoxamid residues (MRID 49813534) were stable in corn (meal, flour, refined oil), sweet corn and soybean (meal and hull) for at least 10 months (303 days). Pethoxamid residues were stable in cotton undelinted seed for at least 5 months (154 days).

A study (MRID 49813542) to validate a method of analysis for pethoxamid (TKC-94) in corn (grain, green plant, and straw) was conducted by Huntingdon Life Sciences in the United Kingdom in 1998. In addition, samples of corn grain, cobs and straw fortified in duplicate by Stahler Agrochemie in 1997 were analyzed to confirm the stability of pethoxamid in crop matrix stored at approximately $\leq -20^{\circ}\text{C}$ for a period of approximately 2 years. The supplemental report (MRID 49813543) indicates the dates of sample fortification, extraction, and analysis in the storage stability study of pethoxamid residues in corn matrices (whole plant/cob, grain and straw). The method was validated from the range of 0.01-1.0 ppm and the recovery range was 74-102% grain, 84-108% in green plant and 80-106% in straw. The results of the study showed that residues of pethoxamid are stable for at least 2 years in corn grain, green plant and straw stored at ≤ -20 °C.

A freezer storage stability study (MRID 49813544) with pethoxamid in soybean (green plant, seed, and straw) at 0.01, 0.1, and 1.0 ppm was submitted, and samples were placed in frozen storage at ≤ -20 °C. Stored samples and freshly fortified samples were analyzed for residues of pethoxamid at nominal intervals of 0 and 8 months. The study was conducted by Huntingdon Life Sciences in the United Kingdom in 1998. The supplemental report (MRID 49813545) indicates the dates of sample fortification, extraction, and analysis in the storage stability study of pethoxamid residues in soybean. Acceptable concurrent recoveries (70-100%) were obtained from samples of soybean (green plant, seed, and straw) fortified with pethoxamid at 0.1-1.0 ppm. The results of the study showed that residues of pethoxamid are stable for at least 8 months in soybean (green plant, seed, and straw) stored at ≤ -20 °C.

TABLE 5.2.1. Summary of Storage Conditions.			
Matrix	Storage Temperature (°C)	Actual Storage Duration	Interval of Demonstrated Storage Stability
Corn	≤-20	24 months	Residues of pethoxamid in whole plant/cob, grain and straw were stable during frozen storage for up to 2 years. Also, oilseed rape seed was stable for up to 12 months.
Soybean		8 months	Residues of pethoxamid in soybean (green plant, seed, and straw) were stable during frozen storage for up to 8 months. Another study soybean seed was stable for up to 12 months.
Cotton		5 months	Residues of pethoxamid in cotton undelinted seed were stable during frozen storage for at least 5 months (154 days).

Conclusions. The freezer storage stability studies are scientifically acceptable. An acceptable method was used for residue quantitation. HED concludes that based on the submitted data, residues of pethoxamid are stable during frozen storage for at least 5 months in cotton undelinted seed, for at least 12 months in soybean, and for at least 2 years in corn. The submitted data support the storage interval for the field trials.

5.3 Residue Data

5.3.1 Crop Field Trials (860.1500)

DER References: 49813546.der (corn)
 49813548.der (cotton)
 49813547.der (soybeans)

Field, Pop, & Sweet Corn

Seventeen trials for pethoxamid on field, pop, and sweet corn were conducted in Canada and/or United States encompassing NAFTA Growing Zones 1 (PA; 1 trial), 2 (NC; 1 trial), 5 (IA, IL, MB, MI, MN, MO, NE, ON, and WI; 13 trials), 6 (OK; 1 trial), and 8 (TX; 1 trial); three trials were conducted with popcorn in Zone 5 (IA, IL, and NE); and 15 trials were conducted with sweet corn in Zones 1 (NY and PA; 2 trials), 2 (NC; 1 trial), 3 (FL; 1 trial), 5 (IA, IL, MB, MI, MO, NE, and ON; 7 trials), 7 (SK; 1 trial), 10 (CA; 1 trial), 11 (WA; 1 trial), and 12 (OR; 1 trial) during the 2014 growing season. At four pairs of field corn test sites (in IA, IL, MN, and NE), trials were determined to be replicates; therefore, residues were averaged, and each pair of sites was taken to be one independent trial, which is reflected in the above trial count. Each site consisted of one untreated plot and one treated plot reflecting a preplant or preemergence application to the soil followed by a foliar broadcast application targeting the 40-inch growth stage (prior to beginning of tasseling). Applications were made with a 5.0 lb ai/gal emulsifiable-concentrate (EC) formulation of pethoxamid at 1.42-1.56 lb ai/A/application, with 32- to 65-day retreatment intervals, for total seasonal rates of 2.88-3.12 lb ai/A (2X). At one field corn site, an additional plot was treated at an exaggerated for a total application rate of 15.0 lb ai/A (10X) for the collection of field corn grain for possible processing. An adjuvant (nonionic surfactant or crop oil concentrate) was added to the spray mixture for all foliar applications, and for preemergence/preplant applications at five trial sites.

Field corn forage was harvested at the late dough to early dent stage, and grain and stover were harvested at maturity, at PHIs of 42-78 days for forage and 71-125 days for grain and stover. For popcorn, grain and stover were harvested at maturity at a PHI of 97-107 days. For sweet corn, forage, kernel plus cob with husk removed (K+CWHR), and stover were harvested at the milk stage at a PHI of 14-42 days; stover was dried for 2-12 days prior to collection. Trial results show the residues were below the LOQ in/on all samples of field corn grain harvested at a PHI of 71-125 days, all samples of popcorn grain and stover harvested at a PHI of 97-107 days, and all samples of sweet corn K+CWHR harvested at a PHI of 14-42 days. In the residue decline studies, residues of pethoxamid were below the LOQ (<0.01 ppm) in/on field corn forage, grain, and stover; popcorn grain and stover; and sweet corn forage, K+CWHR, and stover samples from all sampling intervals; therefore, residue decline could not be evaluated. The results from these studies are discussed below and summarized in Table 5.3.1(a).

Cotton

Eleven supervised field trials were conducted with cotton in NAFTA Growing Zones 2 (GA), 4 (AR, LA and MO), 6 (OK and TX), 8 (TX), and 10 (CA) during the 2014 growing season. In CA, trials were determined to be replicates; therefore, residues were averaged, and the pair of sites was taken to be one independent trial. Each site consisted of one untreated plot and one treated plot reflecting a preplant or preemergence application to the soil followed by a foliar broadcast application targeting the first white flower growth stage (~BBCH 60). Applications were made with a 5.0 lb ai/gal EC formulation of pethoxamid at 1.43-1.56 lb ai/A/application, with 50- to 84-day retreatment intervals, for total seasonal rates of 2.96-3.05 lb ai/A (2X). An adjuvant (nonionic surfactant or crop oil concentrate) was added to the spray mixture for all foliar applications, and for the preplant/preemergence application at two sites. Duplicate treated samples of seed cotton were harvested at maturity by hand (7 trials) or using stripper equipment (4 trials) at a PHI of 67-185 days and ginned to produce undelinted cotton seed (all trials) and gin byproducts (at 3 stripper harvest equipment trials). At one trial, additional undelinted cotton seed and gin byproducts samples were collected 12 days before and 7, 14, and 22 days after nominal harvest to assess residue decline.

Residues of pethoxamid were below the LOQ (<0.01 ppm) in/on all undelinted seed samples collected at a PHI of 67-185 days. In the corresponding gin byproduct samples, collected at three trials reflecting a PHI of 67-112 days, individual sample (and per-trial average) residues of pethoxamid were <0.01-0.047 (<0.01-0.039) ppm. In the residue decline study, residues of pethoxamid were below the LOQ (<0.01 ppm) in/on cotton undelinted seed samples from all sampling intervals; therefore, residue decline could not be evaluated.

Soybean

Eighteen field trials for pethoxamid on soybeans were conducted in Canada and/or the United States encompassing NAFTA Growing Zones 2 (NC and SC; 2 trials, 4 (AR, LA, and MO; 3 trials), and 5 (IA, IL, MB, MI, MN, MO, NE, and WI; 13 trials) during the 2014 growing season. At three pairs of test sites (in IA, IL, and MN), trials were determined to be replicates; therefore, residues were averaged, and each pair of sites was taken to be one independent trial, which is reflected in the above trial count. Each site consisted of one untreated plot and one treated plot reflecting a preplant or preemergence application to the soil followed by a foliar broadcast application targeting the R1 growth stage (first flowers open; ~BBCH 60). Applications were

made with a 5.0 lb ai/gal EC formulation of pethoxamid at 1.96-2.10 lb ai/A/application with 39- to 70-day retreatment intervals, for total seasonal rates of 3.96-4.12 lb ai/A (3X). At one site, an additional plot was treated at an exaggerated rate a total application rate of 20.0 lb ai/A (13X) for the collection of soybean seed for processing. An adjuvant (nonionic surfactant or crop oil concentrate) was added to the spray mixture for all foliar applications. Duplicate treated samples of soybean forage and hay were harvested at BBCH 60-70 at a PHI of 6-7 days; hay was dried for 1-10 days prior to collection. Duplicate treated samples of soybean seed were harvested at maturity at a PHI of 86-112 days. At two trials, additional forage and hay samples were harvested at PHIs of 0, 14, and 21 days, and additional seed samples were collected 6-7 days before, and 7 and 14 days after nominal harvest to assess residue decline.

Trial results show that residues of pethoxamid were highest in/on forage (0.154-23.3 ppm) and hay (0.083-21.4 ppm) harvested at a PHI of 6-7 days. Residues of pethoxamid were nondetectable (<0.01 ppm) in/on all soybean seed samples harvested at a PHI of 86-112 days, including samples from the exaggerated-rate (20X) plot. In the residue decline studies, residues of pethoxamid were below the LOQ (<0.01 ppm) in/on soybean seed samples from all sampling intervals.

Table 5.3.1(a). Summary of Residues from Field Trials with Pethoxamid.

Crop Matrix	Analyte	Application Rate (lb ai/acre) [kg ai/ha]	PHI (days)	n ¹	Residues (ppm)						
					Min. ²	Max. ²	LAFT ³	HAFT ³	Median ³	Mean ³	SD ³
Corn (proposed use = 2.88-3.12 lb ai/acre total application rate; 14-42, 42-78, 71-125-days PHI)											
Field corn forage	Pethoxamid	2.90-3.05 [3.25-3.42]	42-78	13	<0.01	0.015	<0.01	0.014	0.01	0.01	0.001
Field corn grain	Pethoxamid		71-125	13	<0.01	<0.01	<0.01	<0.01	0.01	0.01	N/A
Field corn stover	Pethoxamid		71-125	13	<0.01	0.022	<0.01	0.019	0.01	0.01	0.002
Popcorn grain	Pethoxamid	2.97-2.99	97-107	3	<0.01	<0.01	<0.01	<0.01	0.01	0.01	N/A
Popcorn stover	Pethoxamid	[3.33-3.35]	97-107	3	<0.01	<0.01	<0.01	<0.01	0.01	0.01	N/A
Sweet corn forage	Pethoxamid	2.88-3.12 [3.23-3.50]	14-42	15	<0.01	0.391	<0.01	0.350	0.01	0.047	0.101
Sweet corn K+CWHR	Pethoxamid		14-42	15	<0.01	<0.01	<0.01	<0.01	0.01	0.01	N/A
Sweet corn stover	Pethoxamid		14-42	15	<0.01	0.759	<0.01	0.467	0.01	0.046	0.118
Cotton (proposed use = 2.96-3.05 lb ai/acre total application rate, 67-185, 67-112 days PHI)											
Cotton undelinted	Pethoxamid	2.96-3.05 [3.32-3.42]	67-185	11	<0.01	<0.01	<0.01	<0.01	0.01	0.01	N/A
Gin byproducts	Pethoxamid	2.96-3.00 [3.32-3.36]	67-112	3	<0.01	0.047	<0.01	0.039	0.014	0.02	0.016
Soybean (proposed use = 3.96-4.12 lb ai/acre total application rate, 6-7, 86-112 days PHI)											
Soybean forage	Pethoxamid	3.96-4.12 [4.44-4.62]	6-7	18	0.154	23.3	0.245	21.1	1.45	3.70	5.50
Soybean hay	Pethoxamid		6-7	18	0.083	21.4	0.295	16.1	2.99	4.48	4.76
Soybean seed	Pethoxamid		86-112	18	<0.01	<0.01	<0.01	<0.01	0.01	0.01	N/A

¹ n = number of field trials.

² Values based on total number of samples.

³ Values based on per-trial averages. LAFT = Lowest Average Field Trial, HAFT = Highest Average Field Trial, SD = Standard Deviation. For computation of the LAFT, HAFT, median, mean, and standard deviation, values < LOQ are assumed to be at the LOQ. N/A = Not applicable.

Conclusions. The number and locations of field trials are in accordance with OCSPP Guideline 860.1500 and the geographic distribution of the field trials by NAFTA growing zones is summarized in Appendix D. Sufficient numbers of trials were conducted in geographically appropriate regions to support the establishment of tolerances. All commodities were analyzed for residues of pethoxamid using the data-collection methods described in Section 5.1: EAS

Method No. AGR/MOA/PTX-8. Residue field trials were conducted for corn, cotton, and soybean at preplant or preemergence application to the soil followed by a foliar broadcast application. However, the post-emergence use was no longer supported by the registrant. The field trials for corn, cotton and soybean are above the registrant proposed maximum annual application rate at 1.5 lb ai/A; however, the maximum seasonal rates are 2.88 -4.12 lb ai/A (3X) in/on corn, cotton, and soybean. Residues of pethoxamid were nondetectable (<0.01 ppm) in/on all soybean seed, cotton undelinted seed, and corn grain.

5.3.2 Field Rotational Crops (860.1900)

Residue data were not submitted for field crop rotational studies for pethoxamid. However, in the confined rotational studies, pethoxamid was rapidly metabolized resulting in no detectible residue on spinach, carrots, turnips, spinach, and wheat.

Conclusions. The label supports a 30-day plant back interval for all rotated crops.

5.3.3 Processed Food and Feed (860.1520)

DER References: 49813546- 49813548.der

Processing studies were conducted at the exaggerated-rate on corn, soybean, and cotton. For example, cotton was applied at 5.0 lb ai/gal EC formulation of pethoxamid at 7.47-7.50 lb ai/A/application for a total seasonal rate of 15.0 lb ai/A (~10x the corresponding field trial rates). Residues of pethoxamid were nondetectable in/on corn grain and all soybean seed samples at the exaggerated-rate and residues in the cotton undelinted seeds before processing and after processing to hulls, meal, and refined oil were all below the LOQ; therefore, processing factors were not calculated.

Conclusions. The submitted processing studies are acceptable. The data indicate that pethoxamid does not concentrate in corn grain, soybean seed, and cotton undelinted seed resulting in quantifiable residues following application of pethoxamid at exaggerated rates up to 7-10x the maximum proposed seasonal application rate. The available data indicate that tolerances are not required for the processed commodities.

5.3.4 Meat, Milk, Poultry and Eggs (860.1480)

A livestock feeding study was not provided by the petitioner. The metabolism data on poultry and ruminant confirm that residues of pethoxamid are not expected in edible livestock tissue, milk or eggs above 0.01 ppm based on the proposed uses. Rather than citing 40 CFR 180.6(a)(3) as rationale for not establishing tolerances for residues of pethoxamid in livestock commodities, HED is recommending that tolerances be listed for residues in livestock commodities at 0.01 ppm on meat, milk, poultry and eggs in order to harmonize with proposed Canadian MRLs. If new uses are proposed for livestock feeding commodities, then a feeding study may be required to support those uses.

5.3.5. Food Handling (860.1460)

At this time, there are no food-handling uses associated with pethoxamid.

5.3.6 Water, Fish, and Irrigated Crops (860.1400)

At this time, there are no water, fish, or irrigated crop uses associated with pethoxamid.

5.4 Food Residue Profile

Adequate residue chemistry data have been provided for pethoxamid. The submitted studies showed that quantifiable residues of pethoxamid are not expected in human foods or livestock feed when the available data are evaluated against the proposed use pattern. Likewise, quantifiable residues of pethoxamid are not expected in livestock commodities.

6.0 Tolerance Derivation

HED concurs that the metabolism data on poultry and ruminant, and dietary burden confirm that residues of pethoxamid are not expected in edible livestock tissue, milk or eggs above 0.01 ppm based on the proposed uses. However, PMRA is recommend establishing tolerances at the limit of quantitation (0.01 ppm) for residues in livestock commodities and the registrant, FMC would like to harmonize with the proposed Canadian MRLs. All other issues raised for discussion by RAB1 and focus group reviewers during the meeting have been addressed.

7.0 ChemSAC Reference

This memorandum was reviewed by the Chemistry Science Advisory Council (ChemSAC) on 04/10/2019 and has been revised to reflect the recommendations of that group (see minutes from 05/15/2019).

M. Negussie. Pethoxamid. Report of the Residues of Concern Knowledgebase Subcommittee. 06/20/2019. D450748.

Appendix A. Summary of Metabolites and Degradates

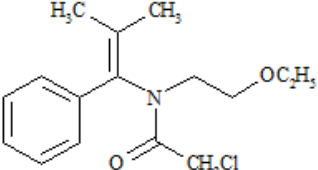
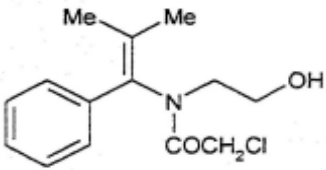
Table A. Summary of Metabolites and Degradates.			
Chemical Name Structure	Matrix	Percent TRR (ppm) Matrices- Major Residue (>10% TRR)	Matrices- Minor Residue <10% TRR (ppm)
Pethoxamid 2-chloro-N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propen-1-yl)acetamide  Derek report: ◆ Chromosome damage in vitro in mammal is PLAUSIBLE • Alert matched: 061 alpha-Halo carbonyl compound ◆ Skin sensitization in mammal is PLAUSIBLE • Alert matched: 413 Alkyl halide	Soybean immature whole plant		1.1% TRR (0.363 ppm)
	Corn immature whole plant		1.0% TRR (0.066 ppm)
		77.1% TRR (13.96 ppm)	
	Poultry Liver		0.4% TRR (0.001 ppm)
	Poultry Eggs		1.7% TRR (0.001 ppm)
	Poultry Abdominal Fat		2.7% TRR (0.001 ppm)
	Poultry Subcutaneous Fat		7.3% TRR (0.004 ppm)
	Rotational Crops:		
	Carrot Root at 30-day PBI	11.3% TRR (0.008 ppm)	
	Carrot Root at 120-day PBI		3.8% TRR (0.001 ppm)
MET-1 2-chloro-N-(2-hydroxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide  Derek Report: ◆ Chromosome damage in vitro in mammal is PLAUSIBLE • Alert matched: 061 alpha-Halo carbonyl compound ◆ Skin sensitization in mammal is PLAUSIBLE • Alert matched: 413 Alkyl halide	Goat Liver	10.9% TRR (0.06 ppm)	
	Goat Urine	39.3% TRR (27.6 ppm)	
MET-2	poultry liver		0.5% TRR (0.001 ppm)
	poultry eggs		1.4% TRR (0.001 ppm)

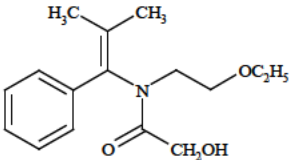
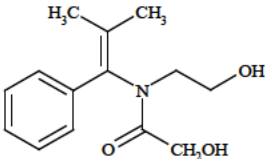
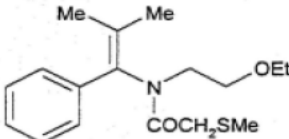
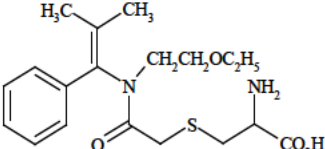
Table A. Summary of Metabolites and Degradates.			
Chemical Name Structure	Matrix	Percent TRR (ppm) Matrices- Major Residue (>10% TRR)	Matrices- Minor Residue <10% TRR (ppm)
N-(2-ethoxyethyl)-2-hydroxy-N-(2-methyl-1-phenyl-1-propenyl)acetamide 	poultry abdominal fat		3.1% TRR (0.001 ppm)
MET-3 2-hydroxy-N-(2-hydroxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide 	poultry liver		2.7% TRR (0.007 ppm)
	poultry eggs		2.8% TRR (0.001 ppm)
	poultry breast muscle		3.8% TRR (0.001 ppm)
MET-6 N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-(methylthio)acetamide  No Derek alert	Drinking Water	Maximum %Applied (day)	Final %Applied (day)
	Aerobic Aquatic	Lake 12.0% (14 d)	Lake 5.2% (102 d)
		River 10.3% (31 d)	River 4.5% (102 d)
	Anaerobic Aquatic	Lake 9.6% (61, 101 d)	Lake 9.6% (101 d)
		River 15.2% (101 d)	River 15.2% (101 d)
MET-30 S-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]-D,L-cysteine  No Derek alert	soybean immature whole plant	20.3% TRR (6.403 ppm)	
	corn immature whole plant		7.5% TRR (0.498 ppm)
	potato immature foliage		3.4% TRR (0.612 ppm)
	poultry liver		0.4% TRR (0.001 ppm)
	poultry eggs		3.3% TRR (0.001 ppm)
	goat liver	21.6% TRR (0.120 ppm)	
	goat urine	14.7% TRR (10.3 ppm)	
	Rotational Crops:		
	turnip root at 54 DAP		9.8% TRR (0.019 ppm)
	turnip root at 79 DAP		1.8% TRR (0.001 ppm)

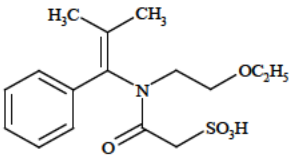
Table A. Summary of Metabolites and Degradates.			
Chemical Name Structure	Matrix	Percent TRR (ppm) Matrices- Major Residue (>10% TRR)	Matrices- Minor Residue <10% TRR (ppm)
	turnip tops at 54 DAP		4.5% TRR (0.026 ppm)
	turnip tops at 79 DAP		7.5% TRR (0.047 ppm)
	spinach at 47 DAP		3.4% TRR (0.027 ppm)
	spinach at 70 DAP		7.5% TRR (0.038 ppm)
	spinach at 98 DAP		6.0% TRR (0.025 ppm)
	wheat forage at 70 DAP	11.3% TRR (0.054 ppm)	
	wheat straw at 126 DAP		4.3% TRR (0.026 ppm)
	wheat straw at 146 DAP		6.1% TRR (0.109 ppm)
	wheat grain at 126 DAP		2.0% TRR (0.003 ppm)
MET-42 N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-sulfoacetamide  No Derek alert	potato immature foliage	10.0% TRR (0.207 ppm)	
	poultry liver		3.4% TRR (0.009 ppm)
	poultry eggs		8.2% TRR (0.004 ppm)
	poultry breast muscle		2.7% TRR (0.001 ppm)
	poultry thigh muscle		3.7% TRR (0.001 ppm)
	Rotational Crops		
	turnip root at 54 DAP		5.7% TRR (0.011 ppm)
	turnip tops at 54 DAP	14.2% TRR (0.082 ppm)	
	turnip tops at 79 DAP	21.0% TRR (0.130 ppm)	
	turnip tops at 105 DAP	26.4% TRR (0.132 ppm)	
	spinach at 47 DAP		9.1% TRR (0.072 ppm)
	spinach at 70 DAP		7.7% TRR (0.039 ppm)
	spinach at 98 DAP		7.9% TRR (0.032 ppm)
	wheat forage at 70 DAP	14.2% TRR (0.068 ppm)	
	wheat straw at 126 DAP		9.9% TRR (0.060 ppm)
	wheat straw at 146 DAP	12.9% TRR (0.231 ppm)	
	wheat grain at 126 DAP		3.5% TRR (0.005 ppm)
	Drinking Water	Maximum %Applied (day)	Final %Applied (day)
	Aerobic Soil	ssl 10.38% (10 d)	ssl 4.95% (120 d)
		sl 4.82% (30 d)	sl 2.05% (120 d)
		ssl 5.57% (59 d)	ssl 2.91% (120 d)
		cl 7.39% (10 d)	cl 1.71% (120 d)

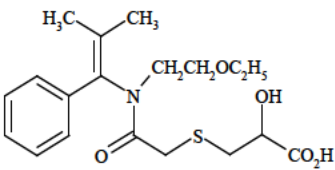
Table A. Summary of Metabolites and Degradates.			
Chemical Name Structure	Matrix	Percent TRR (ppm) Matrices- Major Residue (>10% TRR)	Matrices- Minor Residue <10% TRR (ppm)
		Ssl 11.53% (90 d)	Ssl 8.41% (120 d)
	Aerobic Soil	Lake 2.6% (102 d)	Lake 2.6% (102 d)
		River 2.2% (31, 102 d)	River 2.2% (102 d)
	Anaerobic Aquatic	Lake 2.3% (61 d)	Lake 1.1% (101 d)
		River 2.9% (101 d)	River 2.9% (101 d)
	Terrestrial Field Dissipation Study	IA 68.3 g peq/ha	IA 12.3 g peq/ha
		NY 73 g peq/ha	NY 8 g peq/ha
		CA 161 g peq/ha	CA 22 g peq/ha
		TX 157 g peq/ha	TX 24 g peq/ha
MET-41 3-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoyl methylthio]-D,L-lactic acid 	Rotational Crops:		
	turnip root at 54 DAP		2.1% TRR (0.004 ppm)
	turnip root at 79 DAP		3.2% TRR (0.002 ppm)
	turnip root at 105 DAP		5.9% TRR (0.003 ppm)
	turnip tops at 54 DAP		2.9% TRR (0.017 ppm)
	turnip tops at 79 DAP		1.2% TRR (0.007 ppm)
	turnip tops at 105 DAP		1.2% TRR (0.006 ppm)
	spinach at 47 DAP		1.7% TRR (0.013 ppm)
	spinach at 70 DAP		3.1% TRR (0.016 ppm)
	spinach at 98 DAP		2.0% TRR (0.008 ppm)
	wheat forage at 70 DAP		3.6% TRR (0.017 ppm)
	wheat straw at 126 DAP		2.8% TRR (0.017 ppm)
	wheat straw at 146 DAP		2.4% TRR (0.043 ppm)
	wheat grain at 126 DAP		1.3% TRR (0.002 ppm)
	wheat grain at 146 DAP		0.6% TRR (0.001 ppm)
	wheat chaff at 146 DAP		0.7% TRR (0.005 ppm)
MET-43 N-(2-ethoxyethyl)-2-mercapto-N-(2-methyl-1-phenyl-1-propenyl)acetamide	soybean immature whole plant		2.8 TRR (0.896 ppm)
	corn immature whole plant		5.9% TRR (0.387 ppm)

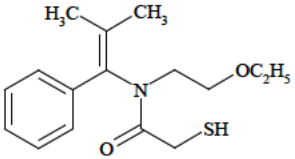
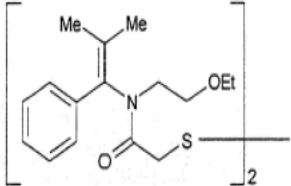
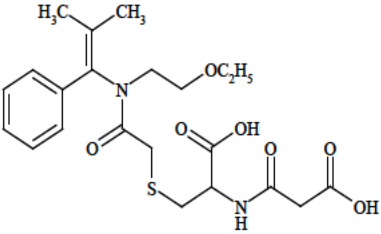
Table A. Summary of Metabolites and Degradates.			
Chemical Name Structure	Matrix	Percent TRR (ppm) Matrices- Major Residue (>10% TRR)	Matrices- Minor Residue <10% TRR (ppm)
			
MET-44 bis S-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl] 	potato immature foliage		7.9% TRR (0.163 ppm)
MET-47 N-(2-carboxyacetyl)-S-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]-D,L-cysteine 	soybean immature whole plant	17.4% TRR (5.505 ppm)	
	poultry liver		3.4% TRR (0.009 ppm)
	poultry eggs		3.9% TRR (0.002 ppm)
MET-48 3-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethylthio]-2-(α-D-glucopyranosyloxy)propionic acid	Rotational Crops		
	turnip root at 54 DAP		1.1% TRR (0.002 ppm)
	turnip tops at 105 DAP		4.1% TRR (0.021 ppm)
	spinach at 47 DAP		7.0% TRR (0.055 ppm)
	spinach at 70 DAP		8.6% TRR (0.044 ppm)
	spinach at 98 DAP		8.2% TRR (0.034 ppm)
	wheat forage at 70 DAP		3.6% TRR (0.017 ppm)

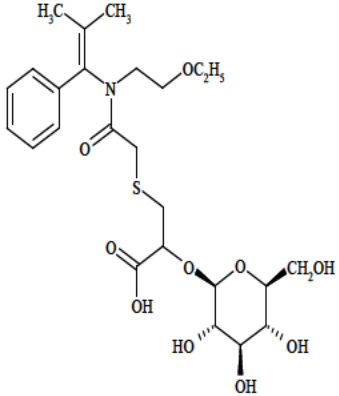
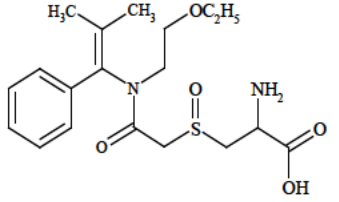
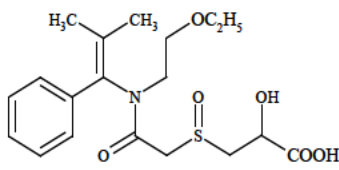
Table A. Summary of Metabolites and Degradates.			
Chemical Name Structure	Matrix	Percent TRR (ppm) Matrices- Major Residue (>10% TRR)	Matrices- Minor Residue <10% TRR (ppm)
			
MET-49 S-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]-D,L-cysteine-S-oxide 	Rotational Crops:		
	turnip tops at 79 DAP		6.0% TRR (0.037 ppm)
	turnip tops at 105 DAP		4.8% TRR (0.024 ppm)
	spinach at 47 DAP		5.5% TRR (0.043 ppm)
	spinach at 70 DAP		4.6% TRR (0.023 ppm)
	spinach at 98 DAP		2.1% TRR (0.009 ppm)
	wheat forage at 70 DAP		2.6% TRR (0.012 ppm)
	wheat forage at 146 DAP		1.3% TRR (0.023 ppm)
	wheat grain at 126 DAP;		0.9% TRR (0.001 ppm)
	wheat chaff at 126 DAP		1.1% TRR (0.004 ppm)
	wheat chaff at 146 DAP		2.4% TRR (0.018 ppm)
MET-50 3-((2-((2-ethoxyethyl)(2-methyl-1-phenylprop-1-en-1-yl)amino)-2-oxoethyl)sulfinyl)-2-hydroxypropanoic acid  No Derek alert	Rotational Crops		
	turnip root at 54 DAP		8.7% TRR (0.017 ppm)
	turnip root at 79 DAP		2.8% TRR (0.002 ppm)
	turnip root at 105 DAP		5.4% TRR (0.003 ppm)
	turnip tops at 54 DAP	15.3% TRR (0.089 ppm)	
	turnip tops at 79 DAP	12.1% TRR (0.075 ppm)	
	turnip tops at 105 DAP	17.7% TRR (0.089 ppm)	
	spinach at 47 DAP	12.2% TRR (0.096 ppm)	
	spinach at 70 DAP	10.3% TRR (0.053 ppm)	
	spinach at 98 DAP		4.5% TRR (0.018 ppm)
	wheat forage at 70 DAP		7.5% TRR (0.036 ppm)

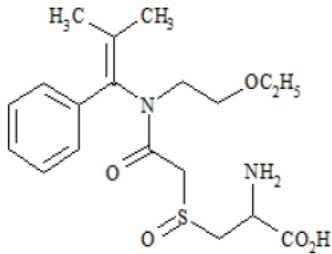
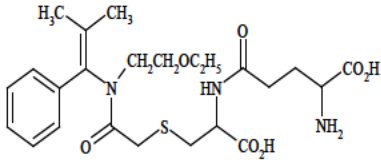
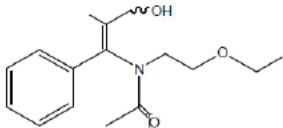
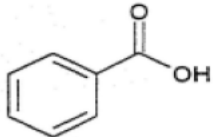
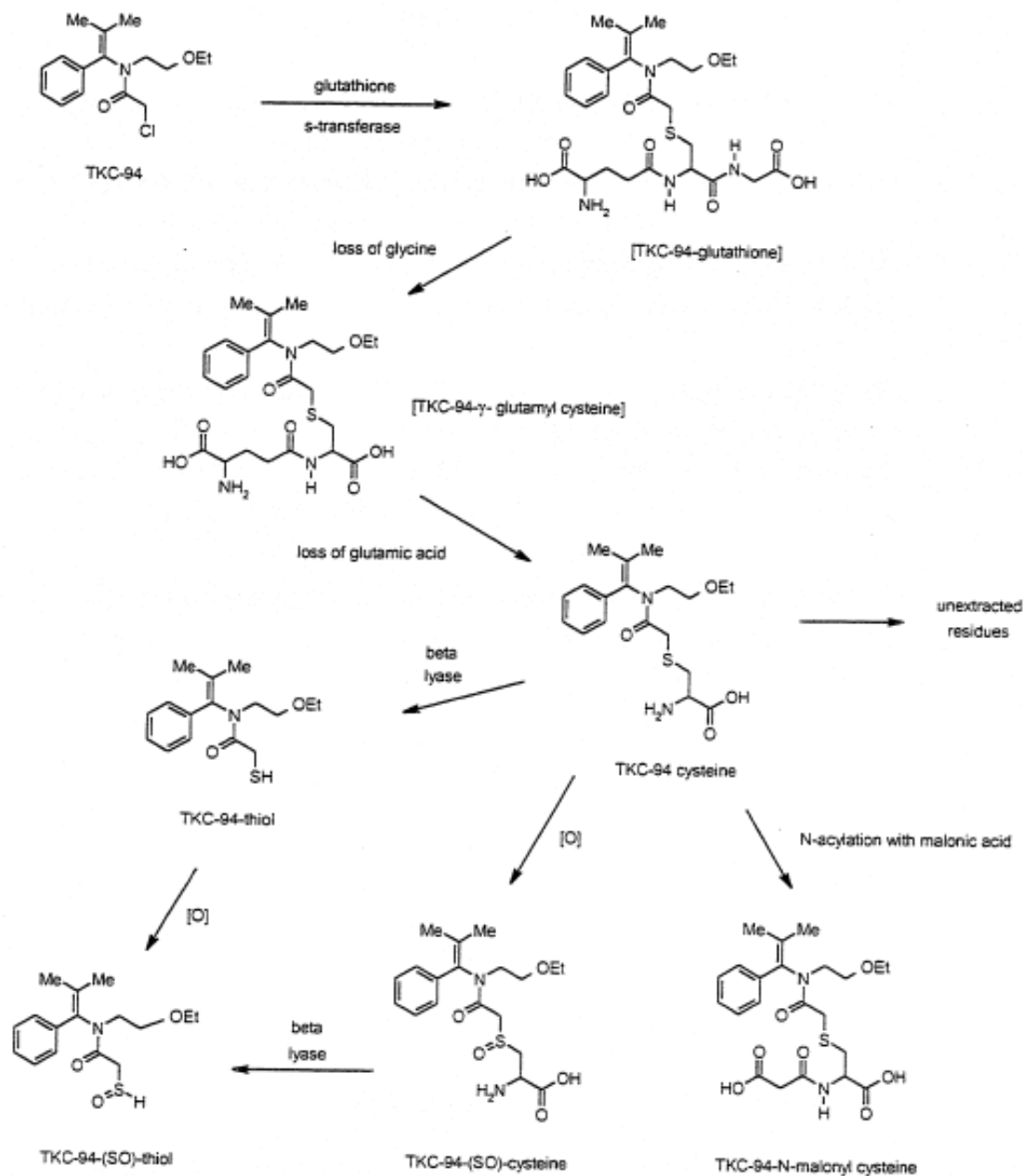
Table A. Summary of Metabolites and Degradates.			
Chemical Name Structure	Matrix	Percent TRR (ppm) Matrices- Major Residue (>10% TRR)	Matrices- Minor Residue <10% TRR (ppm)
	wheat straw at 126 DAP		4.8% TRR (0.029 ppm)
	wheat straw at 146 DAP		5.5% TRR (0.098 ppm)
	wheat grain at 126 DAP		4.6% TRR (0.006 ppm)
TKC-94-SO-cysteine 	wheat chaff at 126 DAP		4.1% TRR (0.014 ppm)
TKC-94-glutamyl cysteine (MET-130) 	wheat chaff at 146 DAP		6.0% TRR (0.046 ppm)
	poultry liver		2.7% TRR (0.007 ppm)
	poultry eggs		6.9% TRR (0.003 ppm)
MET-102 	Drinking Water	Maximum %Applied (day)	Final %Applied (day)
	Photolysis in Water	Buffer 21.05% (6 d)	Buffer 15.9% (16 d)
No Derek alert			
MET-33 benzoic acid 	Drinking Water	Maximum %Applied (day)	Final %Applied (day)
	Photolysis in Water	Buffer 31.6% (16 d)	Buffer 31.6% (16 d)
Carbon Dioxide $O=C=O$	Drinking Water	Maximum %Applied (day)	Final %Applied (day)
	Aerobic Soil	ssl 37.60% (120 d)	ssl 37.60% (120 d)
		sl 39.32% (120 d)	sl 39.32% (120 d)
		ssl 38.14% (120 d)	ssl 38.14% (120 d)

Table A. Summary of Metabolites and Degradates.			
Chemical Name Structure	Matrix	Percent TRR (ppm) Matrices- Major Residue (>10% TRR)	Matrices- Minor Residue <10% TRR (ppm)
		cl 47.84% (120 d)	cl 47.84% (120 d)
		ssl 25.73% (120 d)	ssl 25.73% (120 d)

Appendix B. Metabolic Pathways

Figure B.1. Proposed Metabolic Pathway of Pethoxamid in Plants (Soybean)



Compounds in brackets [] were not detected in this study

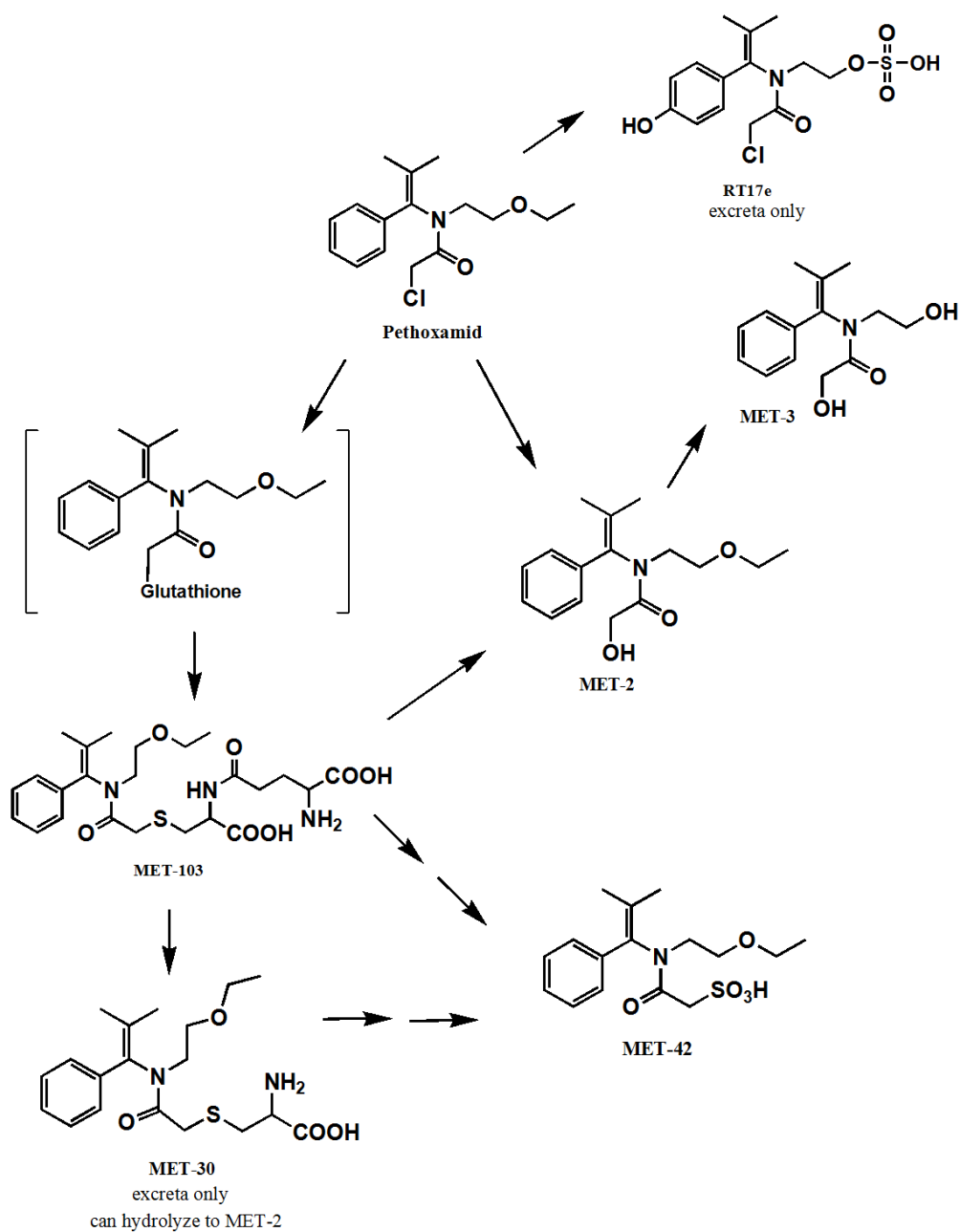
Figure B.2. Proposed Metabolic Pathway of Pethoxamid in Poultry

Figure B.3. Proposed Metabolic Pathway of Pethoxamid in Ruminants.

NOTE: Waiting on the registrant to provide diagrams without watermarks; therefore, diagram has been omitted.

Figure B.4. Metabolic Profile of Pethoxamid in Soil and Rotational Crops.

NOTE: Waiting on the registrant to provide diagrams without watermarks; therefore, diagram has been omitted.

Appendix C. Summary of Residues in Metabolism Studies

Plant Metabolism Studies

Soybean

Table C.1. Summary of Characterization and Identification of Radioactive Residues in Soybean Matrices Following Application of [¹⁴C]Pethoxamid at 1.1 lb ai/A (1.2 kg ai/ha).¹

Compound ²	Preemergence application		Foliar application	
	Mature leaves (127-day PHI)		Immature whole plants (8-day PHI) ²	
	TRR = 0.444 ppm		TRR = 31.576 ppm	
	% TRR	ppm	% TRR	ppm
Pethoxamid	ND	--	1.1	0.363
TKC-94-SO-cysteine	ND	--	6.4	2.034
TKC-94-cysteine (MET-30)	ND	--	20.3	6.403
TKC-94-N-malonyl cysteine (MET-47)	ND	--	17.4	5.505
TKC-94-thiol (MET-43)	ND	--	2.8	0.896
TKC-94-SO-thiol	ND	--	1.6	0.498
Discrete unknowns	52.0	0.231	18.1	5.763
Multiple minor unknowns	38.6	0.172	28.9	9.007
HCl/NaOH hydrolysate(s)	3.9	0.017	1.8	0.564
Total extractable	94.5	0.420	98.4	31.073
Total identified	--	--	49.6	15.699
Total unidentified	90.6	0.403	47.0	14.770
Total bound residues (PES)	5.5	0.024	1.6	0.502
% Accountability ³	100		100	

¹ ND =Not detected. Ppm are reported in pethoxamid equivalents, and TRR values were calculated by summing extractable radioactivity (following solvent extraction and hydrolysis as applicable) and nonextractable radioactivity.

² For 8-day PHI whole plants, in addition to the listed compounds, which were identified and quantitated following partitioning of the combined ACN/water extract with ethyl acetate, the following compounds were tentatively identified but not quantitated following acid hydrolysis of the combined ACN/water extract: benzoic acid and N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)oxamic acid (MET-31), and putative structures were provided for two additional metabolites

³ Total (ppm)/TRR (ppm)×100

Corn**Table C.2. Summary of Characterization and Identification of Radioactive Residues in Corn Matrices Following Application of [¹⁴C]Pethoxamid at 1.1 lb ai/A (1.2 kg ai/ha).¹**

Compound ²	Preemergence app.		Foliar application					
	Mature leaves (106-day PHI)		Immature whole plants ² (8-day PHI)		Immature leaves (33-day PHI)		Mature leaves (78-day PHI)	
	TRR = 0.111 ppm		TRR = 6.600 ppm		TRR = 1.963 ppm		TRR = 0.550 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Pethoxamid	ND	--	1.0	0.066	ND	--	ND	--
TKC-94-γ-glutamyl cysteine (MET-130)	ND	--	4.8	0.317	ND	--	ND	--
TKC-94-cysteine (MET-30)	ND	--	7.5	0.498	ND	--	ND	--
TKC-94-N-malonyl cysteine (MET-47)	ND	--			ND	--	ND	--
TKC-94-thiol (MET-43)	ND	--	5.9	0.387	ND	--	ND	--
Unknown 1 (Rt ~41 min)	3.7	0.004	ND	--	3.5	0.068	3.4	0.019
Unknown 2 (Rt ~42 min)	5.2	0.006	ND	--	4.5	0.088	3.5	0.019
Unknown 3 (Rt ~47 min)	15.1	0.017	12.3	0.815	17.6	0.346	13.6	0.075
TKC-94-SO-lactic acid	NA	--	NA	--	8.0	0.158	NA	--
TKC-94-S-lactic glucoside (MET-48)					9.1	0.179		
Minor unknowns (2)					0.3	0.007		
Unknown 4 (Rt ~56 min)	1.3	0.001	ND	--	4.3	0.085	1.1	0.006
Unknown 5 (Rt ~28 min)	3.2	0.004	ND	--	ND	--	15.7	0.086
Unknown 6 (Rt ~17 min)	9.9	0.011	ND	--	ND	--	3.3	0.018
Unknown 7 (Rt ~20 min)	7.9	0.009	ND	--	ND	--	4.5	0.025
Unknown (Rt ~54 min)	ND	--	5.6	0.373	ND	--	ND	--
Multiple minor unknowns	41.2	0.045	57.7	3.806	56.6	1.110	34.9	0.192
HCl/NaOH hydrolysate(s)	--	--	4.1	0.274	10.9 ³	0.214	--	-
Total extractable	87.5	0.097	98.9	6.536	97.4	1.911	80.0	0.440
Total identified	--	--	19.2	1.268	17.1	0.337	--	--
Total unidentified	87.5	0.097	79.7	5.268	80.3	1.574	80.0	0.440
Total bound residues (PES)	12.6	0.014	1.0	0.064	2.6	0.052	20.0	0.110
% Accountability ⁴	100		100		100		100	

¹ ND = Not detected. NA = Not applicable; this component was not investigated in the subject matrix. Ppm are reported in pethoxamid equivalents, and TRR values were calculated by summing extractable radioactivity (following solvent extraction and hydrolysis as applicable) and nonextractable radioactivity.

² For 8-day PHI whole plants, in addition to the listed compounds, which were identified and quantitated following partitioning of the combined ACN/water extract with ethyl acetate, benzoic acid was identified at 33.7% and 59.4% TRR following acid hydrolysis of the combined ACN/water extract with 2 M and 6 M HCl, respectively, and metabolite MET-31 and its geometric isomer were tentatively identified in the 2 M HCl hydrolysate, together accounting for 16.2% TRR.

³ A small quantity of benzoic acid was identified but not quantitated in the 2 M HCl hydrolysate of immature leaves.

⁴ Total (ppm)/TRR (ppm)×100

Potato Foliage**Table C.3. Summary of Characterization and Identification of Radioactive Residues in Potato Foliage Following Foliar Application of [¹⁴C]Pethoxamid at 1.21 lb ai/A (1.36 kg ai/ha).¹**

Compound	Immature foliage (0-day PHI)		Immature foliage (37-day PHI)		Mature foliage (76-day PHI)	
	TRR = 18.102 ppm		TRR = 2.069 ppm		TRR = 1.521 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Pethoxamid	77.1	13.960	ND	--	ND	--
Pethoxamid cysteine conjugate (MET-30)	3.4	0.612	ND	--	ND	--
Pethoxamid-SO-cysteine (M42)	ND	--	10.0	0.207	ND	--
Pethoxamid-SO-lactic acid glycoside (M44)	ND	--	7.9	0.163	7.0	0.107
Region 2 (Rt 47.50 min)	11.0	1.996	ND	--	ND	--
Minor unknowns	6.5	1.173	75.5	1.562	80.7	1.228
Acid/base hydrolysates	1.9	0.349	4.6	0.096	9.1	0.138
Total extractable	98.0	17.741	93.4	1.933	87.8	1.335
Total identified	80.5	14.572	17.9	0.371	7.0	0.107
Total characterized	1.8	0.349	4.7	0.096	9.0	0.138
Total unidentified	17.5	3.169	75.5	1.562	80.7	1.228
Total bound residues (PES)	0.1	0.012	1.9	0.040	3.2	0.048
% Accountability ²	100		100		100	

¹ ND = Not detected. Ppm are reported in pethoxamid equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Total (ppm)/TRR (ppm)×100

Potato Tubers**Table C.4. Summary of Characterization and Identification of Radioactive Residues in Potato Tubers Following Foliar Application of [¹⁴C]Pethoxamid at 1.21 lb ai/A (1.36 kg ai/ha).¹**

Compound	Immature tuber (37-day PHI)		Mature tuber (76-day PHI)	
	TRR = 0.059 ppm		TRR = 0.013 ppm	
	% TRR	ppm	% TRR	ppm
Region 1 (Rt 4.25 min)	ND	--	15.4	0.002
Region 23 (Rt 45.75 min)	13.6	0.008	ND	--
Minor unknowns	73.2	0.043	61.6	0.008
Total extractable	86.4	0.051	76.9	0.010
Total identified	NA	--	NA	--
Total characterized	NA	--	NA	--
Total unidentified	86.4	0.051	76.9	0.010
Total bound residues (PES)	13.6	0.008	23.1	0.003
% Accountability ²	100		100	

¹ ND = Not detected. NA = Not applicable. Ppm are reported in pethoxamid equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Total (ppm)/TRR (ppm)×100

Goat**Table C.5. Summary of Characterization and Identification of Radioactive Residues in Goat Liver, Kidney, and Urine Following Dosing with [¹⁴C]Pethoxamid at 9.4 ppm.¹**

Compound/Fraction	Liver		Kidney		Urine (Days 1-5)	
	TRR = 0.555 ppm		TRR = 0.147 ppm		70.15% AD	
	% TRR	ppm	% TRR	ppm	%TRR	%AD
MET-30 (TKC-94-cysteine; GL13/GU16)	21.6	0.120	ND	--	14.7	10.3
MET-1 glucuronide conjugate (GL12/GU13&GU14)	10.9	0.060	ND	--	39.3	27.6
Region GL1/GK1/GU1 (TLC origin)	5.3	0.029	16.5 ²	0.024	0.7	0.5
Region GL3/GK2/GU3 (polar)	3.4	0.019	10.7 ²	0.016	2.0	1.4
Region GL4/GK3/GU4 (polar)	3.7	0.021	12.1 ²	0.018	0.9	0.6
Region GL6/GK5/GU6	5.9	0.033	16.1 ²	0.024	2.1	1.5
Region GL7/GK6/GU7	6.8 ³	0.038	1.7	0.002	1.0	0.7
Region GL9/GK9/GU10	18.4 ⁴	0.102	11.2 ²	0.016	3.7	2.6
Region GL10/GU11			ND	--	8.5	6.0
Region GL11/GK10/GU12	1.9 ⁵	0.011	5.3 ⁵	0.008	2.8	2.0
Minor unknowns ⁶	9.2	0.052	12.8	0.020	22.1	15.4
Non-specific radioactivity	6.4	0.036	6.3	0.009	2.2	1.5
0.1 M NaOH hydrolysate	1.2	0.007	2.7	0.004	NA	--
Total extractable	94.7	0.526	95.4	0.140	NA	--
Total identified	32.5	0.180	NA	--	54.0	37.9
Total unidentified	61.0	0.271	90.1	0.133	46.0	32.2
Total bound residues (PES)	5.4	0.030	4.5	0.007	NA	--
% Accountability ⁷	100.1		99.9		100.0	

¹ ND = Not detected; NA = not applicable. Ppm are reported in pethoxamid equivalents, and TRR values were normalized by the study author based on recovery.

² Acid hydrolysis (0.5 M HCl, 37 °C, 18 hr) of the kidney protease/ACN extract substantially decreased the levels of GK1 (5.2% TRR), GK2 (8.6% TRR), GK3 (2.1% TRR), and GK5 (8.7% TRR), and increased the levels of less polar components, GK4 (18.4% TRR, 0.027 ppm) and GK9 (13.0% TRR, 0.019 ppm).

³ Tentatively identified as MI-E in liver, but identification could not be confirmed.

⁴ Found to be comprised of six components, each accounting for ≤4.8% TRR (≤0.027 ppm) in liver; no quantitative data were provided for urine.

⁵ Tentatively identified as MET-32 in liver and kidney, but identification could not be confirmed.

⁶ Comprised of minor single or multicomponent unknowns each ≤2.4% TRR in liver, ≤4.5% TRR in kidney, and ≤4.9% TRR in urine.

⁷ Total (ppm)/TRR (ppm)×100.

Poultry

Table C.6. Summary of Characterization and Identification of Radioactive Residues in Poultry Eggs and Tissues Following Dosing with [¹⁴C]Pethoxamid at 16.6 ppm.¹

Compound/Fraction	Liver		Eggs (Day 8)		Breast Muscle		Thigh muscle		Abdominal fat		Subcutaneous fat	
	TRR = 0.268 ppm		TRR = 0.043 ppm		TRR = 0.020 ppm		TRR = 0.027 ppm		TRR = 0.032 ppm		TRR = 0.053 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	%TRR	ppm	%TRR	ppm
Pethoxamid	0.4	0.001	1.7	0.001	ND	--	ND	--	2.7	0.001	7.3	0.004
MET-42	3.4	0.009	8.2	0.004	2.7	0.001	3.7	0.001	ND	--	ND	--
MET-3	2.7	0.007	2.8	0.001	3.8	0.001	ND	--	ND	--	ND	--
MET-103			6.9	0.003	ND	--	ND	--	ND	--	ND	--
MET-30	0.4	0.001	3.3	0.001	ND	--	ND	--	ND	--	ND	--
MET-47	3.4	0.009	3.9	0.002	ND	--	ND	--	ND	--	ND	--
MET-2	0.5	0.001	1.4	0.001	ND	--	ND	--	3.1	0.001	3.4	0.002
Minor unknowns ²	35.9	0.095	39.1	0.017	33.5	0.006	30.3	0.008	28.6	0.010	15.6	0.008
0.1 M HCl hydrolysate	NA ³	--	2.3	0.001	5.0	0.001	3.7	0.001	18.8	0.006	17.0	0.009
0.2 M KOH hydrolysate			7.0	0.003	25.0	0.005	22.2	0.006	18.8	0.006	24.5	0.013
Precipitate ⁴	15.3	0.041	NA	--	NA	--	NA	--	NA	--	NA	--
Protease soluble ⁴	17.2	0.046	18.6	0.008	NA	--	29.6	0.008	NA	--	5.7	0.003
Buffer soluble	3.7	0.010	<0.1	<0.001	NA	--	<0.1	<0.001	NA	--	11.3	0.006
Aqueous-soluble	5.2	0.014	NA	--	NA	--	NA	--	NA	--	NA	--
Organosoluble (EtOAc/hexane) ⁵	11.2	0.030	NA	--	NA	--	NA	--	12.5	0.004	11.3	0.006
Total extractable	99.3	0.266	95.3	0.041	70.0	0.014	88.8	0.024	84.5	0.027	96.2	0.051
Total identified	10.8	0.028	28.2	0.013	6.5	0.002	3.7	0.001	5.8	0.002	10.7	0.006
Total characterized	42.6	0.114	18.6	0.008	NA	--	29.6	0.008	NA	--	5.7	0.003
Total unidentified	45.9	0.122	48.4	0.021	63.5	0.012	56.2	0.015	78.7	0.026	79.7	0.042
Total bound residues (PES)	0.7	0.002	4.7	0.002	30.0	0.006	11.1	0.003	15.6	0.005	3.8	0.002
% Accountability ⁶	100		100		100		100.6		100.1		100	

¹ ND = Not detected; NA = not applicable. Ppm are reported in pethoxamid equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Comprised of minor single or multicomponent HPLC unknowns, with each single component present at: ≤5.6% TRR (≤0.015 ppm) in liver; ≤8.6% TRR (≤0.004 ppm) in eggs; ≤14.0% TRR (≤0.003 ppm) in breast muscle; ≤7.4% TRR (≤0.002 ppm) in thigh muscle; ≤21.1% TRR (≤0.007 ppm) in abdominal fat; and ≤5.3% TRR (≤0.003 ppm) in subcutaneous fat.

³ Combined and subjected to partitioning with ethyl acetate which yielded aqueous and organosoluble fractions and the precipitate below.

⁴ Characterized as associated with or likely associated with protein.

⁵ Including 10.1% TRR (0.027 ppm) in liver characterized as being associated with fatty acid-related components.

⁶ Total (ppm)/TRR (ppm)×100.

*Turnip (Rotational Crops)***Table C.7. Summary of Characterization and Identification of Radioactive Residues in Rotational Turnip Tops From a 30-day PBI Following Application of [phenyl-U-¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹**

Compound	54 DAP First harvest		79 DAP Second harvest		105 DAP Third harvest	
	TRR = 0.58 ppm		TRR = 0.62 ppm		TRR = 0.50 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
MET-30	4.5	0.026	7.5	0.047	ND	--
MET-41	2.9	0.017	1.2	0.007	1.2	0.006
MET-42	14.2	0.082	21.0	0.130	26.4	0.132
MET-48	ND	--	ND	--	4.1	0.021
MET-49	ND	--	6.0	0.037	4.8	0.024
MET-50	15.3	0.089	12.1	0.075	17.7	0.089
Minor unknowns ²	58.3	0.336	43.7	0.270	39.83	0.201
Total extractable	95.2	0.55	91.5	0.57	94.0	0.47
Total identified	36.9	0.214	47.8	0.296	54.2	0.272
Total unidentified	58.3	0.336	43.7	0.270	39.8	0.201
Total bound residues (PES)	4.9	0.028	8.5	0.053	6.0	0.030
% Accountability ³	100.1		100.0		100.0	

¹ ND = Not detected. Ppm are reported in pethoxamid equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Single or multicomponent unknowns with each component present at <10% TRR, except for one unknown in 105-DAP tops (P5: 11.5% TRR, 0.058 ppm).

³ Total (ppm)/TRR (ppm)×100

*Spinach (Rotational Crops)***Table C.8. Summary of Characterization and Identification of Radioactive Residues in Rotational Spinach From a 30-day PBI Following Application of [phenyl-U-¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹**

Compound	47 DAP First harvest		70 DAP Second harvest		98 DAP Third harvest	
	TRR = 0.79 ppm		TRR = 0.51 ppm		TRR = 0.41 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
MET-30	3.4	0.027	7.5	0.038	6.0	0.025
MET-41	1.7	0.013	3.1	0.016	2.0	0.008
MET-42	9.1	0.072	7.7	0.039	7.9	0.032
MET-48	7.0	0.055	8.6	0.044	8.2	0.034
MET-49	5.5	0.043	4.6	0.023	2.1	0.009
MET-50	12.2	0.096	10.3	0.053	4.5	0.018
Minor unknowns ²	54.9	0.434	48.8	0.249	63.8	0.262
Aqueous extractable	NA	--	NA	--	1.8	0.007
Enzyme extractable	NA	--	NA	--	5.6	0.023
Total extractable	93.8	0.74	94.7	0.48	95.1	0.39
Total identified	38.9	0.306	41.8	0.213	30.7	0.126
Total characterized	NA	--	NA	--	5.6	0.023
Total unidentified	54.9	0.434	48.8	0.249	65.6	0.269
Total bound residues (PES)	6.3	0.050	5.3	0.027	4.9	0.020
% Accountability ³	100.1		100.0		100.0	

¹ ND = Not detected. NA = Not applicable. Ppm are reported in pethoxamid equivalents, and TRR values were calculated by

summing extractable and nonextractable residues.

² Single or multicomponent unknowns with each component present at <10% TRR, with exception of one unknown in 47-DAP spinach (P19: 14.9% TRR, 0.118 ppm), one unknown in 70-DAP spinach (P5: 11.9% TRR, 0.061 ppm), and one unknown in 98-DAP spinach (P5: 12.3% TRR, 0.050 ppm).

³ Total (ppm)/TRR (ppm)×100

Wheat Forage (Rotational Crops)

Table C.9. Summary of Characterization and Identification of Radioactive Residues in Rotational Wheat Forage and Straw From a 30-day PBI Following Application of [phenyl-U-¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹

Compound	Wheat forage		Wheat straw			
	70 DAP First harvest		126 DAP Second harvest		146 DAP Third harvest	
	TRR = 0.48 ppm		TRR = 0.61 ppm		TRR = 1.79 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
MET-30	11.3	0.054	4.3	0.026	6.1	0.109
MET-41	3.6	0.017	2.8	0.017	2.4	0.043
MET-42	14.2	0.068	9.9	0.060	12.9	0.231
MET-48	3.6	0.017	ND	--	ND	<0.002
MET-49	2.6	0.012	ND	--	1.3	0.023
MET-50	7.5	0.036	4.8	0.029	5.5	0.098
Minor unknowns ²	47.7	0.227	49.3	0.300	40.7	0.730
Aqueous extractable	NA	--	NA	--	12.5	0.224
Enzyme extractable	NA	--	NA	--	7.1	0.127
Post enzyme extracts/hydrolysates	NA	--	NA	--	5.0	0.090
Total extractable	90.5	0.43	71.1	0.43	93.5	1.66
Total identified	42.8	0.204	21.8	0.132	28.2	0.504
Total characterized	NA	--	NA	--	7.1	0.127
Total unidentified	47.7	0.227	49.3	0.300	53.2	0.954
Total bound residues (PES)	9.6	0.046	28.9	0.18	6.5	0.116
% Accountability ³	100.1		100.0		100.0	

¹ ND = Not detected. NA = Not applicable. PPM are reported in pethoxamid equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Single or multicomponent unknowns with each component present at <10% TRR, except for one unknown in 126-DAP wheat straw (P19: 15.8% TRR, 0.096 ppm).

³ Total (ppm)/TRR (ppm)×100

Appendix D. Field Trial Geographic Distribution

Corn

Table D.1. Trial Numbers and Geographical Locations.																
Crop	No. Trials	NAFTA Growing Zone														Total
		1	2	3	4	5	5B	6	7	7A	8	9	10	11	12	
Field corn	Sub.	1	1			13 ¹		1			1 ²					17
	U.S. Req. ³	1/1	1/1			17/12		1/1								20/15
	Can. Req. ⁴					9	7									16
Popcorn	Sub.					3										3
	U.S. Req. ⁵															3
	Can. Req. ⁴					3										3
Sweet corn	Sub.	2	1	1		7			1 ⁶				1	1	1	15
	U.S. Req. ³	2/1	1/1	1/1		5/3							1/1	1/1	1/1	12/9
	Can. Req. ⁴					4	3			1						8

¹ HED has determined that four pairs of trials conducted in Zone 5 are each replicates.

² The trial conducted in Zone 8 was located near the border with Zone 6.

³ As per Table 5 of 860.1500 for field and sweet corn; the second number reflects a 25% reduction in the number of field trials allowed for the crop as a representative commodity in support of a crop group/subgroup tolerance as applicable (refer to Tables 2 and 3) or when application results in no quantifiable residues.

⁴ As per Table 1.2 of PMRA Regulatory Directive DIR2010-05 for corn, grain; corn, pop; and corn, sweet.

⁵ As per Table 1 of 860.1500 for popcorn. Field trial locations are not specified for crops requiring ≤3 trials; however, 91% of the U.S. production of popcorn occurs in Zone 5 (Table 6 of 860.1500).

⁶ The trial conducted in Zone 7 was irrigated to represent Zone 7A.

Soybean

Table D.2. Trial Numbers and Geographical Locations.																
Crop	No. Trials	NAFTA Growing Zone														Total
		1	2	3	4	5	5B	6	7	8	9	10	11	12		
Soybean	Sub.		2		3	13 ¹									18	
	U.S. Req. ²		2/2		3/2	15/11									20/15	
	Can. Req. ³					13	3								16	

¹ HED has determined that three pairs of trials conducted in Zone 5 are each replicates.

² As per Table 5 of 860.1500 for soybean; the second number reflects a 25% reduction in the number of field trials allowed for the crop as a representative commodity in support of a crop group/subgroup tolerance as applicable (refer to Tables 2 and 3) or when application results in no quantifiable residues.

³ As per Table 1.2 of PMRA Regulatory Directive DIR2010-05 for soybean.

Cotton

Table D.3. Trial Numbers and Geographical Locations.														
Crop	No. Trials	NAFTA Growing Zone												Total
		1	2	3	4	5	6	7	8	9	10	11	12	
Cotton	Sub.		1		3		2 ¹		3		2 ²			11
	Req. ³		1/1		3/2		1/1		4/3		3/2			12/9

¹ The study author noted that one trial conducted in Zone 6 was ~25 miles from the border between Zones 6 and 8 and intended to represent both zones.

² HED has determined that two trials conducted in Zone 10 are replicates.

³ As per Table 5 of 860.1500 for cotton; the second number reflects a 25% reduction in the number of field trials allowed for the crop as a representative commodity in support of a crop group/subgroup tolerance as applicable (refer to Tables 2 and 3) or when application results in no quantifiable residues. Per OCSPP 860.1000 Supplement (6/30/08), for cotton gin byproducts, cotton must be harvested by commercial equipment to provide an adequate representation of plant residue for the ginning

process; three field trials for harvesting of stripper cotton are needed (no data are needed for picker cotton).

Appendix E. International Residue Limits Table

Residue Definition:					
US		Canada ¹	Mexico ²	Codex	Other ³
40 CFR 180.xxx Plants and Livestock: pethoxamid, 2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide		Plants and Livestock: pethoxamid, 2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide		None	
Commodity ^l	Tolerance (ppm) /Maximum Residue Limit (mg/kg)				
	US	Canada	Mexico	Codex	EU
Corn, field, forage	0.01	--			
Corn, field, stover	0.01	--			
Corn, field, grain	0.01	0.01			0.01
Corn, pop, stover	0.01	--			
Corn, pop, grain	0.01	0.01			
Corn, sweet, forage	0.01	--			
Corn, sweet, stover	0.01	--			
Corn, sweet, kernel plus cob with husk removed	0.01	0.01			0.01
Cotton, undelinted seed	0.01	0.01			0.01
Cotton, gin byproducts	0.01	--			
Soybean, forage	0.01	--			
Soybean, hay	0.01	--			
Soybean, seed	0.01	0.01			0.01
Milk	0.01	0.01			0.01
Eggs	0.01	0.01			
Cattle, fat	0.01	0.01			0.01
Cattle, meat	0.01	0.01			
Cattle, meat byproducts	0.01	0.01			
Goat, fat	0.01	0.01			0.01
Goat, meat	0.01	0.01			
Goat, meat byproducts	0.01	0.01			
Hog, fat	0.01	0.01			0.01
Hog, meat	0.01	0.01			
Hog, meat byproducts	0.01	0.01			
Horse, fat	0.01	0.01			
Horse, meat	0.01	0.01			
Horse, meat byproducts	0.01	0.01			
Poultry, fat	0.01	0.01			0.01
Poultry, meat	0.01	0.01			
Poultry, meat byproducts	0.01	0.01			
Sheep, fat	0.01	0.01			0.01
Sheep, meat	0.01	0.01			
Sheep, meat byproducts	0.01	0.01			
Completed by: Sheila Piper 2/4/2019					

¹ Canadian MRLs are being proposed concurrently with the U.S. tolerances and are harmonized for primary crop and livestock commodities. For livestock feed stuffs, disharmony is caused since Canada does not establish MRLs on feed stuffs.

² Mexico adopts US tolerances and/or Codex MRLs for its export purposes.

³ Tolerances are established for the European Commission and the European Food Safety Authority (EFSA; 8/2017) for plants and livestock commodities. For tolerance enforcement, the parent, pethoxamid is the residue of concern in plant commodities. In livestock commodities, a residue definition can currently not be set since the toxicological relevance of a major metabolite in livestock products (MET-30) would need to be addressed.

Appendix F. MRIDs

OCSPP 860 Series Guideline	MRID Number	Title
860.1300	49813527	Nature of the Residue in Plants- Soybean
860.1300	49813528	Nature of the Residue in Plants- Maize
860.1300	49813529	Nature of the Residue in Plants- Potato
860.1300 860.1380	49813530	Nature of the Residue in Livestock- Hen Storage Stability-Hen
860.1300	49813531	Nature of the Residue in Livestock- Ruminant
860.1340	49813534	Residue Analytical Method- Plants (Development and Validation)
860.1340	49813535	Residue Analytical Method- Animal
860.1340	49813536	ILV-Residue Analytical Method- Animal
860.1340	49813537	Independent Laboratory Validation Method
860.1340	49813538	Residue Analytical Method Validation (Waiver Request)
860.1360	49813539	Multiresidue Method
860.1380	49813540	Storage Stability-Crops
860.1380	49813541	Storage Stability-Crops
860.1380	49813542	Storage Stability-Maize
860.1380	49813543	Storage Stability-Maize
860.1380	49813544	Storage Stability-Soybeans
860.1380	49813545	Storage Stability-Soybeans
860.1500 860.1520	49813546	Magnitude of the Residue in RAC-Corn (field, sweet and popcorn) Magnitude of the Residue in Processed Food/Feed-Corn (field, sweet and popcorn)
860.1500 860.1520	49813547	Magnitude of the Residue in RAC-Soybeans Magnitude of the Residue in Processed Food/Feed-Soybean
860.1500 860.1520	49813548	Magnitude of the Residue in RAC-Cotton Magnitude of the Residue in Processed Food/Feed-Cotton
860.1850	49813552	Confined Rotational Crop
860.1900	49813553	Field Accumulation in Confined Rotational Crops

DATA EVALUATION RECORD

PETHOXAMID

Study Type: OCSPP 860.1340, Residue Analytical Method

Study Type: OCSPP 860.1360, Multiresidue Method

EPA Contract No. EP-W-16-018

Task Assignment Form No. 21-2-009 (MRIDs 49813534-49813538)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by



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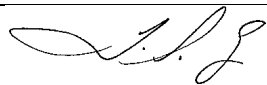
Primary Reviewer:
Kelly Luck, M.Sc.

Signature: 
Date: 2/2/18

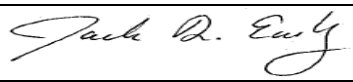
Secondary Reviewer:
Sandra Hastings

Signature: 
Date: 2/6/18

Quality Assurance:
Tracy S. Lang, M.S.

Signature: 
Date: 2/20/18

Project Manager:
Jack D. Early, M.S.

Signature: 
Date: 2/21/18

Volume 3

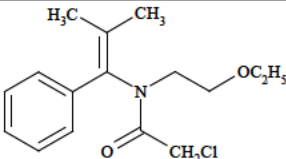
Annex B

PETHOXAMID

B.5 METHODS OF ANALYSIS

B.5.2 ANALYTICAL METHODS FOR THE DETERMINATION OF RESIDUES (ALL COMPONENTS INCLUDED IN THE RESIDUE DEFINITION PROPOSED)

Table B.5.2-1. Overview of the Analytical Methods for the Determination of Pethoxamid Residues.					
Data Requirement	Matrix	Analytes	Method Type [Method ID]	Limit of Quantitation (ppm)	Reference
Enforcement Method - Plant Commodities	Crop commodities	Pethoxamid	LC/MS/MS [AGR/MOA/PTX-8]	0.01	Study No. S13-00341 MRID 49813538 PMRA# Not available Study No. 2793 MRID 49813534 PMRA# Not available
Data-Gathering Method - Plant Commodities	No study relevant to this data requirement was submitted under the current task.				
ILV of Enforcement Method - Plant Commodities	Head lettuce and rape seed	Pethoxamid	LC/MS/MS [AGR/MOA/PTX-8]	0.01	Study No. S13-03972 MRID 49813537 PMRA# Not available
Radiovalidation of Methods - Plant Commodities	No study relevant to this data requirement was submitted under the current task.		N/A	N/A	
Enforcement Method- Livestock Commodities	Eggs, milk, fat, kidney, liver, and muscle	Pethoxamid	LC/MS/MS [S15-03519]	0.01	Study No. S15-03519 MRID 49813535 PMRA# Not available
Data-Gathering Method - Livestock Commodities	No study relevant to this data requirement was submitted under the current task.				
ILV of Enforcement Method - Livestock Commodities	Milk, liver, and muscle	Pethoxamid	LC/MS/MS [S15-03519]	0.01	Study No. S15-03520 MRID 49813536 PMRA# Not available
Radiovalidation of Methods - Livestock Commodities	No study relevant to this data requirement was submitted under the current task.				
Multiresidue Method Testing	No data pertaining to FDA multiresidue methods were submitted under the current task.		FDA-Pesticide Analytical Manual Multiresidue Methods		

Table B.5.2-2. Chemical Structure of Analyte Addressed by Methods for the Determination of Pethoxamid.		
Method ID	Chemical Name	Chemical Structure
Plant method: AGR/MOA/PTX-8 Livestock method: S15-03519	Common name: Pethoxamid Chemical name: 2-chloro-N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propen-1-yl)acetamide	

B.5.2.1 Analytical Methods for Plant Matrices (Annex IIA 4.3, Annex IIIA 5.3)

B.5.2.1.1 Post-Registration Method (Enforcement)

Document ID: MRID Nos. 49813534, 49813537, and 49813538
PMRA No. Not available

Report: Sears, K. (2015) Method Validation and 10-Month Storage Stability of Pethoxamid in Various Crops. Study Numbers: 1495 PXA; 2793. Unpublished study prepared by Pyxant Labs Inc., sponsored by Cheminova A/S, and submitted by FMC Corporation. 600 p. (MRID 49813534)

Winter, O. and Winkler, K. (2013) Independent Laboratory Validation of an Analytical Method for the Determination of Pethoxamid Residues in Lettuce (Head) and Rape Seed (Seed). Study Numbers: 968 PXA; S13-03972; CHE-1301V; G13-0222. Unpublished study prepared by Eurofins Agroscience Services Chem GmbH, sponsored by Cheminova A/S, and submitted by FMC Corporation. 45 p. (MRID 49813537)

Tessier, V. (2013) Validation of an Analytical Method for the Determination of Pethoxamid Residues in Various Crops. Study Numbers: 741 PXA; S13-00341. Unpublished study prepared by Eurofins Agroscience Services Chem SAS, sponsored by Cheminova A/S, and submitted by FMC Corporation. 74 p. (MRID 49813538)

Guidelines: EPA OCSPP Harmonized Test Guideline 860.1340 Residue Analytical Method (August 1996)
PMRA Regulatory Directive DIR98-02 – Residue Chemistry Guidelines, Section 3 – Residue Analytical Method
EU SANCO 825/00/rev. 7 (17/3/04)
OECD Guidance Document on Pesticide Residue Analytical Methods

GLP Compliance: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Acceptability: The study is considered scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP#452109.

Evaluator: Sheila Piper, Chemist, HED, RAB6 *Sheila Piper*

Secondary Evaluator: Robert Lanthier, PMRA

Note: This Data Evaluation Record (DER) was prepared under contract by CDM/CSS-Dynamac Joint Venture (submitted 2/21/18). The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies. The DER has been reviewed by HED and revised as necessary to reflect current Office of Pesticide Programs (OPP) policies.

EXECUTIVE SUMMARY

Method No. AGR/MOA/PTX-8, is being proposed for analysis of pethoxamid in/on maize grain; lettuce; rape seed; orange; field corn forage, grain, and stover; corn meal, flour, and refined oil; sweet corn K+CWHR; cotton undelinted seed, gin byproducts, meal, and hulls; and soybean forage, hay, seed, meal, and hulls commodities for purposes of regulatory enforcement. A method description and method validation data (MRIDs 49813534 and 49813538), and an independent laboratory validation (ILV; MRID 49813537) for a high performance liquid chromatography method with tandem mass spectrometry detection (LC/MS/MS), Eurofins Agrosience Services (EAS) Method No. AGR/MOA/PTX-8, for determination of residues of pethoxamid in crop commodities. The extraction and analysis procedures are based on the QuEChERS method. EAS Method No. AGR/MOA/PTX-8 was used for data collection in the submitted storage stability and crop field trial studies (refer to B.7.6.2.1 and B.7.6.1). The ILV was conducted by Eurofins Agrosience Services Chem GmbH (Hamburg, Germany). MRID 49813534 also includes storage stability data for ten corn, cotton, and soybean commodities, including processed commodities (refer to the B.7.6.2.1 DER for MRID 49813534).

Briefly, samples are extracted with acetonitrile (ACN). QuEChERS extraction salts (magnesium sulfate:sodium chloride:sodium citrate tribasic dihydrate:sodium citrate dibasic sesquihydrate; 4:1:1:0.5, w:w:w:w) are added, and the samples are shaken and centrifuged. A portion of the supernatant is purified by the addition of cleanup salts (primary secondary amine:C18EC sorbent:magnesium sulfate; 1:1:6, w:w:w). Samples are shaken and centrifuged, and an aliquot of the supernatant is diluted with water for analysis by LC/MS/MS, conducted using a reverse-phase C18 column and a gradient mobile phase of ACN and water containing 0.2% acetic acid. The following ion transitions are monitored: m/z 296.2 $\rightarrow$ 131.2 (quantitation) and m/z 296.2 $\rightarrow$ 250.2 (confirmation). The limit of quantitation (LOQ; determined as the lowest level of method validation, LLMV) is 0.01 ppm.

Method validation was conducted at 0.01 and 0.10 ppm using maize grain, lettuce, rape seed, and orange fruit, as well as at 0.01, 0.10 and 1.0 ppm using the following commodities: field corn forage, grain, and stover; corn meal, flour, and refined oil; sweet corn kernel plus cob with husk removed (K+CWHR); cotton undelinted seed, gin byproducts, meal, and hulls; and soybean forage, hay, seed, meal, and hulls. Recovery was measured at both the quantitation and confirmation ion transition for all matrices and fortification levels. At the 0.01-ppm level, the mean recoveries ranged 80-119%, with relative standard deviations (RSDs) within the range of 1.7-18%. At the 0.10-ppm level, the mean recoveries ranged 77-108%, and RSDs ranged 1.0-15%, and at the 1.0-ppm level, mean recoveries ranged 88.4-118%, with RSDs of 0.43-17%.

A successful ILV was conducted using samples of head lettuce and rape seed fortified at 0.01 and 0.10 ppm. The ILV followed method procedures as currently written. Individual recoveries were within the acceptable range of 70-120%. Mean recoveries were within the acceptable limits, and relative standard deviations (RSDs) were <5%. The submission did not include much of the information required for ILVs (e.g., the number of trials required for successful validation, the need for communication with the sponsor, etc.); however, because the method is based on the QuEChERS method, this information is not required.

No radiovalidation data were submitted. Because the extraction solvents used in the plant metabolism studies (refer to B.7.1.1-B.7.1.3) are very similar to those used in the proposed enforcement method, no radiovalidation data will be required for crop commodities.

Storage stability studies conducted with the method validation and ILV studies demonstrated that pethoxamid residues are stable in the final extracts of maize grain, lettuce, rape seed, and orange fruit during at least 7 days of refrigerated storage.

The effect of matrix was also evaluated with the method validation and ILV studies. For the method validation, the data showed that there was no matrix effect associated with maize grain, lettuce, rape seed, or orange; therefore, solvent calibration standards were used for analysis. However, the ILV laboratory identified a potential matrix effect with rape seed; matrix-matched standards were used in the ILV for both the lettuce and rape seed analyses.

EAS Method No. AGR/MOA/PTX-8 is considered suitable for enforcement purposes.

I. Principle of the Method: EAS Method No. AGR/MOA/PTX-8

Table B.5.2.1.1-1. Summary Parameters for the Post-Registration Analytical Method for the Determination of Pethoxamid Residues in Crop Matrices.	
Method ID	AGR/MOA/PTX-8
Analyte	Pethoxamid
Extraction solvent and technique	Samples are extracted with ACN by shaking with vortex. QuEChERS extraction salts (magnesium sulfate:sodium chloride:sodium citrate tribasic dihydrate:sodium citrate dibasic sesquihydrate; 4:1:1:0.5, w:w:w:w; dispersive SPE citrate extraction tube) are added, and the samples are shaken, vortexed, and centrifuged.
Clean-up strategies	A portion of the supernatant is purified by the addition of cleanup salts (primary secondary amine:C18EC sorbent magnesium sulfate; 1:1:6, w:w:w; SampliQ Dispersive SPE, fatty samples). Samples are shaken, vortexed, and centrifuged, and an aliquot of the supernatant is diluted with water for analysis by LC/MS/MS.
Instrument and detector	LC/MS/MS using an API 4000 mass spectrometer with a reverse-phase column (ACE5 C18) and a gradient mobile phase of ACN and water containing 0.2% acetic acid. The following ion transitions are monitored: m/z 296.2 $\rightarrow$ 131.2 (quantitation) and m/z 296.2 $\rightarrow$ 250.2 (confirmation).
Standardization method	External standardization using a calibration curve of pethoxamid standards at seven concentrations over the range 0.1-10 ng/mL, with a coefficient of determination (r^2) of ≥ 0.990 .
Stability of standard solutions	Stock solutions in ACN:water (9:1, v:v) are to be stored in the dark at 4 °C and prepared every 115 days. Calibration and fortification solutions are to be freshly prepared. The stability of pethoxamid in the final extracts of samples during storage at 4-5 °C for 7-8 days was demonstrated during method validation and ILV (see below).
Retention time	~2.0-2.1 minutes

II. Specificity

The LC/MS/MS method is highly selective for both the quantitation and confirmation of pethoxamid. Analysis of control samples resulted in no significant signals at the expected retention times of the analyte. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of the analyte:

Pethoxamid m/z 296.2 $\rightarrow$ 131.2 (quantitation)
 m/z 296.2 $\rightarrow$ 250.2 (confirmation)

III. Linearity

The linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting. For the least-squares equation which describes the detector response as a function of the standard concentration, calibration curves resulting from the injection of a minimum of seven standards over the concentration range of 0.1-10.0 ng/mL demonstrated linearity, with coefficients of determination (r^2) of at least 0.9853.

IV. Accuracy (Recovery) and Precision (Repeatability)

Radiovalidation. No data addressing the ability of EAS Method No. AGR/MOA/PTX-8 to extract incurred residues were submitted. In the submitted metabolism studies with corn, potato, and soybean (refer to DERs for MRIDs 49813527-49813529; B.7.1.1-B.7.1.3), the majority of radioactivity was extracted from treated immature plants and leaves using ACN and water. No radioactivity was released from corn grain or cobs using ACN, however, these matrices had very low radioactivity levels. For soybean beans and pods, initial ACN extraction did not release significant amounts of radioactivity. Sequential extraction with water released the majority of residues; however, no pethoxamid was found in these extracts. Because the extraction solvents used in the metabolism studies are very similar to those used in the proposed enforcement method, no radiovalidation data will be required for crop commodities.

Method validation. Method validation data were provided in two submissions, MRIDs 49813534 and 49813538. In MRID 49813538, the method was validated by Eurofins Agrosience Services Chem SAS (Vergèze, France) at 0.01 ppm (LOQ) and 0.1 ppm (10xLOQ) in maize grain, lettuce, rape seed, and orange fruit. In MRID 49813534, the method was validated by Pyxant Labs Inc. (Colorado Springs, CO) at 0.01, 0.10 and 1.0 ppm in the following commodities: field corn forage, grain, and stover; corn meal, flour, and refined oil; sweet corn K+CWHR; cotton undelinted seed, gin byproducts, meal, and hulls; and soybean forage, hay, seed, meal, and hulls. In both studies, five samples were fortified with pethoxamid at each fortification level in each matrix, and samples were quantified using both transition ions.

Recovery and repeatability data are presented in Table B.5.2.1.1-2. The individual recoveries were within the range of 67.3-146%. Although one low recovery was observed at the 1.0-ppm fortification level from sweet corn K+CWHR (67.3%) and several high recoveries (121-146%) were observed from field corn stover (1), sweet corn K+CWHR (3), undelinted cotton seed (1), soybean hay (1), and soybean seed (2) fortified at 0.01 ppm, and from undelinted cotton seed fortified at 0.10 ppm, mean recoveries were >70% and RSDs were <20%. At the 0.01-ppm level (LOQ), the mean recoveries ranged 80-119%, with RSDs within the range of 1.7-18%. At the 0.10-ppm level (10xLOQ), the mean recoveries ranged 77-108%, and RSDs ranged 1.0-15%, and at the 1.0-ppm level (100xLOQ), mean recoveries ranged 88.4-118%, with RSDs of 0.43-17%.

As part of the method validation conducted by Eurofins Agrosience Services Chem SAS, the effects of matrix were evaluated. Pethoxamid standards prepared in maize grain, lettuce, rape seed, and orange fruit matrices were analyzed and peak areas were compared to the peak area for a solvent standard at the same concentration (the concentration tested was not reported). The percent differences between the matrix standard and the solvent standard ranged from -0.3 to

8.9%. Based on these results, the laboratory concluded that matrix-matched standards were not required for analysis of maize grain, lettuce, rape seed, and orange fruit samples.

Matrix effects were not evaluated as part of the validation conducted by Pyxant Labs. Solvent calibration standards were used for all analyses.

The stability of pethoxamid in sample final extracts during refrigerated storage was evaluated as part of the method validation conducted by Eurofins Agrosience Services Chem SAS. The final extracts of maize grain, lettuce, rape seed, and orange fruit samples fortified at 0.01 ppm were stored at 4 °C for 7-8 days and reanalyzed, using the quantitation ion transition. After storage, mean recoveries were 84% for maize grain, 85% for lettuce, 84% for rape seed, and 84% for orange fruit. These values compare favorably with the 0-day mean recoveries (reported in Table B.5.2.1.1-2), indicating stability of extracts for at least 7 days of refrigerated storage.

Table B.5.2.1.1-2. Accuracy and Precision Data for the Validation of EAS Method No. AGR/MOA/PTX-8 for the Determination of Pethoxamid Residues in Crop Commodities. ¹					
Matrix	Fortification Level (ppm)	Validation Recovery (%)			RSD (%)
		Individual	Mean	Range	
Pethoxamid: Quantitation ion transition: <i>m/z</i> 296.2 → 131.2					
Maize grain	0.01	85, 82, 78, 82, 81	82	78-85	3
	0.1	76, 78, 76, 79, 76	77	76-79	2
Lettuce	0.01	82, 84, 84, 92, 84	85	82-92	5
	0.1	89, 86, 88, 88, 87	88	86-89	1
Rape seed	0.01	83, 79, 80, 85, 79	81	79-85	3
	0.1	89, 84, 84, 89, 84	86	84-89	3
Orange fruit	0.01	85, 81, 83, 87, 82	84	81-87	3
	0.1	90, 86, 87, 90, 88	88	86-90	2
Field corn forage	0.01	106, 103, 103, 104, 107	105	103-107	1.7
	0.10	104, 106, 102, 102, 99.5	103	99.5-106	2.4
	1.0	114, 117, 118, 112, 112	115	112-118	2.4
Field corn grain	0.01	97.9, 93.3, 93.5, 94.9, 98.6	95.6	93.3-98.6	2.6
	0.10	95.6, 90.7, 93.5, 98.4, 93.2	94.3	90.7-98.4	3.1
	1.0	93.7, 91.6, 88.1, 88.6, 90.4	90.5	88.1-93.7	2.5
Field corn stover	0.01	121, 113, 120, 115, 108	115	108-120; 121	4.6
	0.10	89.3, 105, 101, 103, 108	101	89.3-108	7.1
	1.0	110, 103, 109, 110, 107	108	103-110	2.7
Corn meal	0.01	117, 104, 102, 102, 97.5	105	97.5-117	7.1
	0.10	101, 104, 102, 104, 104	103	101-104	1.4
	1.0	103, 106, 106, 104, 105	105	103-106	1.2
Corn flour	0.01	98.4, 103, 98.4, 99.4, 96.7	99.2	96.7-103	2.4
	0.10	98.8, 98.4, 96.0, 94.9, 97.8	97.2	94.9-98.8	1.7
	1.0	107, 107, 106, 105, 107	106	105-107	0.84
Corn refined oil	0.01	103, 102, 95.7, 99.7, 102	100	95.7-103	2.9
	0.10	96.2, 91.2, 98.7, 99.7, 98.9	96.9	91.2-99.7	3.6
	1.0	93.9, 92.9, 93.4, 93.8, 93.3	93.5	92.9-93.9	0.43
Sweet corn K+CWHR	0.01	99.5, 105, 99.8, 106, 132	108	99.5-106; 132	12
	0.10	99.9, 98.4, 101, 108, 110	103	98.4-110	5.0
	1.0	109, 71.2, 108, 105, 108	100	71.2-109	16
Cotton undelinted seed	0.01	87.2, 120, 88.7, 88.3, 123	101	87.2-120; 123	18
	0.10	121, 117, 89.3, 97.3, 101	105	89.3-117; 121	13
	1.0	115, 106, 101, 103, 104	106	101-115	5.2

Table B.5.2.1.1-2. Accuracy and Precision Data for the Validation of EAS Method No. AGR/MOA/PTX-8 for the Determination of Pethoxamid Residues in Crop Commodities.¹

Matrix	Fortification Level (ppm)	Validation Recovery (%)			RSD (%)
		Individual	Mean	Range	
Cotton gin byproducts	0.01	96.4, 95.7, 100, 106, 82.7	96.2	82.7-106	8.9
	0.10	91.7, 89.5, 96.2, 85.8, 88.9	90.4	85.8-96.2	4.3
	1.0	115, 102, 103, 105, 108	107	102-115	4.9
Cotton seed meal	0.01	117, 108, 115, 112, 117	114	108-117	3.4
	0.10	118, 108, 103, 103, 106	108	103-118	5.8
	1.0	98.2, 96.2, 101, 102, 98.3	99.1	96.2-102	2.4
Cotton seed hulls	0.01	110, 97.7, 86.4, 90.2, 117	100	86.4-117	13
	0.10	92.5, 89.9, 90.7, 91.6, 83.5	89.6	83.5-92.5	4.0
	1.0	95.3, 101, 103, 108, 99.2	101	95.3-108	4.6
Soybean forage	0.01	107, 108, 106, 98.9, 108	106	98.9-108	3.6
	0.10	87.5, 83.6, 87.0, 86.0, 86.3	86.1	83.6-87.5	1.7
	1.0	117, 118, 118, 118, 117	118	117-118	0.47
Soybean hay	0.01	113, 119, 107, 121, 118	116	107-119; 121	4.9
	0.10	105, 105, 103, 106, 100	104	100-106	2.3
	1.0	102, 107, 107, 102, 106	105	102-107	2.5
Soybean seed	0.01	126, 121, 118, 114, 115	119	114-118; 121, 126	4.1
	0.10	102, 98.3, 101, 95.9, 98.5	99.1	95.9-102	2.4
	1.0	111, 108, 109, 108, 104	108	104-111	2.4
Soybean meal	0.01	110, 108, 116, 113, 109	111	108-116	2.9
	0.10	103, 102, 99.8, 99.7, 99.2	101	99.2-103	1.6
	1.0	110, 108, 106, 111, 108	109	106-111	1.8
Soybean hulls	0.01	120, 119, 116, 119, 115	118	115-120	1.8
	0.10	111, 107, 104, 98.7, 102	105	98.7-111	4.5
	1.0	110, 112, 106, 113, 111	110	106-113	2.4
Pethoxamid: Confirmation ion transition: m/z 296.2 $\rightarrow$ 250.2					
Maize grain	0.01	83, 79, 80, 79, 80	80	79-83	2
	0.1	77, 79, 76, 80, 75	77	75-80	3
Lettuce	0.01	84, 85, 85, 91, 86	86	84-91	3
	0.1	88, 86, 89, 89, 87	88	86-89	1
Rape seed	0.01	86, 83, 81, 83, 78	82	78-86	4
	0.1	90, 84, 86, 90, 83	87	83-90	4
Orange fruit	0.01	83, 80, 84, 82, 80	82	80-84	2
	0.1	91, 87, 87, 93, 90	90	87-93	3
Field corn forage	0.01	95.8, 104, 98.7, 93.5, 104	99.2	93.5-104	4.8
	0.10	99.7, 102, 97, 98.2, 95.6	98.5	95.6-102	2.5
	1.0	110, 112, 114, 108, 111	111	108-114	2.0
Field corn grain	0.01	89.7, 85.0, 87.1, 93.0, 88.5	88.7	85.0-93.0	3.4
	0.10	90.3, 86.3, 87.9, 92.9, 89.1	89.3	86.3-92.9	2.8
	1.0	92.4, 90.6, 84.3, 86.6, 88.0	88.4	84.3-92.4	3.6
Field corn stover	0.01	108, 96.8, 98.1, 101, 115	104	96.8-115	7.3
	0.10	81.1, 99.9, 95.4, 96.2, 103	95.1	81.1-103	8.8
	1.0	109, 99.6, 110, 111, 108	108	99.6-111	4.2
Corn meal	0.01	103, 89.0, 89.4, 89.2, 88.5	91.8	88.5-103	6.8
	0.10	94.6, 98.3, 97.3, 99.1, 99.8	97.8	94.6-99.8	2.1
	1.0	101, 103, 105, 101, 103	103	101-105	1.6
Corn flour	0.01	89.7, 98.6, 89.0, 93.1, 87.6	91.6	87.6-98.6	4.8
	0.10	96.8, 94.2, 92.8, 90.0, 92.7	93.3	90.0-96.8	2.7
	1.0	103, 105, 107, 105, 106	105	103-107	1.4

Table B.5.2.1.1-2. Accuracy and Precision Data for the Validation of EAS Method No. AGR/MOA/PTX-8 for the Determination of Pethoxamid Residues in Crop Commodities.¹					
Matrix	Fortification Level (ppm)	Validation Recovery (%)			RSD (%)
		Individual	Mean	Range	
Corn refined oil	0.01	95.2, 92.3, 90.7, 90.2, 91.1	91.9	90.2-95.2	2.2
	0.10	89.8, 84.5, 93.3, 93.1, 93.2	90.8	84.5-93.3	4.2
	1.0	91.2, 90.6, 90.3, 91.8, 91.7	91.1	90.3-91.8	0.73
Sweet corn K+CWHR	0.01	103, 114, 109, 124, 146	119	103-114; 124, 146	14
	0.10	99.5, 96.2, 101, 107, 108	102	96.2-108	4.9
	1.0	104, 67.3, 103, 102, 105	96.3	67.3; 102-105	17
Cotton undelinted seed	0.01	75.7, 104, 76.1, 75.9, 102	86.7	75.7-104	17
	0.10	111, 111, 79.7, 88.0, 92.7	96.5	79.7-111	15
	1.0	110, 104, 98.7, 103, 100	103	98.7-110	4.3
Cotton gin byproducts	0.01	78.6, 72.0, 80.0, 98.9, 84.8	82.9	72.0-98.9	12
	0.10	84.6, 83.4, 89.9, 77.7, 82.4	83.6	77.7-89.9	5.2
	1.0	112, 101, 96.9, 103, 106	104	96.9-112	5.5
Cotton seed meal	0.01	86.3, 86.0, 84.2, 79.5, 78.0	82.8	78.0-86.3	4.6
	0.10	111, 98.6, 92.3, 96.4, 99.5	99.6	92.3-111	7.0
	1.0	98.9, 94.5, 96.8, 99.0, 95.3	96.9	94.5-99.0	2.1
Cotton seed hulls	0.01	92.0, 87.0, 71.8, 77.8, 101	85.9	71.8-101	13
	0.10	86.6, 81.5, 82.0, 86.5, 76.6	82.6	76.6-86.6	5.0
	1.0	92.5, 96.2, 100, 104, 96.4	97.8	92.5-104	4.5
Soybean forage	0.01	99.7, 98.0, 96.5, 92.7, 100	97.4	92.7-100	3.1
	0.10	89.3, 84.2, 88.0, 88.1, 88.3	87.6	84.2-89.3	2.2
	1.0	113, 118, 118, 117, 118	117	113-118	1.9
Soybean hay	0.01	111, 117, 106, 117, 111	112	106-117	4.2
	0.10	97.9, 99.3, 96.7, 98.3, 92.3	96.9	92.3-99.3	2.8
	1.0	100, 104, 107, 99.4, 104	103	99.4-107	3.1
Soybean seed	0.01	102, 98.7, 100, 94.6, 97.6	98.6	94.6-102	2.8
	0.10	88.1, 82.2, 87.2, 80.2, 81.4	83.8	80.2-88.1	4.3
	1.0	110, 107, 109, 106, 104	107	104-110	2.2
Soybean meal	0.01	107, 95.8, 110, 98.3, 102	103	95.8-110	5.7
	0.10	100, 96.0, 95.7, 94.9, 92.7	95.9	92.7-100	2.8
	1.0	109, 108, 107, 109, 109	108	107-109	0.83
Soybean hulls	0.01	104, 102, 102, 102, 98.3	102	98.3-104	2.0
	0.10	92.2, 89.3, 86.0, 79.9, 84.4	86.4	79.9-92.2	5.4
	1.0	108, 109, 106, 112, 106	108	106-112	2.3

¹ The data for maize grain, lettuce, rape seed, and orange fruit were presented in MRID 49813538 and the data for the remaining commodities were presented in MRID 49813534.

Independent laboratory validation (ILV). An ILV study was conducted for EAS Method No. AGR/MOA/PTX-8 by Eurofins Agrosience Services Chem GmbH (Hamburg, Germany). Samples of head lettuce (high water content matrix) and rape seed (high oil content matrix), obtained by the laboratory, were fortified with pethoxamid at 0.01 and 0.10 ppm, and analyzed according to the method procedures described in Table B.5.2.1.1-1. The method was modified slightly to reduce the size of aliquot used for cleanup (due to laboratory equipment requirements).

The results of the ILV are presented in Table B.5.2.1.1-3. Recovery of pethoxamid from fortified head lettuce and rape seed ranged 81-104%. The RSDs ranged 1.0-3.3%.

As part of the validation, the effects of matrix were evaluated. Pethoxamid standards prepared in head lettuce and rape seed matrices at 0.5 ng/mL were analyzed and peak areas were compared to the peak area for a solvent standard at the same concentration. The percent differences between the head lettuce matrix standard and the solvent standard ranged from -4.5 to 10.1%, averaging 6.3 and 5.8% at the quantitation and confirmation ion transitions. For rape seed matrix, the percent differences ranged 17.3-26.1%, averaging 20.9 and 20.6% at the quantitation and confirmation ion transitions. Based on these results, the ILV laboratory concluded that there was no signal enhancement or suppression for head lettuce but that matrix effects >20% were observed for rape seed. The laboratory used matrix-matched standards for the analysis of both lettuce and rape seed samples to compensate for any potential matrix effects.

The stability of pethoxamid in sample final extracts during refrigerated storage was evaluated. All final extracts of head lettuce and rape seed tested in the ILV were stored at 5 ± 4 °C for 7 days and reanalyzed, using the quantitation ion transition. After storage, mean recoveries at the 0.01- and 0.10-ppm fortification levels were, respectively, 93 and 89% for head lettuce, and 78 and 77% for rape seed. These values compare favorably with the 0-day mean recoveries (reported in Table B.5.2.1.1-3), indicating stability of extracts for at least 7 days of refrigerated storage.

No information was provided in the submission concerning: the number of trials required for successful validation; the need for communication with the study sponsor; the number of worker hours and calendar days required to complete analysis of a sample set; or the need for revisions to the method. No critical steps in the method were identified. In addition, the ILV was conducted by the same organization (Eurofins Agroscience Services Chem) that developed the method, although at a different facility. However, because the proposed enforcement method is a QuEChERS method, this information will not be required to support the ILV for the method.

Table B.5.2.1.1-3. Accuracy and Precision Data for the Independent Laboratory Validation of EAS Method No. AGR/MOA/PTX-8 for the Determination of Pethoxamid Residues in Crop Commodities.					
Matrix	Fortification Level (ppm)	Independent Laboratory Validation Recovery (%)			RSD (%)
		Individual	Mean	Range	
Pethoxamid: Quantitation ion transition: m/z 296 $\rightarrow$ 131					
Head lettuce	0.01	95, 100, 101, 104, 102	100	95-104	3.3
	0.10	99, 97, 101, 96, 99	98	96-101	2.0
Rape seed	0.01	81, 82, 81, 82, 83	82	81-83	1.0
	0.10	81, 84, 82, 83, 83	83	81-84	1.4
Pethoxamid: Confirmation ion transition: m/z 296 $\rightarrow$ 250					
Head lettuce	0.01	97, 102, 101, 104, 103	101	97-104	2.7
	0.10	98, 97, 101, 96, 99	98	96-101	2.0
Rape seed	0.01	81, 83, 83, 83, 83	83	81-83	1.1
	0.10	81, 85, 83, 83, 83	83	81-85	1.7

V. Limit of Quantitation

Table B.5.2.1.1-4. Summary of Detection and Quantitation Limits for AGR/MOA/PTX-8.		
Analyte	LOD (ppm) ¹	LOQ (ppm) ²
Pethoxamid	0.003	0.01

¹ LOD = Limit of detection, defined as 1/3 LOQ.

² LOQ = Limit of quantitation, defined as the lowest fortification level where acceptable precision and accuracy data were obtained.

VI. Conclusions

EAS Method No. AGR/MOA/PTX-8 was adequately validated for determining residues of pethoxamid in crop commodities (maize grain; lettuce; rape seed; orange; field corn forage, grain, and stover; corn meal, flour, and refined oil; sweet corn K+CWHR; cotton undelinted seed, gin byproducts, meal, and hulls; and soybean forage, hay, seed, meal, and hulls). The validated LOQ is 0.01 ppm in all commodities tested. A confirmatory method is not needed because two ion transitions are monitored. A successful ILV was conducted with head lettuce and rape seed.

Because the extraction solvents used in the metabolism studies are very similar to those used in the proposed enforcement method, no radiovalidation data will be required for crop commodities.

REFERENCES

Document ID: MRID 49813534; PMRA Study # xxxxxx, PMRA Review # xxxxxx
Report: Bitter, J. (2017) S-2399: Freezer Storage Stability of S-2399 and Metabolites in Processed Fractions. Report No. 201700305; Laboratory Project ID VP-40066. Unpublished study sponsored by Valent USA LLC. 836 p.

B.5.2.1.2 Pre-Registration Method (Data-Gathering)

EAS Method No. AGR/MOA/PTX-8 was used for data collection in the submitted storage stability and crop field trial studies (refer to B.7.6.2.1 and B.7.6.1).

B.5.2.2 Analytical Methods for Foodstuff of Animal Origin (Livestock Matrices; Annex IIA 4.3, Annex IIIA 5.3)

B.5.2.2.1 Post-Registration Method (Enforcement)

Document ID: MRID Nos. 49813535 and 49813536
PMRA No. Not available

Report: Percheron, C. (2015) Pethoxamid - Validation of the Analytical Method for the Determination of Pethoxamid in Different Matrices of Animal Origin. Study Numbers: 1494 PXA; S15-03519. Unpublished study prepared by Eurofins Agroscience Services Chem SAS, sponsored by Cheminova A/S, and submitted by FMC Corporation. 132 p. (MRID 49813535)

Guidelines: Gasso-Brown, D. (2015) Independent Laboratory Validation of the analytical method validated in S15-03519 for the Determination of Pethoxamid in Different Matrices of Animal Origin. Study Numbers: 1518 PXA; S15-03520. Unpublished study prepared by Eurofins Agroscience Services Chem Ltd., sponsored by Cheminova A/S, and submitted by FMC Corporation. 71 p. (MRID 49813536)

GLP Compliance: EPA OCSPP Harmonized Test Guideline 860.1340 Residue Analytical Method (August 1996)
PMRA Regulatory Directive DIR98-02 – Residue Chemistry Guidelines, Section 3 – Residue Analytical Method
EU SANCO 825/00/rev. 7 (17/3/04)
OECD Guidance Document on Pesticide Residue Analytical Methods

Acceptability: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Evaluator: The study is considered scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP# 440771.

Secondary Evaluator: Sheila Piper, Chemist, HED, RAB6 
Robert Lanthier, PMRA

EXECUTIVE SUMMARY

Method [EAS Study Code S15-03519] is being proposed for analysis of livestock commodities for purposes of regulatory enforcement. The method description and method validation data (MRID 49813535), and an ILV (MRID 49813536) for an LC/MS/MS method, EAS Study Code S15-03519, were conducted for determination of residues of pethoxamid in livestock commodities. The extraction and analysis procedures are based on the QuEChERS method and are very similar to those of the proposed enforcement method for crop commodities, EAS

Method No. AGR/MOA/PTX-8. The ILV was conducted by Eurofins Agroscience Services Chem Ltd. (Melbourne, UK).

Briefly, homogenized samples are extracted with water by shaking and vortexing; ACN is added and the mixture is shaken and vortexed. QuEChERS extraction salts (magnesium sulfate:sodium chloride:sodium citrate tribasic dihydrate:sodium citrate dibasic sesquihydrate; 4:1:1:0.5, w:w:w:w) are added, and the samples are shaken, vortexed, and centrifuged. A portion of the supernatant is purified by the addition of cleanup salts (primary secondary amine:C18EC sorbent:magnesium sulfate; 1:1:6, w:w:w). Samples are shaken, vortexed, and centrifuged, and an aliquot of the supernatant is diluted with water for analysis by LC/MS/MS, conducted using a reverse-phase C18 column and a gradient mobile phase of ACN and water containing 0.2% acetic acid. The following ion transitions are monitored: m/z 296.2 $\rightarrow$ 131.2 (quantitation) and m/z 296.2 $\rightarrow$ 250.2 (confirmation). The LOQ (determined as the LLMV) is 0.01 ppm.

Method validation was conducted at 0.01 and 0.1 ppm using eggs, milk, fat, kidney, liver, and muscle. Recovery was measured at both the quantitation and confirmation ion transition for all matrices and fortification levels. At the 0.01-ppm level, the mean recoveries ranged 97-113%, with RSDs within the range of 1-7%. At the 0.1-ppm level (10xLOQ), the mean recoveries ranged 98-113% and RSDs ranged 1-3%.

A successful ILV was conducted using samples of whole milk, liver, and muscle fortified at 0.01 and 0.10 ppm. The ILV followed method procedures as currently written. Individual recoveries were within the acceptable range of 70-120%. Mean recoveries were within the acceptable limits, and relative standard deviations (RSDs) were <5%.

No radiovalidation data were submitted. Because pethoxamid was not found at a level above the LOQ of Method EAS Study Code S15-03519 in any matrix in the livestock metabolism studies (refer to B.7.2.1 and B.7.2.2), radiovalidation of the method using samples from the existing livestock metabolism studies will not be required.

Storage stability studies conducted with the method validation and ILV studies demonstrated that pethoxamid residues are stable in final extracts during refrigerated storage for at least 9 days for eggs and liver; 13 days for milk, fat, and kidney; and 14 days for muscle.

The effect of matrix was also evaluated with the method validation and ILV studies. In both cases, the data showed that there was no matrix effect associated with egg, milk, fat, kidney, liver, or muscle. However, matrix-matched standards were used for all analyses for both the method validation and ILV studies.

Method EAS Study Code S15-03519 is considered suitable for enforcement purposes.

I. Principle of the Method: EAS Study Code S15-03519

Table B.5.2.2.1-1. Summary Parameters for the Post-Registration Analytical Method for the Determination of Pethoxamid Residues in Livestock Matrices.	
Method ID	EAS Study Code S15-03519
Analyte	Pethoxamid
Extraction solvent and technique	Homogenized samples are extracted with water by shaking and vortexing; ACN is added, and the mixture is shaken and vortexed. QuEChERS extraction salts (magnesium sulfate:sodium chloride:sodium citrate tribasic dihydrate:sodium citrate dibasic sesquihydrate; 4:1:1:0.5, w:w:w:w; dispersive SPE citrate extraction tube) are added, and the samples are shaken, vortexed, and centrifuged.
Clean-up strategies	A portion of the supernatant is purified by the addition of cleanup salts (primary secondary amine:C18EC sorbent magnesium sulfate; 1:1:6, w:w:w; SampliQ Dispersive SPE, fatty samples). Samples are shaken, vortexed, and centrifuged, and an aliquot of the supernatant is diluted with water for analysis by LC/MS/MS.
Instrument and detector	LC/MS/MS using an API 4000 mass spectrometer with a reverse-phase column (ACE5 C18) and a gradient mobile phase of ACN and water containing 0.2% acetic acid. The following ion transitions are monitored: m/z 296.2 $\rightarrow$ 131.2 (quantitation) and m/z 296.2 $\rightarrow$ 250.2 (confirmation).
Standardization method	External standardization using a calibration curve of pethoxamid standards at eight concentrations over the range 0.10-25.0 ng/mL, with a coefficient of determination (r^2) of ≥ 0.990 .
Stability of standard solutions	Stock solution at 1,000 $\mu\text{g/mL}$ in ACN:water (90:10, v:v) is reportedly stable at 4 °C for 115 days. Calibration and fortification solutions are to be freshly prepared. The stability of pethoxamid in the final extracts of samples during storage at <10 °C for at least 7 days was demonstrated during method validation and ILV (see below).
Retention times	~2.0 minutes

II. Specificity

The LC/MS/MS method is highly selective for both the quantitation and confirmation of pethoxamid. Analysis of control samples resulted in no significant signals at the expected retention times of the analyte. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of the analyte:

Pethoxamid m/z 296.2 $\rightarrow$ 131.2 (quantitation)
 m/z 296.2 $\rightarrow$ 250.2 (confirmation)

III. Linearity

The linearity of detector response was evaluated using standard solutions in egg, milk, fat, kidney, liver, and muscle matrices. Calibration curves were calculated by linear regression analysis with 1/x weighting. For the least-squares equation which describes the detector response as a function of the standard concentration, calibration curves resulting from the injection of a minimum of eight standards over the concentration range of 0.10-25.0 ng/mL demonstrated linearity, with coefficients of determination (r^2) of at least 0.9975.

IV. Accuracy (Recovery) and Precision (Repeatability)

Radiovalidation. No data addressing the ability of Method EAS Study Code S15-03519 to extract incurred residues were submitted. In the submitted metabolism studies with hen and goat (refer to DERs for MRIDs 49813530 and 49813531; B.7.2.1 and B.7.2.2), the majority of extractable radioactivity was released from hen matrices using ACN and water for eggs, liver,

and muscle, and acetone and hexane for fat. For goat kidney and liver (the only matrices analyzed), initial ACN and water extraction released a minor amount of radioactivity (<15% of total radioactivity), and protease digestion with subsequent ACN extraction released the majority of radioactivity. In these studies, pethoxamid was only found in hen eggs, fat, and liver, at ≤ 0.005 ppm; pethoxamid was not found in any goat matrix. Because pethoxamid was not found at a level above the LOQ of Method EAS Study Code S15-03519, radiovalidation of the method using samples from the existing livestock metabolism studies will not be required.

Method validation. The method was validated by Eurofins Agrosience Services Chem SAS (Vergèze, France) at 0.01 ppm (LOQ) and 0.1 ppm (10xLOQ) in eggs, milk, fat, kidney, liver, and muscle; the type of tissues tested (poultry or beef) was not reported. Five samples were fortified with pethoxamid at each fortification level in each matrix, and samples were quantified using both transition ions.

Recovery and repeatability data are presented in Table B.5.2.2.1-2. The individual recoveries were within the range of 91-117%. At the 0.01-ppm level (LOQ), the mean recoveries ranged 97-113%, with RSDs within the range of 1-7%. At the 0.1-ppm level (10xLOQ), the mean recoveries ranged 98-113%, and RSDs ranged 1-3%.

As part of the method validation, the effects of matrix were evaluated. Pethoxamid standards prepared in egg, milk, fat, kidney, liver, and muscle matrices were analyzed, and peak areas were compared to the peak area for a solvent standard at the same concentration (0.01 ppm) using both ion transitions. The percent differences between the matrix standard and the solvent standard ranged from -6.2 to 7.8%. Based on these results, the laboratory concluded that matrix effects were insignificant for egg, milk, and tissue extracts; however, matrix-matched standards were used for quantitation for all matrices in the method validation.

The stability of pethoxamid in sample final extracts during refrigerated storage was evaluated. The final extracts of egg, milk, fat, kidney, liver, and muscle samples fortified at 0.01 ppm were stored at 4 °C for 9 (egg and liver), 13 (milk, fat, and kidney), or 14 (muscle) days and reanalyzed, using the quantitation ion transition. After storage, mean recoveries in extracts were 106% for egg, 101% for milk, 92% for fat, 105% for kidney, 107% for liver, and 97% for muscle. These values compare favorably with the 0-day mean recoveries (reported in Table B.5.2.2.1-2), indicating stability of extracts during refrigerated storage for at least 9 days for eggs and liver; 13 days for milk, fat, and kidney; and 14 days for muscle.

Table B.5.2.2.1-2. Accuracy and Precision Data for the Validation of EAS Study Code S15-03519.					
Matrix	Fortification Level (ppm)	Validation Recovery (%)			RSD (%)
		Individual	Mean	Range	
Pethoxamid: Quantitation ion transition: m/z 296.2 $\rightarrow$ 131.2					
Eggs	0.01	113, 106, 114, 107, 110	110	106-114	3
	0.1	109, 108, 108, 107, 105	107	105-109	1
Milk	0.01	107, 107, 106, 106, 107	107	106-107	1
	0.1	109, 107, 108, 109, 110	109	107-110	1
Fat	0.01	98, 101, 102, 95, 99	99	95-102	3
	0.1	101, 98, 99, 98, 99	99	98-101	1
Kidney	0.01	112, 113, 115, 112, 112	113	112-115	1
	0.1	113, 115, 114, 112, 113	113	112-115	1

Table B.5.2.2.1-2. Accuracy and Precision Data for the Validation of EAS Study Code S15-03519.					
Matrix	Fortification Level (ppm)	Validation Recovery (%)			RSD (%)
		Individual	Mean	Range	
Liver	0.01	106, 110, 110, 112, 114	110	106-114	3
	0.1	113, 112, 114, 112, 114	113	112-114	1
Muscle	0.01	110, 109, 110, 111, 111	110	109-111	1
	0.1	111, 113, 111, 110, 112	111	110-113	1
Pethoxamid: Confirmation ion transition: m/z 296.2 $\rightarrow$ 250.2					
Eggs	0.01	100, 99, 107, 91, 110	101	91-110	7
	0.1	109, 104, 103, 107, 103	105	103-109	3
Milk	0.01	103, 111, 108, 113, 104	108	103-113	4
	0.1	106, 104, 106, 108, 107	106	104-108	1
Fat	0.01	97, 95, 100, 97, 95	97	95-100	2
	0.1	99, 95, 99, 98, 97	98	95-99	2
Kidney	0.01	116, 108, 113, 111, 113	112	108-116	3
	0.1	112, 113, 113, 109, 110	111	109-113	2
Liver	0.01	100, 112, 115, 117, 117	112	100-117	6
	0.1	110, 112, 115, 110, 113	112	110-115	2
Muscle	0.01	110, 102, 113, 109, 111	109	102-113	4
	0.1	110, 112, 109, 108, 110	110	108-112	1

Independent Laboratory Validation (ILV). An ILV study was conducted for Method EAS Study Code S15-03519 by Eurofins Agrosience Services Chem Ltd. (Melbourne, UK). Samples of bovine whole milk, liver, and muscle, obtained commercially, were fortified with pethoxamid at 0.01 and 0.10 ppm, and analyzed according to the method procedures described in Table B.5.2.2.1-1. The method was modified as needed to optimize instrumental parameters.

The results of the ILV are presented in Table B.5.2.2.1-3. Recovery of pethoxamid from fortified whole milk, liver, and muscle ranged 85-102%. The RSDs ranged 1.7-4.9%.

As part of the validation, the effects of matrix were evaluated. Pethoxamid standards prepared in whole milk, liver, and muscle matrices were analyzed, and peak areas were compared to the peak area for a solvent standard at the same concentration (0.50 ng/mL) using both ion transitions. The percent differences between the matrix standard and the solvent standard ranged from -0.9 to 2.8%. Based on these results, the ILV laboratory concluded that matrix effects were insignificant for milk, liver, and muscle extracts; however, matrix-matched standards were used for quantitation for all matrices in the ILV.

The stability of pethoxamid in sample final extracts during refrigerated storage was evaluated. The final extracts of milk, liver, and muscle fortified at 0.01 ppm were stored at <10 °C for 7 days in the dark and reanalyzed, using the quantitation ion transition. After storage, mean recoveries were 97% for milk, 98% for liver, and 88% for muscle. These values compare favorably with the 0-day mean recoveries (reported in Table B.5.2.2.1-3), indicating stability of extracts for at least 7 days of storage at <10 °C in the dark.

The ILV laboratory reported that the method validation and ILV were carried out at different locations, by different study personnel, and using different instrumentation and stocks of chemicals; no communication with the method developers or others familiar with the method was necessary to carry out the analysis. No critical steps were identified, and no method

revisions were recommended. A set of 13 samples was reported to require a total of 9 hours for completion of analysis: 4.5 hours for sample preparation and extraction, 1 hour for LC/MS/MS instrument setup, 2.5 hours for LC/MS/MS analysis (unattended instrument hours), and 1 hour for data evaluation.

Table B.5.2.2.1-3. Accuracy and Precision Data for the Independent Laboratory Validation of EAS Study Code S15-03519 for Livestock Matrices.					
Matrix	Fortification Level (ppm)	Independent Laboratory Validation Recovery (%)			RSD (%)
		Individual	Mean	Range	
Pethoxamid: Quantitation ion transition: m/z 296.1 $\rightarrow$ 131.0					
Whole milk	0.01	91, 95, 91, 99, 96	94	91-99	3.6
	0.10	94, 92, 94, 93, 85	92	85-94	4.1
Liver	0.01	94, 95, 102, 101, 101	99	94-102	3.8
	0.10	98, 95, 95, 99, 96	97	95-99	1.9
Muscle	0.01	89, 96, 99, 96, 94	95	89-99	3.9
	0.10	95, 95, 96, 92, 93	94	92-96	1.7
Pethoxamid: Confirmation ion transition: m/z 296.1 $\rightarrow$ 250.2					
Whole milk	0.01	91, 94, 90, 99, 95	94	90-99	3.8
	0.10	94, 91, 92, 92, 85	91	85-94	3.8
Liver	0.01	93, 92, 100, 97, 98	96	92-100	3.5
	0.10	97, 94, 95, 98, 95	96	94-98	1.7
Muscle	0.01	87, 93, 99, 96, 91	93	87-99	4.9
	0.10	94, 94, 96, 91, 92	93	91-96	2.1

V. Limit of Quantitation

Table B.5.2.2.1-4. Summary of Detection and Quantitation Limits for EAS Study Code S15-03519.		
Analyte	LOD (ppm) ¹	LOQ (ppm) ²
Pethoxamid	0.002	0.01

¹ LOD = Limit of detection, set at 20% LOQ.

² LOQ = Limit of quantitation, defined as the lowest fortification level where acceptable precision and accuracy data were obtained.

It is noted that the ILV laboratory reported the LOD to be 30% of the LOQ or 0.003 ppm.

VI. Conclusions

Method EAS Study Code S15-03519 was adequately validated for determining residues of pethoxamid in livestock commodities eggs, milk, fat, kidney, liver, and muscle. The validated LOQ is 0.01 ppm in all commodities tested. A confirmatory method is not needed because two ion transitions are monitored. A successful ILV was conducted with milk, liver, and muscle.

No radiovalidation data were submitted. Because pethoxamid was not found at a level above the LOQ of Method EAS Study Code S15-03519 in any matrix in the livestock metabolism studies, radiovalidation of the method using samples from the existing livestock metabolism studies will not be required.

B.5.2.2.2 Pre-Registration Method (Data Gathering)

Not applicable.

B.5.2.3. Multiresidue Methods

The proposed enforcement methods are QuEChERS methods which have been adequately validated. No additional multiresidue method data will be required.

**B.7.1 Metabolism, Distribution, and Expression of Residues in Plants
(Annex IIA 6.2; Annex IIIA, 8.2)**

B.7.1.1 Corn

Document ID: MRID No. 49813528
PMRA No. Not available

Report: Goodyear, A. (2000) (¹⁴C)-TKC-94: Metabolism in Maize. Study Numbers: 100 PXA; 1465/5-D2142. Unpublished study prepared by Covance Laboratories Ltd., sponsored by Cheminova, Inc., and submitted by FMC Corporation. 162 p.

Guidelines: EPA OCSPH Harmonized Test Guideline 860.1300 Nature of the Residue - Plants, Livestock (August 1996)
PMRA Regulatory Directive Dir98-02 – Residue Chemistry Guidelines, Section 2 -Nature of the Residue - Plants, Livestock
OECD Guideline 501 Metabolism in Crops (January 2007)

GLP Compliance: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Acceptability: The study is considered scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP#452109.

Evaluator: Sheila Piper, Chemist, HED, RAB6 *Sheila Piper*

Secondary Evaluator: Robert Lanthier, PMRA

Note: This Data Evaluation Record (DER) was prepared under contract by CDM/CSS-Dynamac Joint Venture (submitted 2/16/18). The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies. The DER has been reviewed by HED and revised as necessary to reflect current Office of Pesticide Programs (OPP) policies.

EXECUTIVE SUMMARY

The metabolism of [phenyl-U-¹⁴C]pethoxamid (specific activity: 45.6 µCi/mg) in greenhouse-grown corn formulated as a 5.0 lb ai/gal (600 g ai/L) emulsifiable concentrate (EC) formulation and applied as a separate soil or foliar application at 1.1 lb ai/A (1.2 kg ai/ha) for each application. Applications were made as: an early postemergence application made to the soil 21 days after planting at BBCH 11 (first true leaves); a preemergence soil application 1 day after planting; or a foliar application to corn plants 29 days after planting at BBCH 14-15 (4- to 5-leaf growth stage). Samples of immature whole plant were collected at preharvest intervals (PHIs) of 14 and 27 days following the postemergence soil application, 36 days following the preemergence application, and 8 days following the foliar application. Samples of immature leaves, stems, and roots were collected 61 days following preemergence application and 33 days following foliar application, and samples of mature grain, cobs, leaves, stems, and roots were collected 106 days following preemergence application and 78 days following foliar application. The in-life and analytical phases of the study were conducted by Covance Laboratories Ltd. (Harrogate, UK).

Total radioactive residues (TRR) in corn matrices were calculated by summing extractable radioactivity (following solvent extraction and hydrolysis as applicable) and nonextractable radioactivity. The highest TRR were observed in 8-day PHI immature whole plants collected following foliar application, and the lowest TRR were found in grain and cobs following soil and foliar applications. TRR in corn matrices were generally lower in immature and mature plants harvested following soil application than following foliar application, and residues in corn plants receiving foliar application declined from first immature harvest to mature harvest. The results obtained following soil applications indicated that radioactivity was taken up from the soil and transported primarily to the plant leaves, while following foliar application only a small portion of the radioactivity was transported from the leaves to other vegetative parts of the plant. Minimal or relatively low radioactivity was found in stems following either treatment, and the study author stated that radioactivity in immature and mature roots following preemergence application may have been artificially high due to adherence of treated soil to the roots. TRR in immature whole plants were 0.011 and 0.008 ppm in samples harvested 14 and 27 days, respectively, following postemergence soil application; 0.035 ppm in samples harvested 36 days following preemergence application; and 6.600 ppm in samples harvested 8 days following foliar application. TRR in immature leaves, stems, and roots, respectively, were 0.049, 0.002, and 0.104 ppm in samples harvested 61 days following preemergence application, and 1.963, 0.008, and 0.021 ppm in samples harvested 33 days following foliar application. In mature corn matrices harvested 106 days following preemergence application and 78 days following foliar application, TRR in grain and cobs were ≤ 0.004 ppm. TRR in leaves, stems, and roots respectively, were 0.111, 0.028, and 0.114 ppm following preemergence application and 0.550, 0.011, and 0.018 ppm following foliar application.

Extraction with acetonitrile (ACN) released 33-61% TRR from immature whole plants collected following postemergence and preemergence soil applications and 74.2% TRR from immature whole plants collected following foliar application. Immature whole plants collected following foliar application, were subjected to additional extraction with water, which released 21% TRR, followed by hydrolysis with 0.1 M HCl and NaOH, which together released 4.1% TRR. In immature corn plants collected as separate matrices following preemergence or foliar application, extraction with ACN released 52-64% TRR from leaves, 40-50% TRR from roots, and either no radioactivity (following soil application) or 50% TRR (following foliar application) from stems, in which radioactivity was very low; hydrolysis with 2 M HCl released an additional 11% TRR from immature leaves following foliar application. In mature corn matrices collected following preemergence or foliar application, extraction with ACN released no radioactivity from seed and cobs, 40-46% TRR from stems, and 33-44% TRR from roots, and extraction with ACN and water together released 80-88% TRR from leaves. Radioactivity remaining in the post-extraction solids (PES) accounted for $<10\%$ TRR or <0.05 ppm in all matrices except: immature and mature roots following preemergence application (50.0-55.8% TRR, 0.052-0.063 ppm) and mature leaves following foliar application (20.0% TRR, 0.110 ppm). Recoveries were normalized for the total amount of radioactivity recovered in the extracts, hydrolysates, and PES; therefore, accountabilities were 100% for each matrix.

Following initial extraction and hydrolysis procedures, only four matrices were selected for further investigation of residues: mature leaves following both soil and foliar applications and 8-day PHI immature whole plants and 33-day PHI immature leaves following foliar application.

Subsamples of these matrices were subjected to further workup, and metabolites and residue components in extracts and hydrolysates (if applicable) were quantified and characterized or identified by high performance liquid chromatography (HPLC) with UV detection and/or thin layer chromatography (TLC). Further identification of residue components in 8-day PHI immature whole plants was conducted using high performance liquid chromatography with mass spectrometry detection (LC/MS). Identifications of pethoxamid and metabolites TKC-94-cysteine (MET-30) and TKC-94-N-malonyl cysteine (MET-47) were confirmed; identifications for remaining metabolites were considered to be tentative.

Only limited study dates were provided in the submission. Corn samples were stored frozen (*ca.* -20 °C) from harvest to analysis. No information was provided concerning when the metabolite profiles were established for leaves and immature whole plants. Based on dated LC/MS chromatograms for the ethyl acetate and aqueous phases of 8-day PHI immature plants, and the date of final HPLC analysis for an isolated metabolite samples were stored for up to 9.9-28.9 months prior to completion of the experimental phase of the study. In addition, analysis of a re-extracted sample of 8-day PHI immature whole plants indicated that metabolite TKC-94-N-malonyl cysteine may have degraded to metabolite TKC-94-cysteine during storage. Residue of pethoxamid are stable during frozen storage in corn grain, green plant and straw for up to 24 months and corn flour and meal for up to 10 months (MRIDs 49813534, 49813542- 49813543).

A total of 19.2% TRR (1.268 ppm) was identified in 8-day PHI immature whole plants. Pethoxamid was a minor residue component, accounting for 1.0% TRR (0.066 ppm). In addition, four metabolites were identified at <10% TRR each: TKC-94-cysteine- γ -glutamyl cysteine (MET-103) at 4.8% TRR (0.317 ppm), TKC-94-cysteine (MET-30) and TKC-94-N-malonyl-cysteine (MET-47), together accounting for 7.5% TRR (0.498 ppm), and TKC-94-thiol (MET-43) at 5.9% TRR (0.387 ppm). The major residue component, which accounted for 12.3% TRR (0.815 ppm), was an unknown designated Unknown 3, that was found to be nearly entirely comprised of metabolites TKC-94-SO-lactic acid and TKC-94-S-lactic glucoside (MET-48) during analysis of 33-day PHI immature leaves. Following strong acid hydrolysis of the plant extract, benzoic acid was found at 33.7-59.4% TRR in the 2 M and 6 M HCl hydrolysates, and an oxamic acid metabolite (MET-31) and its geometric isomer were tentatively identified, together accounting for 16.2% TRR in the 2 M HCl hydrolysate. Remaining radioactivity was comprised of one more discrete unknown (5.6% TRR, 0.373 ppm) and multiple minor unknowns that were not further resolved but together accounted for 57.7% TRR (3.806 ppm).

A total of 17.1% TRR (0.337 ppm) was identified in 33-day PHI immature leaves; no residue components were identified in mature leaves. Seven discrete unknowns, designated Unknowns 1-7, were found in mature leaves from both preemergence and foliar applications, and Unknowns 1-4 were found in immature leaves following foliar application. Unknown 3 was a major residue component in all samples, accounting for 13.6-15.1% TRR (0.017-0.075 ppm) in mature leaves and 17.6% TRR (0.346 ppm) in immature leaves. In immature leaves, the unknown was found to be comprised of TKC-94-SO-lactic acid at 8.0% TRR (0.158 ppm), TKC-94-S-lactic glucoside (MET-48) at 9.1% TRR (0.179 ppm), and two minor unknown components. Unknowns 6 and 7 were major residue components in mature leaves following preemergence application, at 9.9% TRR (0.011 ppm) and 7.9% TRR (0.009 ppm), respectively, and Unknown 5 was a major residue component in mature leaves following foliar application, at 15.7% TRR

(0.086 ppm). Further investigation of Unknown 5 indicated that, like Unknown 3, it was comprised of two major components and two minor components; however, identification attempts were unsuccessful. Remaining unknowns individually accounted for $\leq 5.2\%$ TRR (≤ 0.088 ppm) in mature and immature leaves, and multiple minor unknowns that were not further resolved together accounted for 34.9-56.6% TRR (0.045-1.110 ppm). The study author stated that the distribution of the unknowns in immature and mature leaves suggested that Unknown 5 is formed primarily as a plant metabolite and that contribution from the soil route is small, while Unknowns 6 and 7 appear to be soil metabolites that have been taken up by the plants. Because the respective amounts of Unknowns 1-4 were comparable in plants following both preemergence and foliar applications, the study author concluded that these metabolites resulted primarily from pethoxamid, either taken up from the soil or following application to the leaves. In addition to these components, a small amount of benzoic acid was identified, but not quantitated, in the 2 M HCl hydrolysate of immature leaves.

Based on the results of the corn metabolism study, the study author concluded that pethoxamid was rapidly absorbed and metabolized in corn. Due to the predominance of sulfur-containing metabolites, the study author proposed that pethoxamid was metabolized primarily via glutathione conjugation, with initial displacement of the chlorine atom by the sulfhydryl group of glutathione, likely in a reaction catalyzed by glutathione-S-transferase. The glutathione conjugate (not found in the study) was metabolized initially by loss of glycine to yield TKC-94- γ -glutamyl cysteine (MET-130), and then by loss of glutamic acid to yield TKC-94-cysteine (MET-30). The presence of TKC-94-thiol (MET-43), TKC-94-N-malonyl cysteine (MET-47), TKC-94-SO-lactic acid, and TKC-94-S-lactic acid glucoside suggested extensive further metabolism of TKC-94-cysteine, possibly via β -lyase to form TKC-94-thiol and by malonyl transferase to form the N-malonyl conjugate.

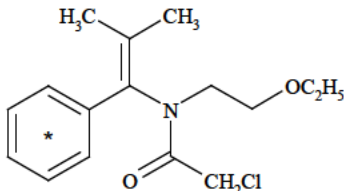
I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

The radiolabeled test substance, dissolved in ACN, was isotopically diluted with non-labeled pethoxamid and formulated as a 5.0 lb ai/gal (600 g ai/L) EC formulation using a formulation blank containing non-labeled pethoxamid, formulation additives, and water.

Table B.7.1.1-1. Pethoxamid Nomenclature.	
Common name	Pethoxamid
Identity	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide
CAS registry number	106700-29-2
Company experimental name	TKC-94
Radiolabeled compound	[phenyl- U - ^{14}C]pethoxamid
Lot/Batch #	CFQ9901
Radiochemical purity	>98%
Specific activity as received	102.5 μ Ci/mg (3.79 MBq/mg)
Specific activity of dose	45.6 μ Ci/mg (101,232 dpm/ μ g)

Table B.7.1.1-1. Pethoxamid Nomenclature.	
Position of radiolabel	

2. Test Crop

Table B.7.1.1-2. Crop Information.				
Crop/Crop Group	Variety	Growth Stage at Application	Growth Stage at Harvest	Harvested Commodities
Corn/Cereal grain, group 15, and Cereal grain, forage, fodder and straw, group 16	239 Lot 31FA	<u>Postemergence soil:</u> BBCH 11 (first true leaves)	Immature and mature (growth stages not further specified)	<u>Postemergence soil:</u> Immature whole plants (14- and 27-day PHIs)
		<u>Preemergence soil:</u> 1 day after planting		<u>Preemergence soil:</u> Immature whole plants (36-day PHI) Immature leaves, stems, and roots (61-day PHI) Mature grain, cobs, leaves, stems, and roots (106-day PHI)
		<u>Foliar:</u> BBCH 14-15 (4- to 5-leaf stage)		<u>Foliar:</u> Immature whole plant (8-day PHI) Immature leaves, stems, and roots (33-day PHI) Mature grain, cobs, leaves, stems, and roots (78-day PHI)

3. Soil Type

Corn was grown in a mixture of multipurpose compost, sharp sand, and topsoil mixed in a ratio of ~3:2:1. The physicochemical properties of the soil are presented in Table B.7.1.1-3.

Table B.7.1.1-3. Soil Physicochemical Properties.							
Soil Type	pH	Organic Matter (%)	Sand (%)	Silt (%)	Clay (%)	Moisture Holding Capacity (% at 1/3 bar)	Cation Exchange Capacity (meq/100 g)
Loamy sand	6.1	6.4	83.7	9.3	7.0	Not reported	Not reported

B. STUDY DESIGN

Experimental Conditions

Corn plants were grown in 45-L plastic containers in a greenhouse. The corn plants were maintained under conditions suitable for plant growth during the study. Greenhouse temperatures ranged 7-45 °C, water was applied to the soil as necessary, leaves were misted with water occasionally, and fertilizers and pesticides were used as needed. Supplementary lighting was used periodically to increase light levels.

The radiolabeled test substance was formulated as a 5.0 lb ai/gal (600 g ai/L) EC formulation, and applied to corn seeded and grown in separate containers at 1.1 lb ai/A (1.2 kg ai/ha) for each

application type. A single soil application was made to corn seeded on different dates to establish an early postemergence application made 21 days after planting at BBCH 11 (first true leaves; two containers) and a preemergence application made 1 day after planting (four containers). A foliar application was made to separate corn plants 29 days after planting at BBCH 14-15 (4- to 5-leaf growth stage; four containers). The study author indicated that the postemergence soil application was made as part of a “preliminary” study, and the preemergence and foliar applications were made as part of the main study; however, samples from all three treatments were analyzed. For the soil applications, the test substance was applied dropwise by pipette over the soil surface. For the foliar application, a plastic bag was placed over the plants in each container and sealed at the base with tissues, and the test substance was applied through holes cut in the ends of the bag using a compressed air sprayer. The study author noted that a full leaf canopy covered the area of each study container at the time of the foliar treatment; thus, the majority of the spray was applied to the leaf surface.

Table B.7.1.1-4. Use Pattern Information.	
Chemical name	[phenyl-U- ¹⁴ C]pethoxamid
Application method	The test substance was formulated as a 5.0 lb ai/gal (600 g ai/L) EC formulation and applied as a single postemergence or preemergence soil application by pipetting dropwise to the soil or as a single foliar application using a compressed air sprayer.
Application rate	1.1 lb ai/A (1.2 kg ai/ha) for each application
Number of applications	A single postemergence soil, preemergence soil, or foliar application
Timing of applications	Postemergence soil: 21 days after planting Preemergence soil: 1 day after planting Foliar: 29 days after planting
PHI	Postemergence soil: 14 and 27 days for immature whole plants Preemergence soil: 36 days for immature whole plants; 61 days for immature leaves, stems, and roots; and 106 days for mature grain, cobs, leaves, stems, and roots Foliar: 8 days for immature whole plants; 33 days for immature leaves, stems, and roots; and 78 days for mature grain, cobs, leaves, stems, and roots

Sampling

Samples of immature plants (foliage) were collected 14 and 27 days following postemergence application, 36 days following preemergence application, and 8 days following foliar application. Samples of immature plants, separated into leaves and stems, were collected 61 and 33 days following preemergence and foliar applications, respectively. Mature corn grain, cobs, leaves, and stems were harvested 106 days following preemergence application and 78 days following foliar application.

Immature whole plants were harvested by cutting at the soil surface. Immature leaf samples were collected following preemergence and foliar application by separating the leaf from the stem at the leaf junction. Plant samples were then cut into small pieces forming composite samples of leaf, stem, and roots. For mature plants, leaves and stems were harvested as above, corn ears were broken from the stems, and the husks were removed and combined with the leaves. Roots were collected by digging up a number of root systems and removing as much soil as possible by tapping and shaking.

All samples were placed in frozen storage (*ca.* -20 °C) on the day of harvest. Shortly after harvest, six corn ears from each treatment type were removed from the freezer, and grain was

removed from the cobs using a metal spatula while maintaining the samples frozen using dry ice. Composite samples of grain and cob were prepared for each treatment group. For all matrices, samples were homogenized in the presence of dry ice prior to extraction.

TRR Determination

Radioactivity was determined in extracts and hydrolysates by LSC and in PES fractions by combustion/LSC. TRR were calculated by summing extractable radioactivity (following solvent extraction and hydrolysis as applicable) and nonextractable radioactivity. The limit of quantitation for TRR determinations was not reported.

Extraction and Analysis

Samples of immature and mature corn matrices were extracted with ACN, and the extract was collected by centrifugation. No further details were provided concerning these procedures.

Subsamples of mature leaves following preemergence and foliar application, and 8-day PHI immature whole plants and 33-day PHI immature leaves following foliar application were subjected to additional workup for further investigation of radioactive residues.

Subsamples of mature leaves were sequentially extracted with duplicate aliquots of ACN and water, and the extracts were collected by centrifugation, then filtered, combined, and partitioned twice with hexane. The resulting ACN/water phase was filtered, concentrated by rotary evaporation to remove ACN, and partitioned with hexane followed by ethyl acetate. The final aqueous phase was concentrated by rotary evaporation for chromatography.

A subsample of 8-day PHI immature whole plants (Subsample 1) was extracted sequentially with triplicate aliquots of ACN and water, and the extracts were collected by centrifugation. The first and second ACN and water extracts contained the majority of the extractable radioactivity, and like extracts were combined and partitioned with hexane, initially as separate ACN and water extracts and then as a combined extract. The resulting ACN/water phase was concentrated to near dryness and reconstituted in ACN:water (1:1, v:v). The PES were subjected to acid and base hydrolysis with 0.1 M HCl followed by 0.1 M NaOH (conditions not specified), and the hydrolysates were collected by centrifugation but were not further analyzed. A subsample of the concentrated ACN/water extract was subjected to acid hydrolysis by acidifying with concentrated HCl to achieve an overall concentration of 2 M HCl and heating at 60 °C for 3 hours then at 80 °C for 24 hours. The resulting hydrolysate was partitioned with ethyl acetate, and the ethyl acetate phase was centrifuged to remove particulate matter, concentrated under a stream of nitrogen to remove solvent, then re-dissolved in ACN. A second subsample of the concentrated ACN/water extract was subjected to base hydrolysis, using concentrated NaOH to achieve 2 M NaOH and then heating at 80 °C for 24 hours. The resulting hydrolysate was reserved for TLC analysis.

Additional material was prepared using a larger subsample of 8-day PHI immature whole plants (Subsample 2) subjected to the extraction and hydrolysis procedures described above except that the subsample was extracted 2x each with ACN and water, and separate aliquots of the

concentrated ACN/water extract following hexane partitioning were subjected to: (1) acid hydrolysis with 2 M HCl (at 80 °C for 24 hours) and 6 M HCl (at 95 °C for 20 hours) followed by ethyl acetate partitioning; and (2) partitioning 2x with ethyl acetate, with the initial aqueous phase acidified to pH 4 prior to the second partitioning step and the ethyl acetate phases combined, centrifuged to remove particulates, concentrated under nitrogen, and re-dissolved in ACN.

Immature leaves were also subjected to additional workup including sequential extraction with ACN and water and acid hydrolysis with 2 M HCl at reflux; however, no further details were provided.

Flowcharts of the extraction and hydrolysis procedures is presented in Figures B.7.1.1-1 (leaves) B.7.1.1-2 (immature whole plants, Subsample 1) and B.7.1.1-3 (immature whole plants, Subsample 2).

Figure B.7.1.1-1. Flowchart for Extraction of Mature Leaves.

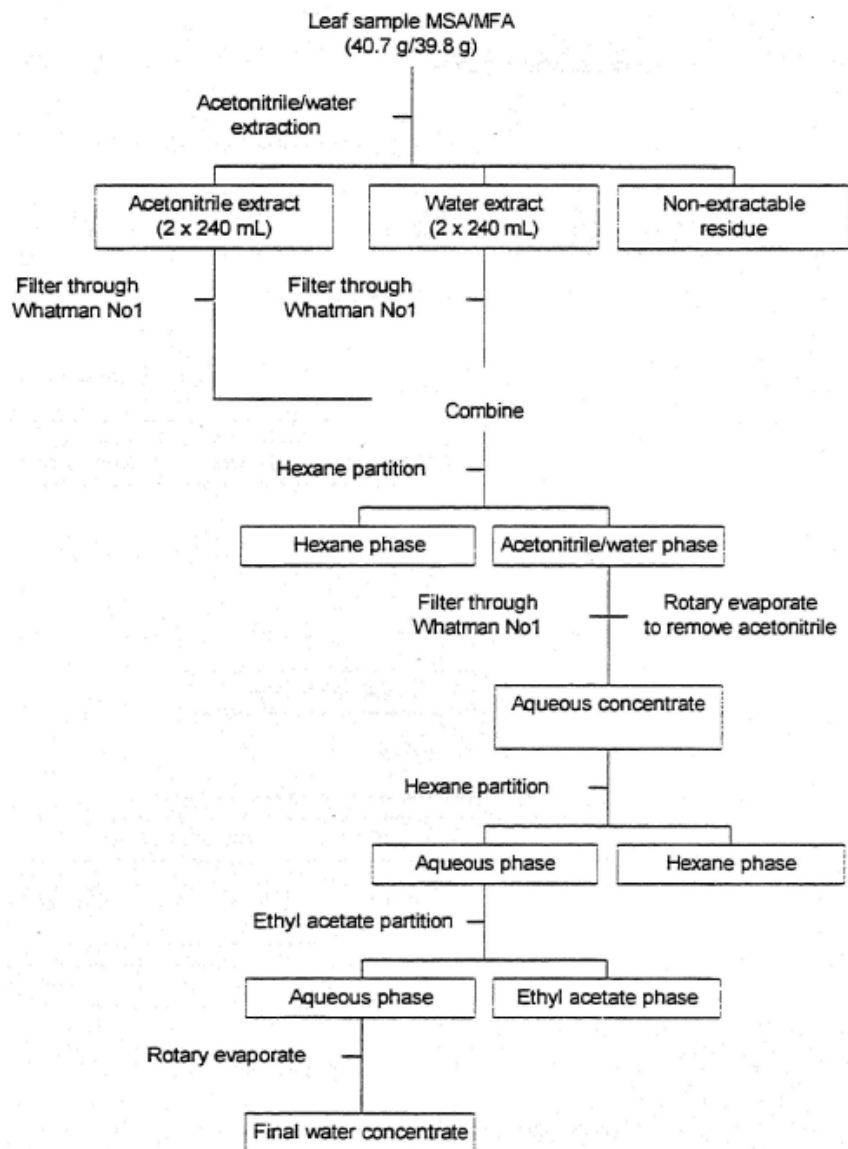


Figure B.7.1.1-2. Flowchart for Extraction and Hydrolysis of 8-day PHI Whole Immature Plant (Subsample 1).

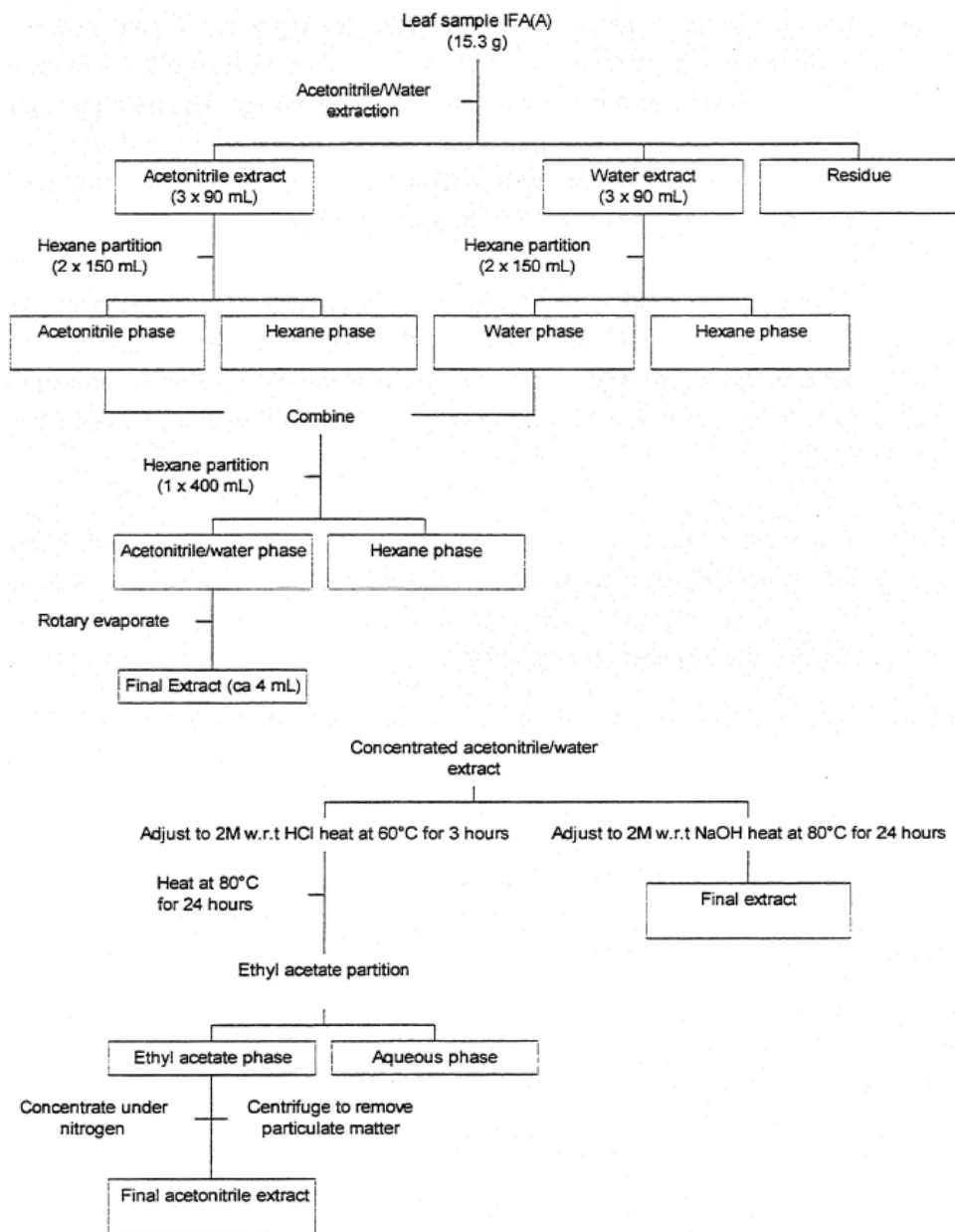
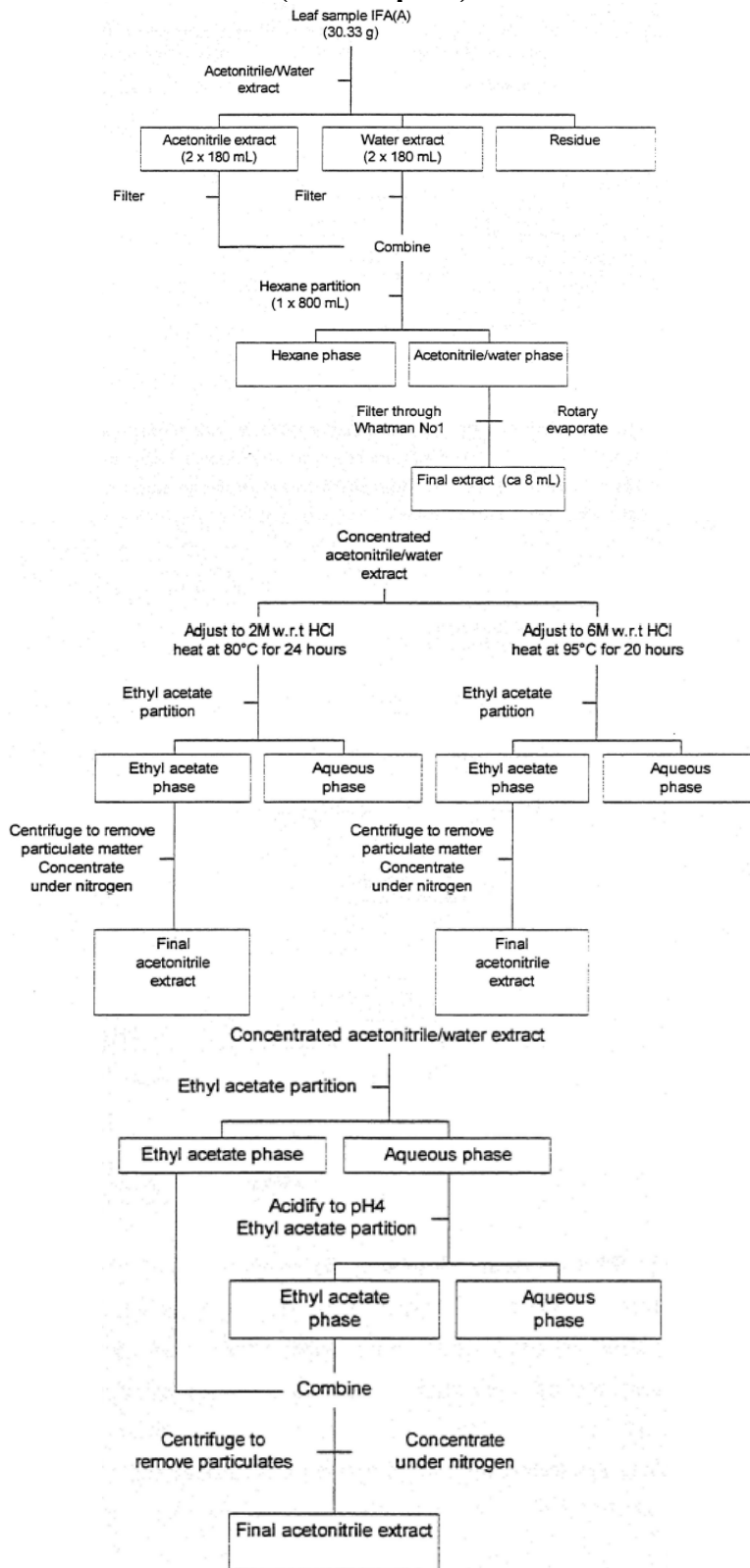


Figure B.7.1.1-3. Flowchart for Extraction and Hydrolysis of 8-day PHI Whole Immature Plant (Subsample 2).



Identification and Characterization

Extracts and hydrolysates of 8-day PHI immature whole plants, 33-day PHI immature leaves, and 106- and 78-day PHI mature leaves were analyzed by HPLC using a system equipped with a Spherisorb ODS2 column (Methods 1-5) or Zorbax NH2 column (Methods 6 and 7), a UV detector (254 nm), a flow-through radiodetector, and a fraction collector, and utilizing mobile phase gradients of: water and ACN, each containing 1% acetic acid (Method 1); 0.01 M phosphate buffer (pH 2.5) and ACN (Method 3); or 0.01 M formate buffer (pH 3.0) and ACN (Methods 4-7).

The combined ACN/water extract of 8-day PHI whole plants was also analyzed by TLC using silica gel plates (K6F) and solvent systems of hexane:ethyl acetate:triethylamine (10:10:1, v:v:v; Method 1); ethyl acetate:formic acid (100:1, v:v; Method 2); or ACN:water (17:3, v:v; Method 4). The 2 M NaOH hydrolysate of 8-day PHI whole plants was also analyzed by TLC Method 4. Radioactivity was detected and quantitated using phosphorimaging and a linear analyzer.

Identification of TKC-94-cysteine (MET-30), and TKC-94-N-malonyl cysteine (MET-47) in 8-day PHI whole plants was confirmed by co-chromatography with reference standards using HPLC Method 5 and freshly prepared extracts generated from a larger plant sample. Refer to Table B.7.1.1-13 for identified residue components and to Appendix I for the names and structures of reference standards used in the study.

To elucidate metabolite structures and confirm identification of parent and certain metabolites, the following fractions were analyzed by LC/MS: the ethyl acetate and aqueous phases that were generated following partitioning of the combined ACN/water extract of 8-day PHI whole plants; the 2 and 6 M HCl hydrolysates the combined ACN/water extract of 8-day PHI whole plants; and Unknown 3 isolated from the combined ACN/water extract of 33-day PHI leaves. LC/MS Method 1, which was used for analysis of intact metabolites in the ethyl acetate and aqueous phases of 8-day PHI whole plants, utilized the HPLC parameters of Method 4, with the addition of a Finnigan LCQ Ion Trap mass spectrometer operated in the positive ionization mode using electrospray ionization and full scan mass spectroscopy. LC/MS Method 2, which was used for analysis of the 2 M and 6 M HCl hydrolysates, utilized the HPLC parameters of Method 1, and a VG Quattro triple quadrupole mass spectrometer operated in the positive and negative ionization modes with electrospray ionization and full scan mass spectroscopy. LC/MS Method 3, which was used for analysis of isolated Unknown 3, utilized the HPLC parameters of Method 7, with the addition of a Finnigan LCQ Ion Trap mass spectrometer operated in the positive ionization mode using APCI and full scan mass spectrometry.

II. RESULTS AND DISCUSSION

A. Total Radioactive Residues

The TRR in corn matrices are summarized in Table B.7.1.1-5.

Table B.7.1.1-5. TRR in Corn Matrices.						
Matrix	Application type ¹					
	Postemergence soil		Preemergence soil		Foliar	
	PHI (days)	Residues ² (ppm)	PHI (days)	Residues ² (ppm)	PHI (days)	Residues ² (ppm)
Immature: Whole plants (foliage)	14	0.011	36	0.035	8	6.600
	27	0.008				
<i>Whole plants</i> ³			61	0.020	33	1.055
Leaves				0.049		1.963
Stems				0.002		0.008
Roots				0.104		0.021
Mature: <i>Whole plants</i> ³			106	0.042	78	0.134
Grain				0.004		0.002
Cob				0.004		0.004
Leaves				0.111		0.550
Stems				0.028		0.011
Roots				0.114		0.018

¹ Postemergence soil application was made 21 days after planting; preemergence application was made 1 day after planting; and foliar application was made 29 days after planting.

² Reported in parent equivalents. Calculated by summing extractable radioactivity (following solvent extraction and hydrolysis as applicable) and nonextractable radioactivity.

³ TRR for italicized fractions were calculated by the study author from the amount in each separated plant fraction (excluding roots).

B. Extraction, Characterization, and Distribution of Residues in Corn

The distribution of radioactivity in corn matrices is presented in Tables B.7.1.1-6 (immature matrices, postemergence soil application) B.7.1.1-7 (immature matrices, preemergence application), B.7.1.1-8 (mature matrices, preemergence application), B.7.1.1-9 (immature matrices, foliar application), and B.7.1.1-10 (mature matrices, foliar application). All samples of immature and mature corn matrices were extracted initially with ACN. Following initial analysis, only four matrices were selected for further investigation of residues: mature leaves collected following preemergence and foliar applications and 8-day PHI immature whole plants and 33-day PHI leaves collected following foliar application. No further investigations were conducted on grain or cobs due to very low radioactivity, and chromatography was not attempted on the stem extracts of mature samples due to low residues and difficulties that were encountered in analyzing the residues in leaves (see below).

We note that raw data supporting chromatographic analyses were not provided in the submission; therefore, the study reviewer could not verify any of the reported quantitative data for HPLC or TLC analyses.

Soil Application

Table B.7.1.1-6. Distribution of Radioactive Residues in Immature Corn Matrices Following Postemergence Soil Application of [¹⁴C]Pethoxamid at 1.1 lb ai/A (1.2 kg ai/ha).¹				
Metabolite Fraction	Whole plants (14-day PHI)		Whole plants (27-day PHI)	
	TRR = 0.011 ppm		TRR = 0.008 ppm	
	%TRR	ppm	%TRR	ppm
ACN	50.0	0.005	33.3	0.003
PES	50.0	0.005	66.7	0.006

¹ Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

Table B.7.1.1-7. Distribution of Radioactive Residues in Immature Corn Matrices Following Preemergence Soil Application of [¹⁴C]Pethoxamid at 1.1 lb ai/A (1.2 kg ai/ha).¹								
Metabolite Fraction	Whole plants (36-day PHI)		Leaves (61-day PHI)		Stems (61-day PHI)		Roots (61-day PHI)	
	TRR = 0.035 ppm		TRR = 0.049 ppm		TRR = 0.002 ppm		TRR = 0.104 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN	60.9	0.021	52.2	0.026	ND	--	50.0	0.052
PES	39.1	0.014	47.8	0.023	100	0.002	50.0	0.052

¹ ND = Not detected. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

Table B.7.1.1-8. Distribution of Radioactive Residues in Mature Corn Matrices Following Preemergence Soil Application of [¹⁴ C]Pethoxamid at 1.1 lb ai/A (1.2 kg ai/ha). ¹						
Metabolite Fraction	Grain (106-day PHI)		Cobs (106-day PHI)		Leaves (106-day PHI)	
	TRR = 0.004 ppm		TRR =0.004 ppm		TRR = 0.111 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN	ND	--	ND	--	54.9	0.061
Water					32.6	0.036
Combined ACN/water					87.5	0.097
Unknown 1 (Rt ~41 min)					3.7	0.004
Unknown 2 (Rt ~42 min)					5.2	0.006
Unknown 3 (Rt ~47 min)					15.1	0.017
Unknown 4 (Rt ~55 min)					1.3	0.001
Unknown 5 (Rt ~28 min)					3.2	0.004
Unknown 6 (Rt ~16 min)					9.9	0.011
Unknown 7 (Rt ~20 min)					7.9	0.009
Multiple minor unknowns ²					41.2	0.045
PES	100	0.004	100	0.004	12.6	0.014
Metabolite Fraction	Stems (106-day PHI)		Roots (106-day PHI)			
	TRR = 0.028 ppm		TRR = 0.114 ppm			
	%TRR	ppm	%TRR	ppm		
ACN	46.2	0.013	44.2	0.050		
PES	53.8	0.015	55.8	0.063		

¹ ND = Nondetectable. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Remaining radioactive regions; total calculated by the study reviewer by subtraction.

Foliar Application

Table B.7.1.1-9. Distribution of Radioactive Residues in Immature Corn Matrices Following Foliar Application of [¹⁴C]Pethoxamid at 1.1 lb ai/A (1.2 kg ai/ha).¹								
Metabolite Fraction	Whole plants (8-day PHI)		Leaves (33-day PHI)		Stems (33-day PHI)		Roots (33-day PHI)	
	TRR = 6.600 ppm		TRR = 1.963 ppm		TRR = 0.008 ppm		TRR = 0.021 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN	74.2	4.900	63.8	1.252	50.0	0.004	40.0	0.008
Water	20.6	1.362	22.7	0.445				
Combined ACN/water ²	94.8	6.262	86.5	1.697				
Unknown 1 (Rt ~46 min)			3.5	0.068				
Unknown 2 (Rt ~47 min)			4.5	0.088				
Unknown 3 (Rt ~51 min)			17.6 ³	0.346				
TKC-94-SO-lactic acid			8.0	0.158				
TKC-94-S-lactic glucoside (MET-48)			9.1	0.179				
Minor unknowns (2)			0.3	0.007				
Unknown 4 (Rt ~57 min)			4.3	0.085				
Multiple minor unks. ⁴			56.6	1.110				
(i) Ethyl acetate	28.9	1.910						
Pethoxamid	1.0	0.066						
TKC-94-thiol	5.9	0.387						
Unknown (Rt ~54 min)	1.8	0.121						
Multiple minor unks. ⁴	20.2	1.336						
(i) Water	65.9	4.352						
TKC-94-γ-glutamyl cysteine	4.8	0.317						
TKC-94-cysteine & TKC-94-N-malonyl cysteine	7.5	0.498						
Unknown (Rt ~54 min)	3.8	0.252						
Unknown 3 (Rt ~47 min)	12.3	0.815						
Multiple minor unks. ⁴	37.5	2.470						
(ii) 2 M HCl	79.4	5.247						
Unknown (Rt ~5 min)	12.0	0.795						
Benzoic acid	33.7	2.224						
Unknown (Rt ~12 min)	8.3	0.545						
Unknown (Rt ~17 min)	8.4 ⁵	0.556						
Unknown (Rt ~18 min)	7.8 ⁵	0.517						
Unknown (Rt ~19 min)	9.2	0.610						
(iii) 6 M HCl	83.6	5.516						
Unknown (Rt ~5 min)	11.2	0.737						
Benzoic acid	59.4	3.921						
Unknown (Rt ~9 min)	6.2	0.411						
Unknown (Rt ~10 min)	6.8	0.447						
0.1 M HCl	1.0	0.069						
0.1 M NaOH	3.1	0.205						

Table B.7.1.1-9. Distribution of Radioactive Residues in Immature Corn Matrices Following Foliar Application of [¹⁴C]Pethoxamid at 1.1 lb ai/A (1.2 kg ai/ha).¹								
Metabolite Fraction	Whole plants (8-day PHI)		Leaves (33-day PHI)		Stems (33-day PHI)		Roots (33-day PHI)	
	TRR = 6.600 ppm		TRR = 1.963 ppm		TRR = 0.008 ppm		TRR = 0.021 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
2 M HCl			10.9 ⁶	0.214				
PES	1.0	0.064	2.6	0.052	50.0	0.004	60.0	0.013

¹ Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable radioactivity (following solvent extraction and hydrolysis as applicable) and nonextractable residues.

² Different aliquots of the combined ACN/water extract of whole plant were subjected to partitioning with ethyl acetate and acid hydrolysis with 2 M or 6 M HCl.

³ Unknown 3 was isolated and subjected to LC/MS analysis for identification of components.

⁴ Remaining radioactive regions; total calculated by the study reviewer by subtraction.

⁵ Following LC/MS analysis, the unknowns eluting at Rts ~17 and ~18 min were tentatively identified as metabolite MET-31 and its geometric isomer.

⁶ A small quantity of benzoic acid was identified but not quantitated in the 2 M HCl hydrolysate of immature leaves.

Table B.7.1.1-10. Distribution of Radioactive Residues in Mature Corn Matrices Following Foliar Application of [¹⁴ C]Pethoxamid at 1.1 lb ai/A (1.2 kg ai/ha). ¹						
Metabolite Fraction	Grain (78-day PHI)		Cobs (78-day PHI)		Leaves (78-day PHI)	
	TRR = 0.002 ppm		TRR =0.004 ppm		TRR = 0.550 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN	ND	--	ND	--	37.0	0.204
Water					43.0	0.236
Combined ACN/water					80.0	0.440
Unknown 1 (Rt ~41 min)					3.4	0.019
Unknown 2 (Rt ~42 min)					3.5	0.019
Unknown 3 (Rt ~47 min)					13.6	0.075
Unknown 4 (Rt ~56 min)					1.1	0.006
Unknown 5 (Rt ~28 min)					15.7	0.086
Unknown 6 (Rt ~17 min)					3.3	0.018
Unknown 7 (Rt ~20 min)					4.5	0.025
Multiple minor unknowns ²					34.9	0.192
PES	100	0.002	100	0.004	20.0	0.110
Metabolite Fraction	Stems (78-day PHI)		Roots (78-day PHI)			
	TRR = 0.011 ppm		TRR = 0.018 ppm			
	%TRR	ppm	%TRR	ppm		
ACN	40.0	0.004	33.3	0.006		
PES	60.0	0.006	66.7	0.012		

¹ ND = Not detected. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Remaining radioactive regions; total calculated by the study reviewer by subtraction.

C. Storage Stability of Residues

Samples were stored frozen (*ca.* -20 °C) between harvest and extraction for analysis. Only limited study dates were provided in the submission. Sample harvest dates were calculated by the study reviewer from reported application dates and PHIs provided by the study author. No

information was provided concerning when the metabolite profiles were established for leaves and immature whole plants. Based on dated chromatograms reflecting LC/MS analysis of the ethyl acetate and aqueous phases of 8-day PHI immature plant, samples were stored for up to 9.9-10.5 months prior to these analyses for characterization and identification of residues. In addition, the study author reported the date of final HPLC analysis for enzyme hydrolysis of an isolated metabolite which reflected a maximum potential storage interval of 28.9 months for the experimental phase of the study. Sample storage conditions and intervals are presented in Table B.7.1.1-11.

The study author noted that, based on the co-chromatography results generated using fresh sample extract prepared for 8-day PHI immature whole plants at some point after metabolite profiling, it appeared that residues of TKC-94-N-malonyl-cysteine may have degraded to TKC-94-cysteine during storage. In addition, it was noted that Unknown 5 likely did not degrade during storage, based on the amount found in fresh extract of 78-day PHI mature leaves prepared an unknown amount of time after the initial extract. Storage stability was not addressed otherwise in the study. However, residue of pethoxamid are stable during frozen storage in corn grain, green plant and straw for up to 24 months (MRIDs 49813542- 49813543)

Table B.7.1.1-11. Summary of Storage Conditions.			
Matrix	Storage Temperature (°C)	Actual Storage Duration	Interval of Demonstrated Storage Stability
Corn immature whole plants	-20	LC/MS analysis ¹ 301-320 days (9.9-10.5 months) TLC analysis of isolated metabolite ² 879 days (28.9 months)	Residues of pethoxamid are stable during frozen storage in/on corn for up to 24 months
Corn leaves		≤879 days (28.9 months) ²	

¹ Calculated by the study reviewer from date of harvest and dated chromatograms.

² Calculated by the study reviewer from date of harvest and the end date provided by the study author for the practical phase of the study.

³ Refer to the DERs for MRIDs 49813542-49813543

D. Identity of Residues in Corn

The characterization and identification of radioactivity in corn is summarized in Table B.7.1.1-12. HPLC analysis of the combined ACN/water extracts of mature leaves following preemergence application and immature and mature leaves following foliar application resolved up to seven discrete unknowns, designated Unknowns 1-7. All seven unknowns were found in mature leaves from both treatments and Unknowns 1-4 were found in immature leaves following foliar application. Unknown 3 was a major residue component in all samples, accounting for 13.6-15.1% TRR (0.017-0.075 ppm) in mature leaves and 17.6% TRR (0.346 ppm) in immature leaves. In addition, Unknowns 6 and 7 were major residue components in mature leaves following preemergence application, at 9.9% TRR (0.011 ppm) and 7.9% TRR (0.009 ppm), respectively, and Unknown 5 was a major residue component in mature leaves following foliar application, at 15.7% TRR (0.086 ppm). Remaining unknowns individually accounted for ≤5.2% TRR (≤0.088 ppm) in mature and immature leaves. The study author stated that the distribution of the unknowns in immature and mature leaves suggested that Unknown 5 is formed primarily as a plant metabolite and that contribution from the soil route is small, while

Unknowns 6 and 7 appear to be soil metabolites that have been taken up by the plants. Because the respective amounts of Unknowns 1-4 were comparable in plants following both preemergence and foliar applications, the study author concluded that these metabolites resulted primarily from pethoxamid, either taken up from the soil or following application to the leaves.

Unknowns 3 and 5 were further investigated in immature and mature leaves, respectively, following foliar application. For unknown 3, the peak was first isolated by preparative chromatography using HPLC Method 3, then evaporated to dryness, reconstituted in water, and cleaned up by solid phase extraction using a C₁₈ cartridge. The sample was eluted with ACN and concentrated for analysis using HPLC Method 7, which indicated that the region contained two major and two minor components. The structures of the major components were investigated by LC/MS, and metabolites TKC-94-SO-lactic acid and TKC-94-S-lactic glucoside (MET-48) were identified based on retention times, mass spectra, and molecular weights. Further investigation of Unknown 5 indicated that it was also comprised of two major and two minor compounds; however, analysis by LC/MS was unsuccessful due to low levels of radioactivity in the sample and the presence of interfering co-extracted plant material.

The majority of characterization and identification efforts were focused on the 8-day PHI immature whole plants collected following foliar application because this matrix bore the highest residues of any matrix in the study, and ~95% of the residues were extractable. Initial analysis of the combined ACN/water extract using HPLC Method 1 characterized the radioactivity in the sample as consisting almost exclusively of polar material. Attempts to further characterize the polar radioactivity via TLC using Methods 1, 2, and 4 were unsuccessful in resolving the compounds present in the sample. A small quantity of parent (0.9% TRR, 0.058 ppm) was identified using HPLC Method 3; otherwise, the radioactivity appeared to be comprised of a large number of unknowns, with only five accounting for >5% TRR individually. The results suggested that the metabolites in the immature plant extract were less polar than those observed in the corresponding mature extracts. The extract was subsequently partitioned with ethyl acetate, and the organic and aqueous phases were analyzed using HPLC Method 4, which was successful in more fully resolving the metabolites in the sample and was utilized in LC/MS analysis to investigate the structures of the residue components.

Further characterization of the residues was conducted following strong acid hydrolysis (2 M and 6 M HCl at 80 and 95 °C, respectively) and base hydrolysis (2 M NaOH at 80 °C) of the combined ACN/water extract. HPLC (Method 1) analysis of the acid hydrolysates indicated that polar radioactivity was converted to a number of less polar species (*ca.* six components between Rts 5-19 min) in the 2 M HCl hydrolysate, with a further simplified profile, consisting of a single major polar component (59.4% TRR, Rt ~7 min.), in the 6 M HCl hydrolysate. TLC Method 4 analysis of the acid and base hydrolysates suggested a greater degree of change in the metabolite profile resulting from acid hydrolysis than base hydrolysis; thus, the base hydrolysate was not further investigated.

Intact metabolites in the ethyl acetate and aqueous phases and hydrolysis products in the acid hydrolysates of 8-day PHI whole plants were investigated by LC/MS. Identification of pethoxamid was confirmed by LC/MS in the ethyl acetate phase, and TKC-94-thiol (MET-43) was tentatively identified based on mass spectra and molecular weight. In the aqueous phase,

TKC-94- γ -glutamyl cysteine (MET-130), TKC-94-cysteine (MET-30), and TKC-94-N-malonyl cysteine (MET-47) were also tentatively identified by LC/MS. TKC-94-cysteine, and TKC-94-N-malonyl cysteine eluted together, with the malonyl-cysteine conjugate eluting as a shoulder on the peak. Identification of TKC-94-cysteine and TKC-94-N-malonyl cysteine was confirmed by HPLC co-chromatography using fresh sample extract prepared for this purpose. A peak corresponding to Unknown 3 in the leaf extracts accounted for 12.3% TRR in the aqueous phase, and a minor unknown eluting at Rt ~54 min was found in both the ethyl acetate and aqueous phases at a total of 5.6% TRR.

On LC/MS of the acid hydrolyzed extracts, benzoic acid and an oxamic acid metabolite (MET-31) and its geometric isomer were tentatively identified. In addition, a possible structure was provided for a fourth region (designated Region 9, 9.2% TRR; refer to Table B.7.1.1-13). A small quantity of benzoic acid was also identified (but not quantitated) in the 2 M HCl hydrolysate of immature leaves. The study author stated that the identification of MET-31 and benzoic acid following acid hydrolysis demonstrated that cleavage of conjugates could be achieved, and that the pethoxamid structure could be completely metabolized under these conditions.

Table B.7.1.1-12. Summary of Characterization and Identification of Radioactive Residues in Corn Matrices Following Application of [¹⁴C]Pethoxamid at 1.1 lb ai/A (1.2 kg ai/ha).¹

Compound ²	Preemergence app.		Foliar application					
	Mature leaves (106-day PHI)		Immature whole plants ² (8-day PHI)		Immature leaves (33-day PHI)		Mature leaves (78-day PHI)	
	TRR = 0.111 ppm		TRR = 6.600 ppm		TRR = 1.963 ppm		TRR = 0.550 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Pethoxamid	ND	--	1.0	0.066	ND	--	ND	--
TKC-94-γ-glutamyl cysteine (MET-130)	ND	--	4.8	0.317	ND	--	ND	--
TKC-94-cysteine (MET-30)	ND	--	7.5	0.498	ND	--	ND	--
TKC-94-N-malonyl cysteine (MET-47)	ND	--			ND	--	ND	--
TKC-94-thiol (MET-43)	ND	--	5.9	0.387	ND	--	ND	--
Unknown 1 (Rt ~41 min)	3.7	0.004	ND	--	3.5	0.068	3.4	0.019
Unknown 2 (Rt ~42 min)	5.2	0.006	ND	--	4.5	0.088	3.5	0.019
Unknown 3 (Rt ~47 min)	15.1	0.017	12.3	0.815	17.6	0.346	13.6	0.075
TKC-94-SO-lactic acid	NA	--	NA	--	8.0	0.158	NA	--
TKC-94-S-lactic glucoside (MET-48)					9.1	0.179		
Minor unknowns (2)					0.3	0.007		
Unknown 4 (Rt ~56 min)	1.3	0.001	ND	--	4.3	0.085	1.1	0.006
Unknown 5 (Rt ~28 min)	3.2	0.004	ND	--	ND	--	15.7	0.086
Unknown 6 (Rt ~17 min)	9.9	0.011	ND	--	ND	--	3.3	0.018
Unknown 7 (Rt ~20 min)	7.9	0.009	ND	--	ND	--	4.5	0.025
Unknown (Rt ~54 min)	ND	--	5.6	0.373	ND	--	ND	--
Multiple minor unknowns	41.2	0.045	57.7	3.806	56.6	1.110	34.9	0.192
HCl/NaOH hydrolysate(s)	--	--	4.1	0.274	10.9 ³	0.214	--	-
Total extractable	87.5	0.097	98.9	6.536	97.4	1.911	80.0	0.440
Total identified	--	--	19.2	1.268	17.1	0.337	--	--
Total unidentified	87.5	0.097	79.7	5.268	80.3	1.574	80.0	0.440
Total bound residues (PES)	12.6	0.014	1.0	0.064	2.6	0.052	20.0	0.110
% Accountability ⁴	100		100		100		100	

¹ ND = Not detected. NA = Not applicable; this component was not investigated in the subject matrix. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable radioactivity (following solvent extraction and hydrolysis as applicable) and nonextractable radioactivity.

² For 8-day PHI whole plants, in addition to the listed compounds, which were identified and quantitated following partitioning of the combined ACN/water extract with ethyl acetate, benzoic acid was identified at 33.7% and 59.4% TRR following acid hydrolysis of the combined ACN/water extract with 2 M and 6 M HCl, respectively, and metabolite MET-31 and its geometric isomer were tentatively identified in the 2 M HCl hydrolysate, together accounting for 16.2% TRR.

³ A small quantity of benzoic acid was identified but not quantitated in the 2 M HCl hydrolysate of immature leaves.

⁴ Total (ppm)/TRR (ppm)×100

E. Proposed Metabolic Pathway

Based on the results of the corn metabolism study, the study author concluded that pethoxamid was rapidly absorbed and metabolized in corn; refer to Figure B.7.1.1-4 for the proposed metabolic pathway. Due to the predominance of sulfur-containing metabolites, the study author proposed that pethoxamid was metabolized primarily via glutathione conjugation, with initial displacement of the chlorine atom by the sulfhydryl group of glutathione, likely in a reaction catalyzed by glutathione-S-transferase. The glutathione conjugate (not found in the study) was metabolized initially by loss of glycine to yield TKC-94-γ-glutamyl cysteine (MET-130), and

then by loss of glutamic acid to yield TKC-94-cysteine (MET-30). The presence of TKC-94-thiol (MET-43), TKC-94-N-malonyl cysteine (MET-47), TKC-94-SO-lactic acid, and TKC-94-S-lactic acid glucoside suggested extensive further metabolism of TKC-94-cysteine, possibly via β -lyase to form TKC-94-thiol and by malonyl transferase to form the N-malonyl conjugate.

Figure B.7.1.1-4. Proposed Metabolic Profile of Pethoxamid in Corn.

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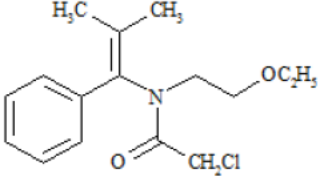
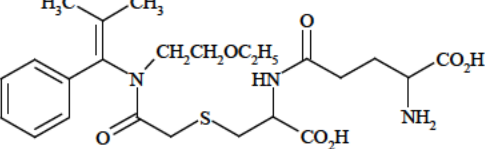
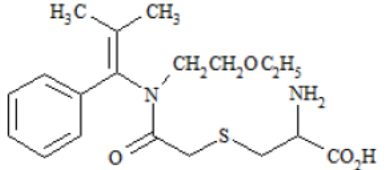
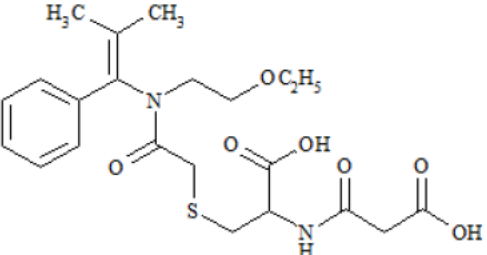
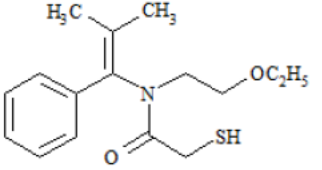
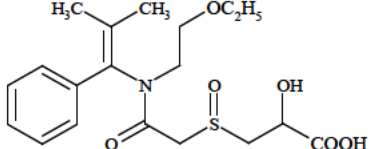
Table B.7.1.1-13. Identification of Compounds Found in the Metabolism Study.¹		
Common Name/Code ²	Chemical Name	Chemical Structure
Pethoxamid	2-chloro-N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propen-1-yl)acetamide	
TKC-94- γ -glutamyl cysteine (MET-103)	None provided	
TKC-94-cysteine (MET-30)	S-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]-D,L-cysteine	
TKC-94-N-malonyl cysteine (MET-47)	N-(2-carboxyacetyl)-S-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]-D,L-cysteine	
TKC-94-thiol (MET-43)	N-(2-ethoxyethyl)-2-mercapto-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
TKC-94-SO-lactic acid	None provided	

Table B.7.1.1-13. Identification of Compounds Found in the Metabolism Study. ¹		
Common Name/Code ²	Chemical Name	Chemical Structure
TKC-94-S-lactic acid glucoside (MET-48)	3-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoyl methylthio]-2-(α -D-glucopyranosyloxy) propionic acid	
Benzoic acid (following strong acid hydrolysis only)	Benzoic acid	
MET-31 (following strong acid hydrolysis only)	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)oxamic acid	
Region 9 (following strong acid hydrolysis only; putative structure)	None provided	

¹ Refer to Appendix I for names and structures of all reference standards used in the study.

² Identification of pethoxamid, TKC-94-cysteine, and TKC-94-N-malonyl cysteine was confirmed; all other metabolites were tentatively identified.

III. CONCLUSIONS

The corn metabolism study is considered scientifically acceptable. Samples of immature and mature corn matrices were harvested following application of [phenyl- U - ^{14}C]pethoxamid at 1.1 lb ai/A (1.2 kg ai/ha) to corn as an early postemergence application made to the soil 21 days after planting; a preemergence application made 1 day after planting; or a foliar application to corn plants at BBCH 14-15 made 29 days after planting. The highest TRR were observed in 8-day PHI immature whole plants collected following foliar application, and the lowest TRR were found in grain and cobs following soil and foliar applications. TRR in corn matrices were generally lower in immature and mature plants harvested following soil application than following foliar application, and residues in corn plants receiving foliar application declined from first immature harvest to mature harvest. TRR in immature whole plants were 0.011 and 0.008 ppm in samples harvested 14 and 27 days, respectively, following postemergence soil application; 0.035 ppm in samples harvested 36 days following preemergence application; and

6.600 ppm in samples harvested 8 days following foliar application. TRR in immature leaves, stems, and roots, respectively, were 0.049, 0.002, and 0.104 ppm in samples harvested 61 days following preemergence application, and 1.963, 0.008, and 0.021 ppm in samples harvested 33 days following foliar application. In mature corn matrices harvested 106 days following preemergence application and 78 days following foliar application, TRR in grain and cobs were ≤ 0.004 ppm. TRR in leaves, stems, and roots respectively, were 0.111, 0.028, and 0.114 ppm following preemergence application and 0.550, 0.011, and 0.018 ppm following foliar application.

Following initial extraction and hydrolysis procedures, only four matrices were selected for further investigation of residues: mature leaves following both soil and foliar applications and 8-day PHI immature whole plants and 33-day PHI immature leaves following foliar application.

A total of 19.2% TRR was identified in 8-day PHI immature whole plants. Pethoxamid was a minor residue component, accounting for 1.0% TRR. In addition, four metabolites were identified at $<10\%$ TRR each: TKC-94-cysteine- γ -glutamyl cysteine (MET-103) at 4.8% TRR, TKC-94-cysteine (MET-30) and TKC-94-N-malonyl-cysteine (MET-47), together accounting for 7.5% TRR, and TKC-94-thiol (MET-43) at 5.9% TRR. The major residue component, which accounted for 12.3% TRR, was an unknown designated Unknown 3, that was found to be nearly entirely comprised of metabolites TKC-94-SO-lactic acid and TKC-94-S-lactic glucoside (MET-48) during analysis of 33-day PHI immature leaves. Following strong acid hydrolysis of the plant extract, benzoic acid was found at 33.7-59.4% TRR in the 2 M and 6 M HCl hydrolysates, and oxamic acid metabolite MET-31 and its geometric isomer were tentatively identified, together accounting for 16.2% TRR in the 2 M HCl hydrolysate. Remaining radioactivity was comprised of one more discrete unknown (5.6% TRR) and multiple minor unknowns that were not further resolved but together accounted for 57.7% TRR.

A total of 17.1% TRR (0.337 ppm) was identified in 33-day PHI immature leaves; no residue components were identified in mature leaves. Seven discrete unknowns, designated Unknowns 1-7, were found in mature leaves from both preemergence and foliar applications, and Unknowns 1-4 were found in immature leaves following foliar application. Unknown 3 was a major residue component in all samples, accounting for 13.6-15.1% TRR in mature leaves and 17.6% TRR in immature leaves. In immature leaves, the unknown was found to be comprised of TKC-94-SO-lactic acid at 8.0% TRR, TKC-94-S-lactic glucoside (MET-48) at 9.1% TRR, and two minor unknown components. Unknowns 6 and 7 were major residue components in mature leaves following preemergence application, at 9.9% TRR and 7.9% TRR, respectively, and Unknown 5 was a major residue component in mature leaves following foliar application, at 15.7% TRR. Further investigation of Unknown 5 indicated that, like Unknown 3, it was comprised of two major components and two minor components; however, identification attempts were unsuccessful. Remaining unknowns individually accounted for $\leq 5.2\%$ TRR in mature and immature leaves, and multiple minor unknowns that were not further resolved together accounted for 34.9-56.6% TRR. In addition to these components, a small amount of benzoic acid was identified, but not quantitated, in the 2 M HCl hydrolysate of immature leaves.

Based on the results of the corn metabolism study, the study author concluded that pethoxamid was rapidly absorbed and metabolized in corn. Due to the predominance of sulfur-containing

metabolites, the study author proposed that pethoxamid was metabolized primarily via glutathione conjugation, with initial displacement of the chlorine atom by the sulfhydryl group of glutathione, likely in a reaction catalyzed by glutathione-S-transferase. The glutathione conjugate (not found in the study) was metabolized initially by loss of glycine to yield TKC-94- γ -glutamyl cysteine (MET-130), and then by loss of glutamic acid to yield TKC-94-cysteine (MET-30). The presence of TKC-94-thiol (MET-43), TKC-94-N-malonyl cysteine (MET-47), TKC-94-SO-lactic acid, and TKC-94-S-lactic acid glucoside suggested extensive further metabolism of TKC-94-cysteine, possibly via β -lyase to form TKC-94-thiol and by malonyl transferase to form the N-malonyl conjugate.

REFERENCES

Document ID: MRID 49813534; PMRA Study # xxxxxx, PMRA Review # xxxxxx
Report: Bitter, J. (2017) S-2399: Freezer Storage Stability of S-2399 and Metabolites in Processed Fractions. Report No. 201700305; Laboratory Project ID VP-40066. Unpublished study sponsored by Valent USA LLC. 836 p.

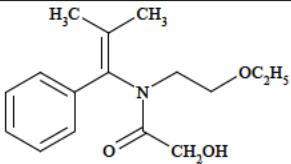
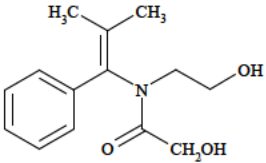
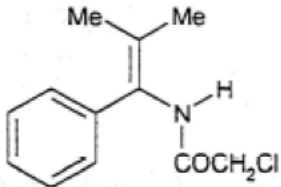
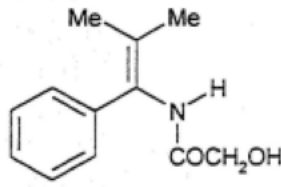
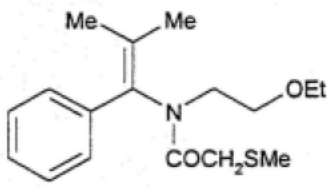
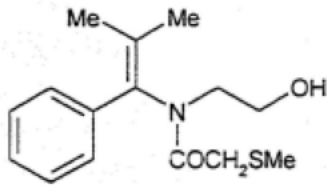
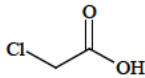
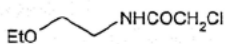
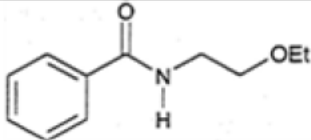
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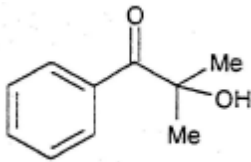
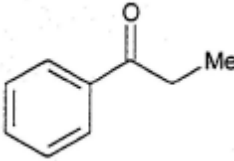
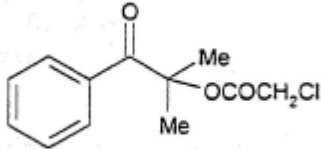
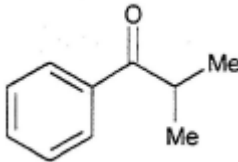
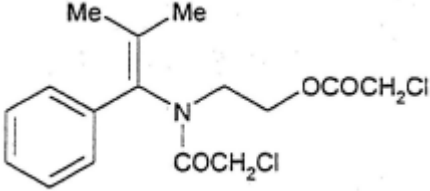
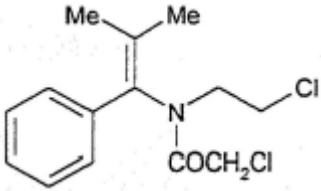
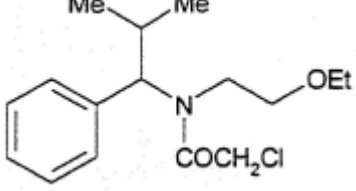
Report: Brewin, S (2000). The Development and Validation of a Method of Analysis for the Determination of Residues in Maize (Grain, Green Plant and Straw. Study No. 59 PXA. Unpublished study prepared by Huntingdon Life Sciences Ltd in United Kingdom sponsored by Cheminova A/S in Denmark and submitted by FMC Corporation. 69 p

Sorensen, L (2000). Supplementary Data to TKC-94 The Development and Validation of a Method of Analysis for the Determination of Residues in Maize (Grain, Green Plant and Straw). Cheminova Doc. No 59 PXA. Unpublished study prepared by Cheminova A/S in Denmark and submitted by FMC Corporation. 46 p.

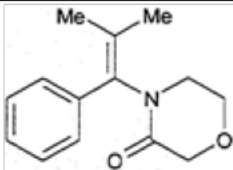
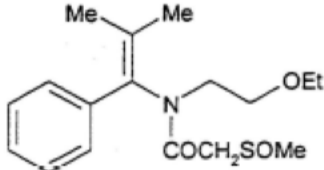
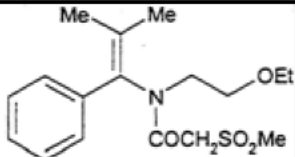
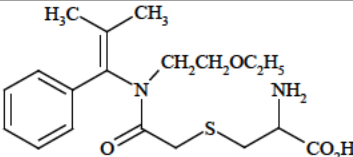
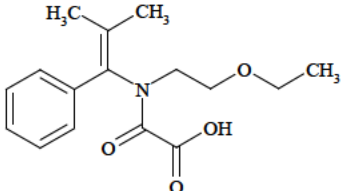
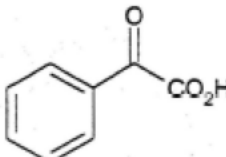
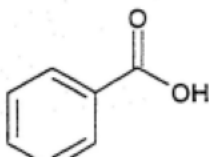
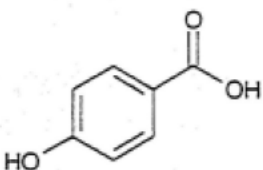
Appendix I. Reference Standards Used in the Study.

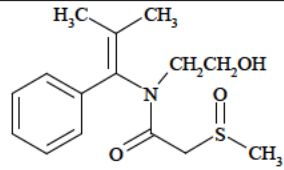
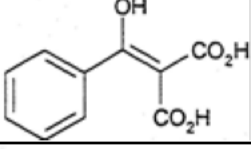
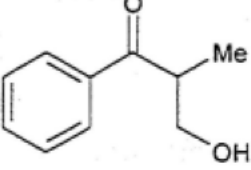
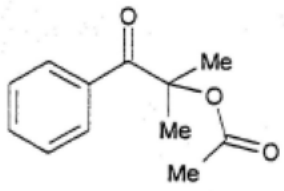
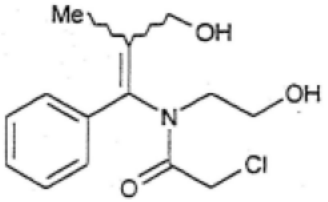
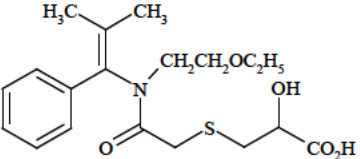
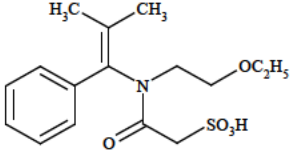
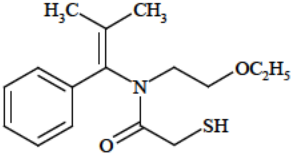
Name	Chemical name	Structure
MET-I	2-chloro-N-(2-hydroxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide	

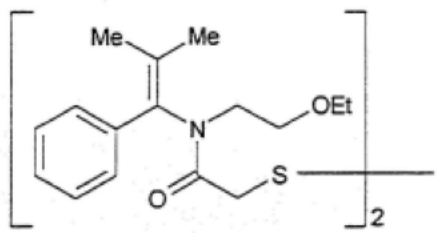
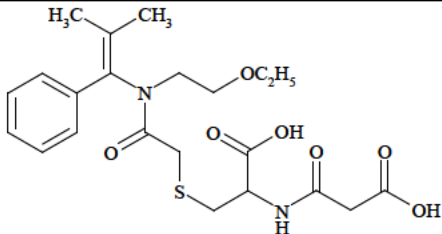
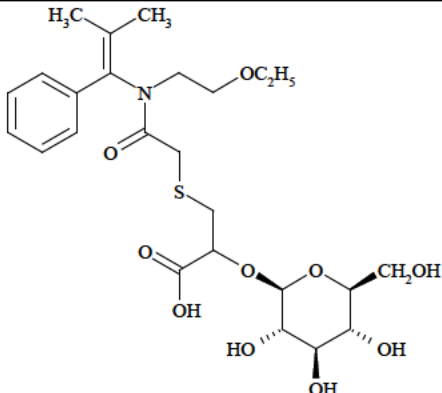
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MET-2	N-(2-ethoxyethyl)-2-hydroxy-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-3	2-hydroxy-N-(2-hydroxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-4	2-chloro-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-5	2-hydroxy-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-6	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-(methylthio)acetamide	
MET-7	N-(2-hydroxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-(methylthio)acetamide	
MET-8	chloroacetic acid	
MET-9	2-chloro-N-(2-ethoxyethyl)acetamide	
MET-10	N-(2-ethoxyethyl)benzamide	

Name	Chemical name	Structure
MET-11	2-hydroxy-2-methylpropiophenone	
MET-12	propiophenone	
MET-13	2-(2-chloroacetoxy)-2-methylpropiophenone	
MET-14	isobutyrophenone	
MET-15	2-chloro-N-[2-(2-chloroacetoxy)ethyl]-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-16	2-chloro-N-(2-chloroethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-17	2-chloro-N-(2-ethoxyethyl)-N-[(2-methyl-1-phenyl)propyl]acetamide	

Name	Chemical name	Structure
MET-18	3-chloro-N-(2-ethoxyethyl)-4-isopropyl-4-phenyl-2-azetidinone	
MET-19	2,2-dichloro-N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-20	2-chloro-2-methylpropiophenone	
MET-21	2-(2,2-dichloroacetoxy)-2-methylpropiophenone	
MET-22	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-23	(E,Z)-2-chloro-N-(2-ethoxyethyl)-N-(1-phenyl-1-propenyl)acetamide	
MET-24	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl)propylamine	
MET-25	2-ethoxyethylamine	
MET-26	N-(2-hydroxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide	

Name	Chemical name	Structure
MET-27	N-(2-methyl-1-phenyl-1-propenyl)morpholine-3-one	
MET-28	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-(methylsulfinyl)acetamide	
MET-29	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-(methylsulfonyl)acetamide	
MET-30	S-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]-D,L-cysteine	
MET-31	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)oxamic acid	
MET-32	phenylglyoxylic acid	
MET-33	benzoic acid	
MET-34	4-hydroxybenzoic acid	

Name	Chemical name	Structure
MET-35	N-(2-hydroxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-(methylsulfinyl)acetamide	
MET-36	2-carboxy-3-hydroxycinnamic acid	
MET-37	3-hydroxy-2-methylpropiophenone	
MET-38	2-acetoxy-2-methylpropiophenone	
MET-40	(E,Z)-2-chloro-N-(2-hydroxyethyl)-N-(3-hydroxy-2-methyl-1-phenyl-1-propenyl)acetamide	
MET-41	3-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoyl methylthio]-D,L-lactic acid	
MET-42	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-sulfoacetamide	
MET-43	N-(2-ethoxyethyl)-2-mercapto-N-(2-methyl-1-phenyl-1-propenyl)acetamide	

Name	Chemical name	Structure
MET-44	bis S-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]	
MET-47	N-(2-carboxyacetyl)-S-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]-D,L-cysteine	
MET-48	3-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethylthio]-2-(α-D-glucopyranosyloxy)propionic acid	

**B.7.1 Metabolism, Distribution, and Expression of Residues in Plants
(Annex IIA 6.2; Annex IIIA, 8.2)**

B.7.1.2 Potato

Document ID: MRID No. 49813529
PMRA No. Not available

Report: Mohajeri, S. and Zane, J. (2015) The Metabolism of [¹⁴C]Pethoxamid in Potatoes (One Radiolabel). Study Numbers: 1747 PXA; 2505W; 2505W-1. Unpublished study prepared by PTRL West, Inc., sponsored by Cheminova, Inc., and submitted by FMC Corporation. 259 p.

Guidelines: EPA OCSPH Harmonized Test Guideline 860.1300 Nature of the Residue - Plants, Livestock (August 1996)
PMRA Regulatory Directive Dir98-02 – Residue Chemistry Guidelines, Section 2 -Nature of the Residue - Plants, Livestock
OECD Guideline 501 Metabolism in Crops (January 2007)

GLP Compliance: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Acceptability: The study is considered scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP#452109.

Evaluator: Sheila Piper, Chemist, HED, RAB6 *Sheila Piper*

Secondary Evaluator: Robert Lanthier, PMRA

Note: This Data Evaluation Record (DER) was prepared under contract by CDM/CSS-Dynamac Joint Venture (submitted 2/16/18). The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies. The DER has been reviewed by HED and revised as necessary to reflect current Office of Pesticide Programs (OPP) policies.

EXECUTIVE SUMMARY

The metabolism of [phenyl-U-¹⁴C]pethoxamid (specific activity: 1.67 MBq/mg) in potatoes grown outdoors was formulated as a 57.3% emulsifiable concentrate (EC) formulation and applied as a single foliar application at 1.21 lb ai/A (1.36 kg ai/ha). The application was made to plants at BBCH 25 (fifth basal side shoots visible), 38 days after planting. Samples of immature foliage were harvested on the day of application, samples of immature foliage and tubers were harvested 37 days after application, and samples of mature foliage and tubers were harvested 76 days after application. The in-life phase of the study was conducted by Excel Research Services, Inc. (Fresno, CA), and the analytical phase of the study was conducted by PTRL West, Inc. (Hercules, CA).

Total radioactive residues (TRR) in potato matrices were determined by combustion/LSC and by summing extractable and nonextractable radioactivity; summed TRR were used for all further determinations. TRR were highest in 0-day foliage and declined with increasing PHI in both foliage and tubers. TRR were: 18.102, 2.069, and 1.521 ppm in foliage harvested 0, 37, and 76 days, respectively, following application; and 0.059 and 0.013 ppm in tubers harvested 37 and 76 days, respectively, following application.

Extraction with acetonitrile (ACN) and water released the majority of the TRR from all potato matrices: 88-98% from foliage, and 77-86% from tubers. For the post-extraction solids (PES) of foliage, sequential mild and strong hydrolyses with HCl, KOH, and H₂SO₄ released a total of 1.9-9.1% TRR. Remaining nonextractable residues accounted for ≤ 0.048 ppm in all matrices. Recoveries were normalized for the total amount of radioactivity recovered in the extracts, hydrolysates, and post-extraction solids (PES); therefore, accountabilities were 100% for each matrix.

Residues were quantified and identified in the ACN/water extracts of potato matrices by high performance liquid chromatography (HPLC) with UV detection and radiodetection. Identification of pethoxamid and the pethoxamid cysteine conjugate (MET-30) in foliage was confirmed by thin layer chromatography (TLC). Metabolites pethoxamid-SO-cysteine (M42) and pethoxamid-SO-lactic acid glycoside (M44) were identified and identities confirmed by high performance liquid chromatography with mass spectrometry detection and tandem mass spectrometry detection (LC/MS and LC/MS/MS). Samples were extracted within 9-19 days of harvest, and the extracts were stored frozen for 1-14 days prior to initial profiling and for up to 632 days for foliage and 625 days for tubers prior to final analysis for residue identification and quantitation. Supporting storage stability data were provided demonstrating the stability of the metabolite profile in the extracts for up to 631 days in foliage and 596 days in tubers. These data are adequate to support the sample storage durations and conditions from the submitted study.

Pethoxamid was the major residue component in 0-day foliage, at 77.1% TRR (13.960 ppm). Pethoxamid cysteine conjugate (MET-30) was also identified in 0-day foliage at 3.4% TRR (0.612 ppm). Pethoxamid-SO-cysteine and pethoxamid-SO-lactic acid glycoside were identified in 37-day foliage at 10.0% TRR (0.207 ppm) and 7.9% TRR (0.163 ppm), respectively, and the SO-lactic acid glycoside was also identified in 76-day foliage at 7.0% TRR (0.107 ppm). Remaining radioactivity in 0-day foliage was comprised of three discrete unknowns present at 1.7-11.0% TRR (0.300-1.996 ppm). In 37- and 76-day foliage, the majority of the remaining radioactivity consisted of multiple minor unknowns (each present at $\leq 4.8\%$ TRR or ≤ 0.100 ppm) that together accounted for 72.5% TRR (1.562 ppm) in 37-day foliage and 80.7% TRR (1.228 ppm) in 76-day foliage.

No metabolites were identified in potato tubers. Radioactivity was characterized as a complex mixture of components, with the major residue component in 37-day tubers accounting for 13.6% TRR (0.008 ppm) and the major component in mature tubers accounting for 15.4% TRR (0.002 ppm). TLC analysis of the extract for 37-day tubers indicated the possible presence of metabolites MET-3, MET-30, MET-42, MET-47, and MET-103, but this was not confirmed using a second system. No correlation with reference standards was observed on TLC analysis of mature tubers.

Acid hydrolysis of the ACN/water extracts of 0-day immature foliage and 37-day immature foliage and tubers released metabolites that were found to be cleaved components of pethoxamid in the rotational crop study submitted under this action (refer to the DER for MRID 49813552, B.7.9.1.1), including RT24H and RT28H (which were tentatively identified/identified in that study) and RT31H, RT51H, and RT54H (which were not identified). These unknowns were not further investigated in this metabolism study.

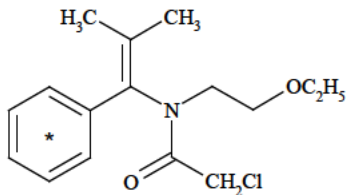
Based on the results of the potato metabolism study, the study authors proposed that the metabolic pathway of pethoxamid proceeds initially by displacement of the chlorine atom by the thiolate anion of glutathione to yield a glutathione conjugate (not found in this study). The glutamic acid and glycine portions of glutathione are then cleaved to form the cysteine conjugate MET-30, which may be metabolized by a number of pathways common to cysteine conjugates, forming many products. MET-30 was oxidized in potato to yield pethoxamid-SO-cysteine, and was converted to its 2-hydroxypropyl analog and conjugated with glucose to form pethoxamid-SO-lactic acid glycoside.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

The radiolabeled test substance, dissolved in toluene, was isotopically diluted with non-labeled pethoxamid. The solvent was evaporated under nitrogen and mixed with blank EC formulation and water to a concentration of 57.3% pethoxamid. Radiochemical purity was measured before and after application to the test plots.

Table B.7.1.2-1. Pethoxamid Nomenclature.	
Common name	Pethoxamid
Identity	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide
CAS registry number	106700-29-2
Company experimental name	TKC-94
Radiolabeled compound	[phenyl- ¹⁴ C]pethoxamid
Lot/Batch #	CFQ41685
Radiochemical purity	98.0% (pre-application), 98.1% (post-application)
Specific activity as received	29 mCi/mmol (3.63 MBq/mg, 2.18 x 10 ⁸ dpm/mg)
Specific activity of dose	1.67 MBq/mg (100,000 dpm/μg)
Position of radiolabels	

2. Test Crop

Table B.7.1.2-2. Crop Information.					
Crop/Crop Group	Variety	Growth Stage at Application	PHI (days)	Growth Stage at Harvest	Harvested Commodities
Potato/Root and tuber vegetable, group 1	Cal White	BBCH 25 (fifth basal side shoots visible)	0	Immature: BBCH 25	Immature foliage
			37	Immature: BBCH 43-45	Immature foliage and tubers
			76	Mature: BBCH 49	Mature foliage and tubers

3. Soil Type

Table B.7.1.2-3. Soil Physicochemical Properties.							
Soil Type	pH	Organic Matter (%)	Sand (%)	Silt (%)	Clay (%)	Moisture Holding Capacity (% at 1/3 bar)	Cation Exchange Capacity (meq/100 g)
Sandy loam	7.4	0.76	66	19	15	Not reported	9.8

B. STUDY DESIGN

Experimental Conditions

Seed potatoes were planted and grown outdoors in wooden boxes (1 m²) filled with sandy loam soil to a depth of 46 cm. The crops were watered and fertilized according to typical agronomic practices. Weather conditions during the field phase of the study were reported to be typical of the area. The test site was located near Madera, CA.

The formulated test substance was applied to potato plants at BBCH 25 at 1.21 lb ai/A (1.36 kg ai/ha) using a manually-operated, trigger-pulled pump sprayer.

The study use pattern is summarized in Table B.7.1.2-4.

Table B.7.1.2-4. Use Pattern Information.	
Chemical name	[phenyl-U- ¹⁴ C]pethoxamid
Application method	The formulated test substance was applied to potato plants at BBCH 25 using a manually-operated trigger-pulled pump sprayer.
Application rate	1.21 lb ai/A (1.36 kg ai/ha)
Number of applications	1
Timing of applications	38 days after planting
PHI	1st harvest: 0 days 2 nd harvest: 37 days 3 rd harvest: 76 days

Sampling

Samples of immature foliage were harvested on the day of application, after the test substance had dried (~1 hour after application). Samples of immature foliage and tubers, intended to simulate collection of an early crop, were harvested 37 days after application, and samples of mature foliage and tubers were harvested 76 days after application. At the first harvest, immature foliage was collected by cutting with pruning shears. At the second and third harvests, immature and mature plants were pulled from the soil, and tubers were collected by hand and brushed to remove soil; leaves and stems were cut into small pieces with pruning shears. Samples were placed in frozen storage (temperature unspecified) immediately after sampling and were delivered frozen to the analytical laboratory, PTRL West, Inc. (Hercules, CA) within 3 days of harvest by Excel Research Services, Inc. or PTRL West, Inc. vehicle (on dry ice). At the analytical laboratory, samples were maintained frozen (*ca.* -20 °C) prior to and following processing and were homogenized in the presence of dry ice for extraction and analysis.

TRR Determination

TRR in the extracts and hydrolysates of rotational crop commodities were determined by direct LSC, and TRR in homogenized crop matrices and PES were determined by combustion/LSC. TRR in samples were also determined by summing radioactivity in extract and PES fractions. The reported limits of quantitation and detection for TRR determinations were 0.004 and 0.002 ppm, respectively.

Extraction and Analysis

Samples of immature and mature potato matrices were extracted sequentially with ACN:water (1:1, v:v; twice) and ACN by shaking for 30 minutes for each extraction. The extracts were isolated by centrifugation and combined for HPLC analysis. The extract of mature tubers was concentrated under nitrogen to remove organic solvent and applied to a weak anion exchange solid phase extraction cartridge (Varian Bond Elut LRC, NH₂ bonded phase), and residues were eluted with water, methanol, and 5% ammonium hydroxide in methanol. The PES for foliage samples from all harvest intervals were subjected to the following sequential acid and base hydrolyses: 0.1 M HCl, 0.1 M KOH, 6 M HCl, and 6 M KOH for 2 hours at ambient temperature; followed by 6 M HCl for 2 hours at reflux, and, for first harvest foliage only, 6 M KOH for 2 hours at reflux and 72% H₂SO₄ for 4 hours (ambient temperature). The hydrolysates were not analyzed by HPLC.

Flowcharts of the extraction and hydrolysis procedures are presented in Figures B.7.1.2-1 and B.7.1.2-2, respectively.

In addition, the combined extracts of 0-day immature foliage and 37-day immature foliage and tubers were subjected to acid hydrolysis in attempts to further characterize residues by converting major components to common moieties. An aliquot of each extract was mixed with 4 N HCl to achieve an overall concentration of 2 N HCl, and the mixture was heated at 100 °C for 2 hours. The hydrolyzed extract was neutralized with 30% (w:v) ammonium hydroxide, concentrated (to remove ammonium hydroxide), and filtered through a 0.45-µm syringe filter for HPLC analysis.

Figure B.7.1.2-1. Flowchart for the Extraction of Potato Matrices.

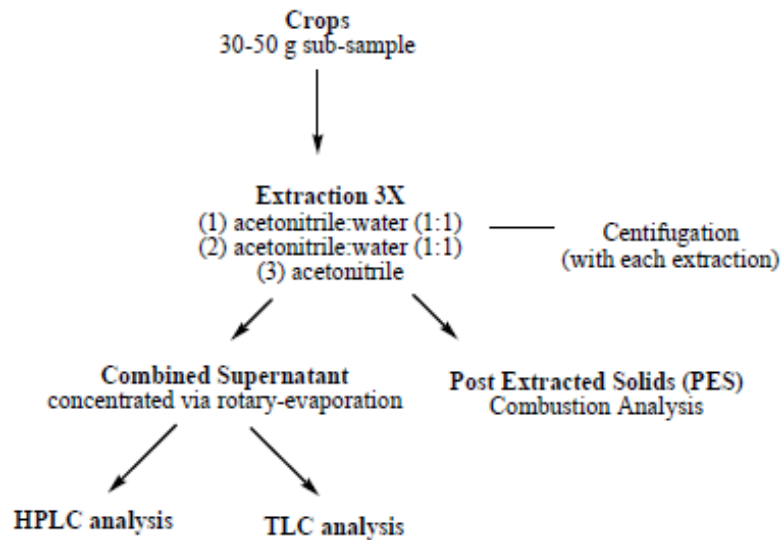
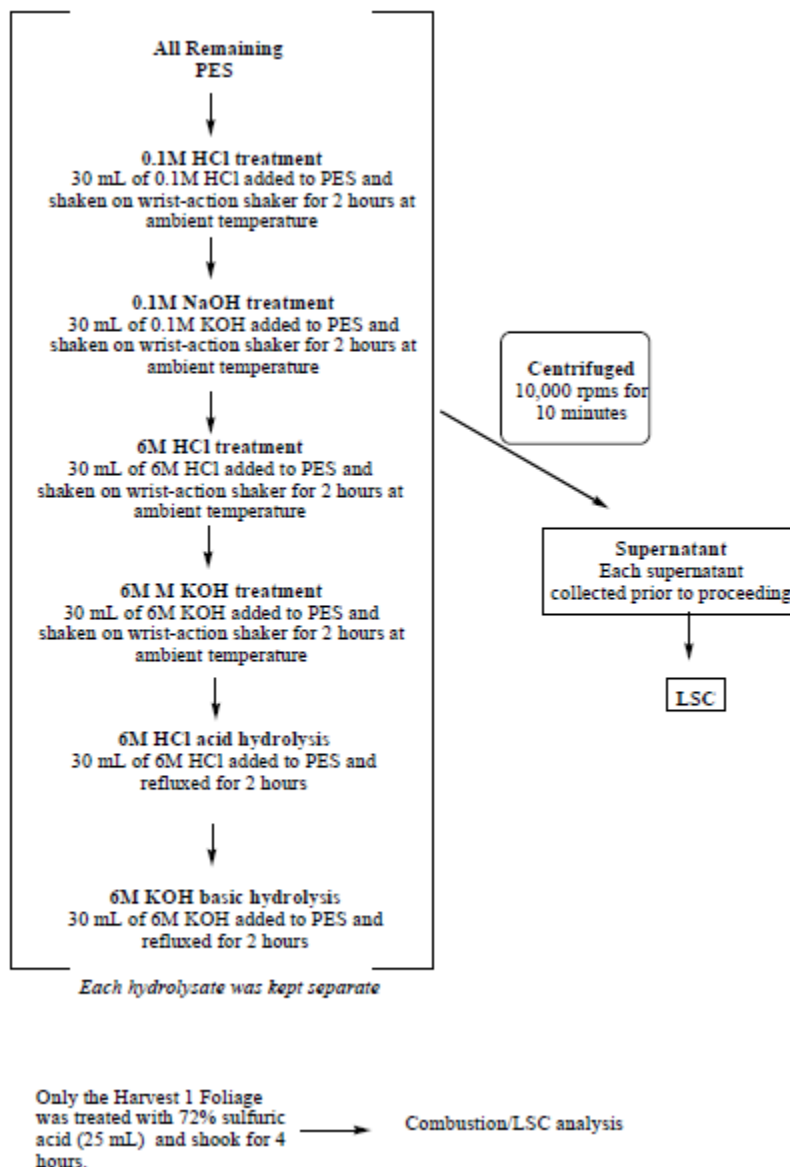


Figure B.7.1.2-2. Flowchart for the Hydrolysis of Post-Extraction Solids of Potato Matrices.



Identification and Characterization

Extracts were analyzed using reverse-phase HPLC with UV detection (254 nm) and radiodetection using either a radioisotope flow monitor or fraction collection. Peak assignments were based on co-elution with reference standards (see Table B.7.1.2-11) injected with each sample. The following HPLC methods were used:

- Method 3 (all extracts, initial profiling): C18 column (Capcell Pak MGII) and gradient mobile phase of water and ACN, each containing 0.1% trifluoroacetic acid;
- Method 6A (all extracts, final profiling): C18 column (Synergy fusion-RP) and gradient mobile phase of ACN and 0.01 M formate buffer (pH 3.0) in water;

- Method 6A (semi-preparative isolation of metabolites): C18 semi-prep column (Capcell Pack MG 5) and gradient mobile phase of ACN and 0.01 M formate buffer (pH 3.0) in water;
- Method 10 (purification of metabolites): C18 column (Synergi Polar-RP) and gradient mobile phase of water and methanol, each containing 0.1% formic acid;
- Method 16 (semi-preparative purification of metabolites): cyano column (SB-CN Semi-Prep) and a gradient mobile phase of water and ACN;
- Method CR (hydrolyzed extracts and select neutral extracts): reverse-phase column (Capcell CR, a 1:20 mixture of SCX and C18) and a gradient mobile phase of 0.1% formic acid in ACN and 10 mM ammonium acetate in water.

The ACN/water extracts of all matrices were also analyzed by TLC. Analyses were conducted on silica gel 60 Å F₂₅₄ plates using a solvent system of chloroform:methanol:acetic acid (70:30:3, v:v:v). Radioactivity was detected using a phosphorimager, and non-labeled standards were determined by quenching of UV light-induced fluorescence.

Metabolites pethoxamid-SO-cysteine and pethoxamid-SO-lactic acid glycoside (initially designated M42/Peak 1 and M44/Peak 2, respectively) were isolated and purified from 37-day immature foliage. Four subsamples of the foliage were extracted as above. The extracts were combined, concentrated by rotary evaporation, and partitioned with ethyl acetate multiple times. The resulting aqueous phase was concentrated, and the metabolites were isolated by semi-preparative HPLC Method 6A with fraction collection. The corresponding fractions for each analyte were combined, then concentrated and neutralized, and the isolated peaks were further purified as follows: (i) HPLC Methods 16 and 10 (cyano and phenyl columns); (ii) SPE on Waters Oasis MAX 500 cartridges, with residues eluted with 25 mM ammonium acetate (pH 6), 5% formic acid in ACN, methanol, 5% formic acid in ACN, methanol and 5% ammonium hydroxide; and (iii) further semi-preparative HPLC using Method 16, followed by concentration, acidification, and cleanup using Method 10. The purified metabolites were reserved for LC/MS and LC/MS/MS analysis.

For LC/MS and LC/MS/MS analysis, HPLC separation was conducted using a reverse phase C18 column (Synergi Fusion-RP) and a gradient mobile phase of ACN and 10 mM ammonium formate buffer (pH 3.1) in water, and full scan MS detection was performed with a Thermo Scientific Q-Exactive mass spectrometer using electrospray ionization with alternating positive/negative polarity.

II. RESULTS AND DISCUSSION

A. Total Radioactive Residues

The TRR in potato samples are summarized in Table B.7.1.2-5. The data indicate that residues declined steadily in foliage and tubers with increasing PHI.

Table B.7.1.2-5. TRR in Potato Matrices.		
Matrix	PHI (days)	Residues ¹ (ppm [¹⁴ C]pethoxamid equivalents)
Immature foliage	0	22.007 (18.102)
Immature foliage	37	2.312 (2.069)
Immature tuber		0.056 (0.059)
Mature foliage	76	1.831 (1.521)
Mature tuber		0.013 (0.013)

¹ Residues in all matrices were determined by combustion/LSC and by summing extractable and nonextractable radioactivity. Summed TRR values (presented in parentheses) were used for all further determinations.

B. Extraction, Characterization, and Distribution of Residues in Potato

The distribution of radioactivity in potato matrices is presented in Table B.7.1.2-6 (foliage) and B.7.1.2-7 (tubers).

Table B.7.1.2-6. Distribution of Radioactive Residues in Potato Foliage Following Application of [¹⁴C]Pethoxamid at 1.21 lb ai/A (1.36 kg ai/ha).¹						
Metabolite Fraction	Immature foliage (0-day PHI)		Immature foliage (37-day PHI)		Mature foliage (76-day PHI)	
	TRR = 18.102 ppm		TRR = 2.069 ppm		TRR = 1.521 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN/water	98.0	17.741	93.4	1.933	87.8	1.335
Pethoxamid	77.1	13.960	ND	--	ND	--
Pethoxamid cysteine conjugate (MET-30)	3.4	0.612	ND	--	ND	--
Pethoxamid-SO-cysteine (M42)	ND	--	10.0	0.207	ND	--
Pethoxamid-SO-lactic acid glycoside (M44)	ND	--	7.9	0.163	7.0	0.107
Region 1 (Rt 46.52 min)	4.8	0.873	ND	--	ND	--
Region 2 (Rt 47.50 min)	11.0	1.996	ND	--	ND	--
Region 4 (Rt 55.72 min)	1.7	0.300	ND	--	ND	--
Region 8 (Rt 16.72 min)	ND	--	2.5	0.051	ND	--
Region 9 (Rt 18.22 min)	ND	--	4.8	0.100	ND	--
Region 14 (Rt 18.30 min)	ND	--	ND	--	3.8	0.058
Region 20 (Rt 24.75 min)	ND	--	3.3	0.068	ND	--
Region 26 (Rt 25.45 min)	ND	--	ND	--	4.5	0.068
Region 29 (Rt 28.62 min)	ND	--	2.7	0.056	ND	--
Minor unknowns ²	ND	--	62.2	1.287	72.4	1.102
PES ³	2.0	0.361	6.6	0.136	12.2	0.186
0.1 N HCl ambient hydrolysis	0.2	0.040	0.5	0.010	0.7	0.011
0.1 N KOH ambient hydrolysis	0.5	0.098	1.2	0.025	3.8	0.058
6 N HCl ambient hydrolysis	0.2	0.028	0.7	0.014	1.1	0.017
6 N KOH ambient hydrolysis	0.3	0.063	1.1	0.023	1.8	0.027
6 N HCl reflux hydrolysis	0.4	0.078	1.2	0.024	1.6	0.025
6 N KOH reflux hydrolysis	0.2	0.040				
72% H ₂ SO ₄ hydrolysis	<<0.1	0.002				
PES	0.1	0.012	1.9	0.040	3.2	0.048

¹ ND = Not detected. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Consisting of: 51 unknowns, plus other extractable components, each present at ≤2.3% TRR (≤0.048 ppm) in 37-day immature foliage; and 52 unknowns, plus other extractable components, each present at ≤3.0% TRR (≤0.046 ppm) in mature foliage.

³ Calculated by the study reviewer by subtraction.

Table B.7.1.2-7. Distribution of Radioactive Residues in Potato Tubers Following Application of [¹⁴C]Pethoxamid at 1.21 lb ai/A (1.36 kg ai/ha).¹				
Metabolite Fraction	Immature tuber (37-day PHI)		Mature tuber (76-day PHI)	
	TRR = 0.059 ppm		TRR = 0.013 ppm	
	%TRR	ppm	%TRR	ppm
ACN/water	86.4	0.051	76.9	0.010
Region 1 (Rt 4.25 min)	3.4	0.002	15.4	0.002
Region 11 (Rt 23.75 min)	5.1	0.003	ND	--
Region 18 (Rt 33.75 min)	5.1	0.003	ND	--
Region 21 (Rt 41.25 or 42.75 min) ²	8.5	0.005	7.7	0.001
Region 22 (Rt 43.25 or 46.25 min) ²	ND	--	7.7	0.001
Region 23 (Rt 45.75 min)	13.6	0.008	ND	--
Region 28 (Rt 53.75 min)	6.8	0.004	ND	--
Minor unknowns ³	44.3	0.026	46.2	0.006
PES	13.6	0.008	23.1	0.003

¹ ND = Not detected. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² The second retention time is for mature tuber.

³ Consisting of: 20 unknowns, plus other extractable components, each present at $\leq 3.4\%$ TRR (≤ 0.002 ppm) in immature tuber; and 37 unknowns plus other extractable components, each present at $\leq 3.1\%$ TRR (≤ 0.0004 ppm) in mature tuber.

C. Storage Stability of Residues

Sample storage conditions and intervals are presented in Table B.7.1.2-8. Extraction with ACN/water was completed within 9-19 days of sample collection. Analyses of the extracts using HPLC Method 3 for initial profiling were completed within 1-14 days of extraction, and the final analyses for quantitation of residues using HPLC Method 6A were completed within 549-632 days of extraction. The final analysis results are reported herein.

To demonstrate the stability of the metabolite profile in the extracts during frozen storage, extracts of all potato matrices were re-analyzed using HPLC Method 3 following storage for 547-631 days for foliage and 548-596 days for tubers. Both quantitative and chromatographic data were provided confirming that the HPLC profiles were very similar for all matrices. Storage stability results for 0-day foliage were nearly identical to initial analysis results. For the 37-day immature samples and 76-day mature samples, although peaks were generally clustered together more closely in the storage stability analysis, the overall profiles were otherwise nearly identical for each matrix.

Although acid hydrolysis of the ACN/water extracts of immature foliage and tubers took place after final profiling (624-658 and 627-661 days after extraction for hydrolysis and analysis, respectively), the demonstrated interval of residue stability in the stored extracts is sufficient to support the investigation. We note that this investigation did not contribute materially to the elucidation of the nature of the residue in potato.

Table B.7.1.2-8. Summary of Storage Conditions.					
Matrix	Storage Temperature (°C)	Actual Storage Duration (days)			Interval of Demonstrated Storage Stability
		Collection to extraction	Extraction to initial profiling (Method 3)	Extraction to final analysis ¹ (Method 6A)	
Potato foliage	ca. -20	9-19	1-14 days	549-632 days	The metabolite profile in the ACN/water extracts was shown to be stable for up to 547-631 days in foliage and 548-596 days in tubers.
Potato tubers		9-19	1-8 days	576-625 days	

¹ Final analysis results were reported for residue identification and quantitation.

D. Identity of Residues in Potato

The characterization and identification of radioactivity in potato matrices is presented in Tables B.7.1.2-9 (foliage) and B.7.1.2-10 (tubers).

Pethoxamid and pethoxamid cysteine conjugate (MET-30) were identified in the ACN/water extract of 0-day immature foliage using HPLC method 6A, and identification was confirmed by TLC co-chromatography. Metabolites pethoxamid-SO-cysteine (M42) and pethoxamid-SO-lactic acid glycoside (M44) were identified in the ACN/water extract of 37-day immature foliage by LC/MS following isolation and purification, with identification confirmed by LC/MS/MS. Pethoxamid-SO-lactic acid glycoside (M44) was also identified by HPLC in mature foliage based on retention time comparisons with the results for 37-day foliage. TLC analysis of the ACN/water extract of 37-day immature foliage indicated that some radioactivity migrated with the reference standards for MET-30, MET-42, MET-50, and MET-103, but the most intense bands of radioactivity did not migrate with any reference standards; no correlation with reference standards was observed on TLC analysis of mature foliage.

No metabolites were identified in potato tubers. HPLC/UV analysis of the ACN/water extract of 37-day immature tubers showed a complex mixture of components, with the major residue component (Rt 45.75 min) accounting for 13.6% TRR (0.008 ppm). In mature tubers, the largest residue component (Rt 4.25 min) accounted for 15.4% TRR (0.002 ppm). TLC analysis of the extract for 37-day tubers indicated the possible presence of metabolites MET-3, MET-30, MET-42, MET-47, and MET-103, but this was not confirmed using a second system. No correlation with reference standards was observed on TLC analysis of mature tubers.

The ACN/water extracts of 0-day immature foliage and 37-day immature foliage and tubers were subjected to acid hydrolysis and analyzed by HPLC Methods 6A and CR. On HPLC analysis of the 37-day foliage and tuber hydrolysates using Method 6A, two major residue components were observed in each matrix, at retention times of 40 and 66 minutes in foliage, and retention times of 37 and 63 minutes in tuber. None of the peaks co-chromatographed with the available reference standards. Hydrolysis of [¹⁴C]pethoxamid (Rt 68 min) and metabolites pethoxamid-SO-cysteine (Rt 42 min) and pethoxamid-SO-lactic acid glycoside (Rt 44 min) under the same conditions were not useful in identifying further residue components. Very little change was observed in pethoxamid: minor transformation products eluted at 62 and 74 minutes, with pethoxamid eluting at 68 minutes. Hydrolysis of pethoxamid-SO-cysteine and pethoxamid-SO-lactic acid glycoside resulted in the formation of at least six new peaks from the cysteine

conjugate (Rts of 46, 52, 58, 62, 66, and 74 min) and a slight shift for the lactic acid glycoside; pethoxamid was not detected in either hydrolysate.

The hydrolyzed extracts of 0- and 37-day foliage and 37-day tuber were analyzed using HPLC Method CR which confirmed that pethoxamid was the major residue component in 0-day foliage (refer to Appendix I). In addition, unknown RT51H was a major residue component in all three matrices (10.2-10.9% TRR), RT24H was a major residue component in 37-day foliage and tuber (10.2-23.5% TRR), and unknown RT54H was a major residue component in 37-day tuber (16.9% TRR). Analysis of hydrolyzed pethoxamid-SO-cysteine and pethoxamid-SO-lactic acid glycoside also indicated the presence of RT24H and RT51H. These components were also observed in the rotational crop study submitted under this action (refer to the DER for MRID 49813552, B.7.9.1.1); a structure for RT24H was proposed in that study based on LC/MS and LC/MS/MS analyses.

Table B.7.1.2-9. Summary of Characterization and Identification of Radioactive Residues in Potato Foliage Following Application of [¹⁴C]Pethoxamid at 1.21 lb ai/A (1.36 kg ai/ha).¹						
Compound	Immature foliage (0-day PHI)		Immature foliage (37-day PHI)		Mature foliage (76-day PHI)	
	TRR = 18.102 ppm		TRR = 2.069 ppm		TRR = 1.521 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Pethoxamid	77.1	13.960	ND	--	ND	--
Pethoxamid cysteine conjugate (MET-30)	3.4	0.612	ND	--	ND	--
Pethoxamid-SO-cysteine (M42)	ND	--	10.0	0.207	ND	--
Pethoxamid-SO-lactic acid glycoside (M44)	ND	--	7.9	0.163	7.0	0.107
Region 2 (Rt 47.50 min)	11.0	1.996	ND	--	ND	--
Minor unknowns	6.5	1.173	75.5	1.562	80.7	1.228
Acid/base hydrolysates	1.9	0.349	4.6	0.096	9.1	0.138
Total extractable	98.0	17.741	93.4	1.933	87.8	1.335
Total identified	80.5	14.572	17.9	0.371	7.0	0.107
Total characterized	1.8	0.349	4.7	0.096	9.0	0.138
Total unidentified	17.5	3.169	75.5	1.562	80.7	1.228
Total bound residues (PES)	0.1	0.012	1.9	0.040	3.2	0.048
% Accountability ²	100		100		100	

¹ ND = Not detected. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Total (ppm)/TRR (ppm)×100

Table B.7.1.2-10. Summary of Characterization and Identification of Radioactive Residues in Potato Tubers Following Application of ¹⁴C]Pethoxamid at 1.21 lb ai/A (1.36 kg ai/ha).¹				
Compound	Immature tuber (37-day PHI)		Mature tuber (76-day PHI)	
	TRR = 0.059 ppm		TRR = 0.013 ppm	
	% TRR	ppm	% TRR	ppm
Region 1 (Rt 4.25 min)	ND	--	15.4	0.002
Region 23 (Rt 45.75 min)	13.6	0.008	ND	--
Minor unknowns	73.2	0.043	61.6	0.008
Total extractable	86.4	0.051	76.9	0.010
Total identified	NA	--	NA	--
Total characterized	NA	--	NA	--
Total unidentified	86.4	0.051	76.9	0.010
Total bound residues (PES)	13.6	0.008	23.1	0.003
% Accountability ²	100		100	

¹ ND = Not detected. NA = Not applicable. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Total (ppm)/TRR (ppm)×100

E. Proposed Metabolic Pathway

Based on the results of the potato metabolism study, the study authors proposed that the metabolic pathway of pethoxamid (refer to Figure B.7.1.2-3) proceeds initially by displacement of the chlorine atom by the thiolate anion of glutathione to yield a glutathione conjugate (not found in this study). The glutamic acid and glycine portions of glutathione are then cleaved to form the cysteine conjugate MET-30, which may be metabolized by a number of pathways common to cysteine conjugates, forming many products. MET-30 was oxidized in potato to yield pethoxamid-SO-cysteine, and was converted to its 2-hydroxypropyl analog and conjugated with glucose to form pethoxamid-SO-lactic acid glycoside.

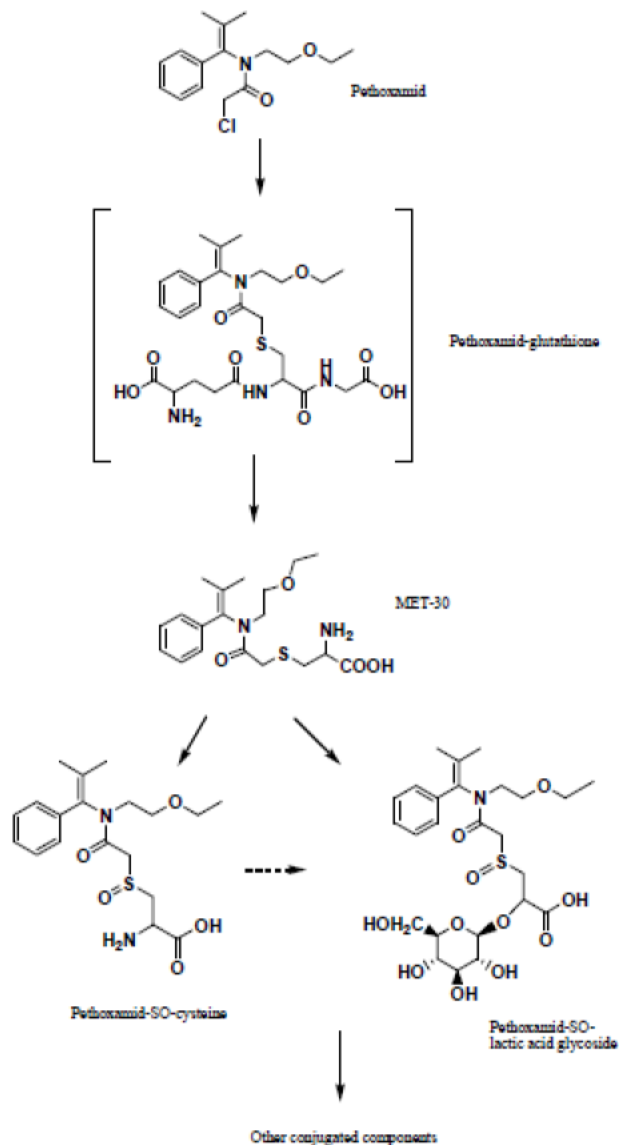
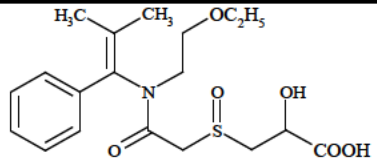
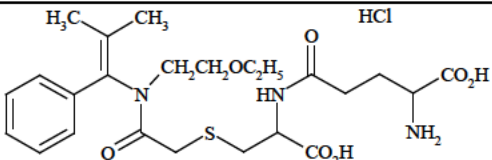
Figure B.7.1.2-3. Proposed Metabolic Profile of Pethoxamid in Potato.


Table B.7.1.2-11. Identification of Compounds from Metabolism Study (both proposed and found).		
Common Name/Code	Chemical Name	Chemical Structure
Pethoxamid	2-chloro-N-(2-ethoxyethyl)-N-(2-methyl-1-phenylprop-1-en-1-yl)acetamide	
Pethoxamid cysteine conjugate (MET-30)	S-(2-((2-ethoxyethyl)(2-methyl-1-phenylprop-1-en-1-yl)-amino)-2-oxoethyl)cysteine	

Table B.7.1.2-11. Identification of Compounds from Metabolism Study (both proposed and found).		
Common Name/Code	Chemical Name	Chemical Structure
Pethoxamid-SO-cysteine (M42)	None provided	
Pethoxamid-SO-lactic acid glycoside (M44)	None provided	
Additional reference standard compounds used in the study but not identified in any matrix		
MET-2	N-(2-ethoxyethyl)-2-hydroxy-N-(2-methyl-1-phenylprop-1-en-1-yl)acetamide	
MET-3	2-hydroxy-N-(2-hydroxyethyl)-N-(2-methyl-1-phenylprop-1-en-1-yl)acetamide	
MET-41	3-((2-((2-ethoxyethyl)(2-methyl-1-phenylprop-1-en-1-yl)amino)-2-oxoethyl)thio)-2-hydroxypropanoic acid	
MET-42	2-[(2-ethoxyethyl)(2-methyl-1-phenyl-1-propen-1-yl)amino]-2-oxoethanesulfonic acid, sodium salt	
MET-47	11-carboxy-6-(2-methyl-1-phenylprop-1-en-1-yl)-7,13-dioxo-3-oxa-9-thia-6,12-diazapentadecan-15-oic acid	

Table B.7.1.2-11. Identification of Compounds from Metabolism Study (both proposed and found).		
Common Name/Code	Chemical Name	Chemical Structure
MET-50	3-((2-((2-ethoxyethyl)(2-methyl-1-phenylprop-1-en-1-yl)amino)-2-oxoethyl)sulfinyl)-2-hydroxypropanoic acid	
MET-103	16-amino-11-carboxy-6-(2-methyl-1-phenylprop-1-en-1-yl)-7,13-dioxo-3-oxa-9-thia-6,12-diazaheptadecan-17-oic acid hydrochloric acid salt	

III. CONCLUSIONS

The potato metabolism study is considered scientifically acceptable. Samples of immature and mature potato matrices were harvested following a single foliar application of [phenyl-U-¹⁴C]pethoxamid at 1.21 lb ai/A (1.36 kg ai/ha) made to plants at BBCH 25. TRR were highest in 0-day foliage and declined with increasing PHI in both foliage and tubers. TRR were: 18.102, 2.069, and 1.521 ppm in foliage harvested 0, 37, and 76 days, respectively, following application; and 0.059 and 0.013 ppm in tubers harvested 37 and 76 days, respectively, following application.

Pethoxamid was identified as the major residue component in 0-day foliage, at 77.1% TRR. Pethoxamid cysteine conjugate (MET-30) was also identified in 0-day foliage at 3.4% TRR. Pethoxamid-SO-cysteine (M42) and pethoxamid-SO-lactic acid glycoside (M44) were identified in 37-day foliage at 10.0% TRR and 7.9% TRR, respectively, and the SO-lactic acid glycoside was also identified in 76-day foliage at 7.0% TRR. Remaining radioactivity in 0-day foliage was comprised of three discrete unknowns present at 1.7-11.0% TRR. In 37- and 76-day foliage, the majority of the remaining radioactivity consisted of multiple minor unknowns (each present at ≤4.8% TRR) that together accounted for 72.5% TRR in 37-day foliage and 80.7% TRR in 76-day foliage.

No metabolites were identified in potato tubers. Radioactivity was characterized as a complex mixture of components, with the major residue component in 37-day tubers accounting for 13.6% TRR and the major component in mature tubers accounting for 15.4% TRR. TLC analysis of the extract for 37-day tubers indicated the possible presence of metabolites MET-3, MET-30, MET-42, MET-47, and MET-103, but this was not confirmed using a second system. No correlation with reference standards was observed on TLC analysis of mature tubers.

Acid hydrolysis of the ACN/water extracts of 0-day immature foliage and 37-day immature foliage and tubers released metabolites that were found to be cleaved components of pethoxamid in the rotational crop study submitted under this action (refer to the DER for MRID 49813552, B.7.9.1.1), including RT24H and RT28H (which were tentatively identified/identified in that study) and RT31H, RT51H, and RT54H (which were not identified). These unknowns were not further investigated in this metabolism study.

Based on the results of the potato metabolism study, the study authors proposed that the metabolic pathway of pethoxamid proceeds initially by displacement of the chlorine atom by the thiolate anion of glutathione to yield a glutathione conjugate (not found in this study). The glutamic acid and glycine portions of glutathione are then cleaved to form the cysteine conjugate MET-30, which may be metabolized by a number of pathways common to cysteine conjugates, forming many products. MET-30 was oxidized in potato to yield pethoxamid-SO-cysteine, and was converted to its 2-hydroxypropyl analog and conjugated with glucose to form pethoxamid-SO-lactic acid glycoside.

REFERENCES

None

Appendix I: Characterization of Radioactive Residues in Immature Potato Foliage and Tuber Following Acid Hydrolysis of the Combined ACN/Water Extracts.

These data are provided for purposes of comparison with the confined rotational crop study submitted under the current action (refer to the DER for MRID 49813552, B.7.9.1.1).

Summary of Characterization of Radioactive Residues in the Hydrolyzed ACN/Water Extracts of Immature Potato Foliage and Tuber Following Application of [phenyl-U-¹⁴C]Pethoxamid at 1.21 lb ai/A (1.36 kg ai/ha).¹						
Compound	Immature foliage (0-day PHI)		Immature foliage (37-day PHI)		Immature tuber (37-day PHI)	
	TRR = 18.102 ppm		TRR = 2.069 ppm		TRR = 0.059 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Pethoxamid	55.1	9.970	ND	--	ND	--
RT24H	ND	--	23.5	0.487	10.2	0.006
RT28H	ND	--	2.0	0.041	6.8	0.004
RT31H	ND	--	3.2	0.066	5.1	0.003
RT51H	10.9	1.969	10.2	0.211	10.2	0.006
RT54H	ND	--	ND	--	16.9	0.010

¹ ND = Not detected. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

**B.7.1 Metabolism, Distribution, and Expression of Residues in Plants
(Annex IIA 6.2; Annex IIIA, 8.2)**

B.7.1.3 Soybean

Document ID: MRID No. 49813527
PMRA No. Not available

Report: Goodyear, A. (2000) (¹⁴C)-TKC-94: Metabolism in Soybean. Study Numbers: 99 PXA; 1465/6-D2142. Unpublished study prepared by Covance Laboratories Ltd., sponsored by Cheminova, Inc., and submitted by FMC Corporation. 143 p.

Guidelines: EPA OCSPH Harmonized Test Guideline 860.1300 Nature of the Residue - Plants, Livestock (August 1996)
PMRA Regulatory Directive Dir98-02 – Residue Chemistry Guidelines, Section 2 -Nature of the Residue - Plants, Livestock
OECD Guideline 501 Metabolism in Crops (January 2007)

GLP Compliance: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Acceptability: The study is considered scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP#452109.

Evaluator: Sheila Piper, Chemist, HED, RAB6 *Sheila Piper*

Secondary Evaluator: Robert Lanthier, PMRA

Note: This Data Evaluation Record (DER) was prepared under contract by CDM/CSS-Dynamac Joint Venture (submitted 2/16/18). The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies. The DER has been reviewed by HED and revised as necessary to reflect current Office of Pesticide Programs (OPP) policies.

EXECUTIVE SUMMARY

FMC Corporation has submitted a study investigating the metabolism of [phenyl-U-¹⁴C]pethoxamid (specific activity: 45.6 µCi/mg) in greenhouse-grown soybeans. The test substance was formulated as a 5.0 lb ai/gal (600 g ai/L) emulsifiable concentrate (EC) formulation and applied as a separate soil or foliar application at 1.1 lb ai/A (1.2 kg ai/ha) for each application. Applications were made as: an early postemergence application to the soil 21 days after planting at BBCH 11 (first true leaves); a preemergence soil application 1 day after planting; or a foliar application to soybean plants 29 days after planting at BBCH 14-15 (4- to 5-leaf growth stage). Samples of immature whole plant were collected at preharvest intervals (PHIs) of 14 and 27 days following the postemergence soil application, 36 days following the preemergence application, and 8 days following the foliar application. Samples of immature leaves, stems, and roots were collected 61 days following preemergence application and 33 days following foliar application, and samples of mature seed, pods (hulls), leaves, stems, and roots were collected 125-134 days following preemergence application and 97-106 days following foliar application. The in-life and analytical phases of the study were conducted by Covance Laboratories Ltd. (Harrogate, UK)

Total radioactive residues (TRR) in soybean matrices were calculated by summing extractable radioactivity (following solvent extraction and hydrolysis as applicable) and nonextractable radioactivity. The highest TRR were observed in 8-day PHI immature whole plants collected following foliar application, and the lowest TRR were found in seed following soil or foliar applications. TRR in soybean matrices were generally lower in immature matrices harvested following soil application than following foliar application but were higher in mature matrices harvested following soil application, despite longer PHIs. The results obtained following soil applications indicated that radioactivity was taken up from the soil and transported primarily to the plant leaves. Likewise, the study suggested that radioactivity resulting from foliar application was transported to other vegetative parts of the plant including the roots. The study author stated that radioactivity in immature and mature roots following preemergence application may have been artificially high due to adherence of treated soil to the roots. TRR in immature whole plants were 0.030 and 0.016 ppm in samples harvested 14 and 27 days, respectively, following postemergence soil application; 0.084 ppm in samples harvested 36 days following preemergence application; and 31.576 ppm in samples harvested 8 days following foliar application. TRR in immature leaves, stems, and roots, respectively, were 0.096, 0.015, and 0.196 ppm in samples harvested 61 days following preemergence application, and 5.674, 0.135, and 0.112 ppm in samples harvested 33 days following foliar application. In mature soybean matrices harvested 125-134 days following preemergence application and 97-106 days following foliar application, TRR in seed and pod, respectively, were 0.029 and 0.107 ppm following preemergence application and 0.011 and 0.028 ppm following foliar application. TRR in leaves, stems, and roots respectively, were 0.444, 0.075, and 0.187 ppm following preemergence application and 0.055, 0.030, and 0.060 ppm following foliar application.

Extraction with acetonitrile (ACN) released 46-61% TRR from immature whole plants collected following postemergence and preemergence soil applications and 71% TRR from immature whole plants collected following foliar application. Immature whole plants collected 8 days following foliar application, were subjected to additional extraction with water, which released 26% TRR, followed by hydrolysis with 0.1 M HCl and NaOH, which together released 1.8% TRR. For immature and mature soybean plants collected as separate matrices, the distribution of radioactivity in the individual matrices was similar for plants harvested following preemergence and foliar applications. In immature leaves, stems, and roots harvested 61 and 33 days following soil and foliar applications, respectively, extraction with ACN released 40-55% TRR from leaves and stems and 36-38% TRR from roots. In mature soybean matrices, extraction with ACN and acetone released little or no radioactivity from seed and pods, while extraction with water released 38-58% TRR, and hydrolysis with 2 M HCl released 14-31% TRR. For mature leaves and stems, extraction with ACN and water, respectively, released 47-60% and 14-30% TRR, while hydrolysis with 2 M HCl released 3.9-14% TRR. For mature roots, extraction with ACN released 31-39% TRR. Radioactivity remaining in the post-extraction solids (PES) accounted for <10% TRR or <0.05 ppm in all matrices except: immature and mature roots following preemergence application (60.7-62.1% TRR, 0.113-0.122 ppm) and immature leaves (59.7% TRR, 3.387 ppm), and stems and roots (45.3-64.2% TRR, 0.061-0.072 ppm) following foliar application. Recoveries were normalized for the total amount of radioactivity recovered in the extracts, hydrolysates, and PES; therefore, accountabilities were 100% for each matrix.

Following initial extraction and hydrolysis procedures, only three matrices were selected for further investigation of residues: mature leaves following both soil and foliar applications and 8-day PHI immature whole plants following foliar application. Subsamples of these matrices were subjected to further workup, and metabolites and residue components in extracts and hydrolysates (if applicable) were quantified and characterized or identified by high performance liquid chromatography (HPLC) with UV detection and/or thin layer chromatography (TLC). Further identification of residue components in 8-day PHI immature whole plants was conducted using high performance liquid chromatography with mass spectrometry and tandem mass spectrometry detection (LC/MS and LC/MS/MS). Identifications of pethoxamid and metabolites TKC-94-cysteine (MET-30) and TKC-94-N-malonyl cysteine (MET-47) were confirmed; identifications of remaining metabolites were considered to be tentative.

Only limited study dates were provided in the submission. Soybean samples were stored frozen (*ca.* -20 °C) from harvest to analysis. No information was provided concerning when the metabolite profiles were established for leaves and immature whole plants. Based on dated LC/MS chromatograms for the ethyl acetate and aqueous phases of 8-day PHI immature plants, and the date of final TLC analysis for an isolated metabolite, samples were stored for up to 10.5-22.9 months prior to completion of the experimental phase of the study. In addition, analysis of a re-extracted sample of 8-day PHI immature whole plants indicated that metabolite TKC-94-N-malonyl cysteine may have degraded to metabolite TKC-94-cysteine during storage. Acceptable storage stability data were submitted indicating that residues of pethoxamid are stable during frozen storage for up to 8 months in soybean forage and straw, and up to 12 months in soybean seed (refer to the DERs for MRIDs 49813540, 49813541, 49813544, and; B.7.6.2).

A total of 49.6% TRR (15.699 ppm) was identified in 8-day PHI immature whole plants. Pethoxamid was a minor residue component, accounting for 1.1% TRR (0.363 ppm). The major residue components were TKC-94-cysteine and TKC-94-N-malonyl-cysteine, accounting for 20.3% TRR (6.403 ppm) and 17.4% TRR (5.505 ppm), respectively. Three other metabolites were identified and quantitated: TKC-94-SO-cysteine at 6.4% TRR (2.034 ppm), TKC-94-thiol (MET-43) at 2.8% TRR (0.896 ppm), and TKC-94-SO-thiol at 1.6% TRR (0.498 ppm). Benzoic acid and an oxamic acid metabolite (MET-31) were tentatively identified but were not quantitated following strong acid hydrolysis of the plant extract. Remaining radioactivity was comprised of six discrete unknowns, each present at 1.4-5.7% TRR (0.461-1.800 ppm), that together accounted for 18.1% TRR (5.763 ppm), and multiple minor unknowns that were not further resolved but together accounted for 28.9% TRR (9.007 ppm).

No residue components were identified in mature leaves. In 127-day PHI mature leaves collected following preemergence application, the residue was composed of nine discrete unknowns, individually present at 4.6-9.3% TRR (0.020-0.041 ppm) and together accounting for 52.0% TRR (0.231 ppm), with multiple minor unknowns, present individually at $\leq 4.5\%$ TRR, together accounting for 38.6% TRR (0.172 ppm). The study author stated that the low levels of radioactivity and large amounts of co-extracted plant material in the sample made mass spectral identification of the unknowns impractical. HPLC analysis of the combined ACN/water extract of 99-day PHI mature leaves (comprising 87.3% TRR, 0.048 ppm) was also conducted. The study author stated that evaluation of the resulting chromatogram was impossible due to the small amounts of radioactivity, but noted that the chromatogram suggested that the sample

contained at least as many components as that of the 127-day PHI mature leaves and that it was unlikely that any single metabolite would exceed 10% TRR or 0.01 ppm.

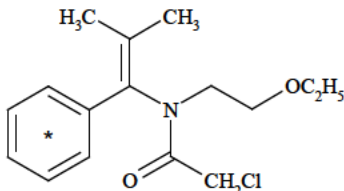
Based on the results of the soybean metabolism study, the study author concluded that pethoxamid was readily absorbed into the soybean plant and rapidly metabolized. Due to the predominance of sulfur-containing metabolites, the study author proposed that pethoxamid was metabolized primarily via glutathione conjugation, with initial displacement of the chlorine atom by the sulfhydryl group of glutathione in a reaction catalyzed by glutathione-S-transferase. The glutathione conjugate (not found in the study) was metabolized initially by removal of glycine to yield TKC-94- γ -glutamyl cysteine (also not found), and then by removal of glutamic acid to yield TKC-94-cysteine (MET-30), which was the major metabolite observed in the immature plant. The presence of TKC-94-thiol (MET-43) and TKC-94-N-malonyl cysteine (MET-47) suggested extensive further metabolism of TKC-94-cysteine, possibly via β -lyase to form TKC-94-thiol and by malonyl transferase to form the N-malonyl conjugate. In addition, sulfur oxidation was a common route of metabolism, with SO derivatives observed for both TKC-94-cysteine and TKC-94-thiol. The appearance of metabolite MET-31 in the acid-hydrolyzed extracts suggests that this metabolite may be present in a conjugated form in the plant.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

The radiolabeled test substance, dissolved in ACN, was isotopically diluted with non-labeled pethoxamid and formulated as a 5.0 lb ai/gal (600 g ai/L) EC formulation using a formulation blank containing non-labeled pethoxamid, formulation additives, and water.

Table B.7.1.3-1. Pethoxamid Nomenclature.	
Common name	Pethoxamid
Identity	2-chloro-N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propen-1-yl)acetamide
CAS registry number	106700-29-2
Company experimental name	TKC-94
Radiolabeled compound	[phenyl-U- ¹⁴ C]pethoxamid
Lot/Batch #	CFQ9901
Radiochemical purity	>98%
Specific activity as received	102.5 μ Ci/mg (3.79 MBq/mg)
Specific activity of dose	45.6 μ Ci/mg (101,232 dpm/ μ g)
Position of radiolabel	

2. Test Crop

Table B.7.1.3-2. Crop Information.				
Crop/Crop Group	Variety	Growth Stage at Application	Growth Stage at Harvest	Harvested Commodities
Soybean/Legume vegetable, group 6	PS9615 Group 1.5	<u>Postemergence soil:</u> BBCH 11 (first true leaves)	Immature and mature (growth stages not further specified)	<u>Postemergence soil:</u> Immature whole plants (14- and 27-day PHIs)
		<u>Preemergence soil:</u> 1 day after planting		<u>Preemergence soil:</u> Immature whole plants (36-day PHI) Immature leaves, stems, and roots (61-day PHI) Mature seed and pods (125- and 134-day PHIs), leaves (127-day PHI), and stems and roots (134-day PHI)
		<u>Foliar:</u> BBCH 14-15 (4- to 5-leaf stage)		<u>Foliar:</u> Immature whole plants (8-day PHI) Immature leaves, stems, and roots (33-day PHI) Mature seed and pods (97- and 106-day PHIs), leaves (99-day PHI), and stems and roots (106-day PHI)

3. Soil Type

Soybeans were grown in a mixture of multipurpose compost, sharp sand, and topsoil mixed in a ratio of ~3:2:1. The physicochemical properties of the soil are presented in Table B.7.1.3-3.

Table B.7.1.3-3. Soil Physicochemical Properties.							
Soil Type	pH	Organic Matter (%)	Sand (%)	Silt (%)	Clay (%)	Moisture Holding Capacity (% at 1/3 bar)	Cation Exchange Capacity (meq/100 g)
Loamy sand	6.1	6.4	83.7	9.3	7.0	Not reported	Not reported

B. STUDY DESIGN

Experimental Conditions

Soybean plants were grown in 45-L plastic containers in a greenhouse. The soybean plants were maintained under conditions suitable for plant growth during the study. Greenhouse temperatures ranged 7-45 °C, water was applied to the soil as necessary, and fertilizers and pesticides were used as needed. Supplementary lighting was used periodically to increase light levels.

The radiolabeled test substance was formulated as a 5.0 lb ai/gal (600 g ai/L) EC formulation, diluted with water, and applied to soybeans seeded and grown in separate containers at 1.1 lb ai/A (1.2 kg ai/ha) for each application type. A single soil application was made to soybeans seeded on different dates to establish an early postemergence application made 21 days after planting at BBCH 11 (first true leaves; two containers) and a preemergence application made 1 day after planting (four containers). A foliar application was made to a separate set of soybean plants 29 days after planting at BBCH 14-15 (4- to 5-leaf growth stage; four containers). The study author indicated that the postemergence soil application was made as part of a “preliminary” study, and the preemergence and foliar applications were made as part of the main

study; however, samples from all three treatments were analyzed. For the soil applications, the test substance was applied dropwise by pipette over the soil surface. For the foliar application, a plastic bag was placed over the plants in each container and sealed at the base with tissues, and the test substance was applied through holes cut in the ends of the bag using a compressed air sprayer. The study author noted that a full leaf canopy covered the area of each study container at the time of the foliar treatment; thus, the majority of the spray was applied to the leaf surface.

Table B.7.1.3-4. Use Pattern Information.	
Chemical name	[phenyl-U- ¹⁴ C]pethoxamid
Application method	The test substance was formulated as a 5.0 lb ai/gal (600 g ai/L) EC formulation and applied as a single postemergence or preemergence soil application by pipetting dropwise to the soil or as a single foliar application using a compressed air sprayer.
Application rate	1.1 lb ai/A (1.2 kg ai/ha) for each application
Number of applications	A single postemergence soil, preemergence soil, or foliar application
Timing of applications	Postemergence soil: 21 days after planting Preemergence soil: 1 day after planting Foliar: 29 days after planting
PHI	Postemergence soil: 14 and 27 days for immature whole plants Preemergence soil: 36 days for immature whole plants; 61 days for immature leaves, stems, and roots; and 125-134 days for mature seed, pods, leaves, stems, and roots Foliar: 8 days for immature whole plants; 33 days for immature leaves, stems, and roots; and 97-106 days for mature seed, pods, leaves, stems, and roots

Sampling

Samples of immature whole plants were collected 14 and 27 days following postemergence application, 36 days following preemergence application, and 8 days following foliar application. Samples of immature plants, separated into leaves, stems, and roots, were collected 61 and 33 days following preemergence and foliar applications, respectively. Mature soybean seed and pods were harvested on two separate occasions from each plot, and the respective samples were combined for analysis: 125 and 134 days following preemergence application and 97 and 106 days following foliar application. Following preemergence and foliar applications, respectively, mature leaves were collected at PHIs of 127 and 99 days, and mature stems and roots were collected at PHIs of 134 and 106 days.

Immature whole plants were harvested by cutting at the soil surface. Immature leaf samples were collected following preemergence and foliar application by separating the leaf from the stem at the leaf junction. Plant samples were then cut into small pieces forming composite samples of leaf, stem, and roots. Harvest of mature soybean matrices from preemergence and foliar application plots was conducted over several days. Ripe (dry) pods and seed were collected on 2 different days, pods and seed were separated, and like matrices were combined. Leaves were harvested from each plot 2 days after the initial seed and pod harvest, leaving the stems and pods intact on the plant to help the soybeans dry and ripen more rapidly, and the final harvest of pods, seed, and mature stems and roots was made 7 days after harvest of the leaves.

Roots were collected by digging up a number of root systems and removing as much soil as possible by tapping and shaking.

All samples were placed in frozen storage (*ca.* -20 °C) on the day of harvest and were homogenized in the presence of dry ice prior to extraction.

TRR Determination

Radioactivity was determined in extracts and hydrolysates by LSC and in PES fractions by combustion/LSC. TRR were calculated by summing extractable radioactivity (following solvent extraction and hydrolysis as applicable) and nonextractable radioactivity. The limit of quantitation for TRR determinations was not reported.

Extraction and Analysis

Samples of immature and mature soybean matrices were extracted with ACN, and the extract was collected by centrifugation. The PES of mature seed, pods, leaves, and stems were sequentially extracted with acetone and water (only water for leaves), then subjected to acid hydrolysis with 2 M HCl (for 16 hours at reflux); the extracts/hydrolysates were collected after each step by centrifugation. No further details were provided concerning these procedures.

Subsamples of mature leaves following preemergence and foliar application, and 8-day PHI immature whole plants following foliar application were subjected to additional workup for further investigation of radioactive residues.

Subsamples of mature leaves were sequentially extracted with duplicate aliquots of ACN and water, and the extracts were collected by centrifugation, then filtered, combined, and partitioned twice with hexane. The resulting ACN/water phase was filtered, concentrated by rotary evaporation to remove ACN, and partitioned with hexane followed by ethyl acetate. The final aqueous phase was concentrated by rotary evaporation for chromatography.

A subsample of 8-day PHI immature whole plants (Subsample 1) was extracted sequentially with triplicate aliquots of ACN and water, and the extracts were collected by centrifugation. The first and second ACN and water extracts contained the majority of the extractable radioactivity, and like extracts were combined and partitioned with hexane, initially as separate ACN and water extracts and then as a combined extract. The resulting ACN/water phase was concentrated to near dryness and reconstituted in ACN:water (1:1, v:v). The PES were subjected to acid and base hydrolysis with 0.1 M HCl followed by 0.1 M NaOH (conditions not specified), and the hydrolysates were collected by centrifugation but were not further analyzed. A subsample of the concentrated ACN/water extract was subjected to acid hydrolysis by acidifying with concentrated HCl to achieve an overall concentration of 2 M HCl and heating at 60 °C for 3 hours then at 80 °C for 24 hours. The resulting hydrolysate was partitioned with ethyl acetate, and the ethyl acetate phase was centrifuged to remove particulate matter, concentrated under a stream of nitrogen to remove solvent, then re-dissolved in ACN. A second subsample of the concentrated ACN/water extract was subjected to base hydrolysis, using concentrated NaOH to

achieve 2 M NaOH and then heating at 80 °C for 24 hours. The resulting hydrolysate was reserved for TLC analysis.

Additional material for chromatography was prepared using a larger subsample of 8-day PHI immature whole plants (Subsample 2) subjected to the extraction and hydrolysis procedures described above except that the subsample was extracted 2x each with ACN and water, and separate aliquots of the concentrated ACN/water extract following hexane partitioning were subjected to: (1) acid hydrolysis with 2 M HCl (at 80 °C for 24 hours) and 6 M HCl (at 95 °C for 20 hours) followed by ethyl acetate partitioning; and (2) partitioning 2x with ethyl acetate, with the initial aqueous phase acidified to pH 4 prior to the second partitioning step and the ethyl acetate phases combined, centrifuged to remove particulates, concentrated under nitrogen, and re-dissolved in ACN. Additional material was prepared for confirmation of metabolites using a third subsample of 8-day PHI whole plants subjected to extraction with acetone, ACN, and water and sequential partitioning with hexane and ethyl acetate.

Flowcharts of the extraction and hydrolysis procedures are presented in Figures B.7.1.3-1 (leaves), B.7.1.3-2 (immature whole plants, Subsample 1), and B.7.1.3-3 (immature whole plants, Subsample 2).

Figure B.7.1.3-1. Flowchart for Extraction of Mature Leaves.

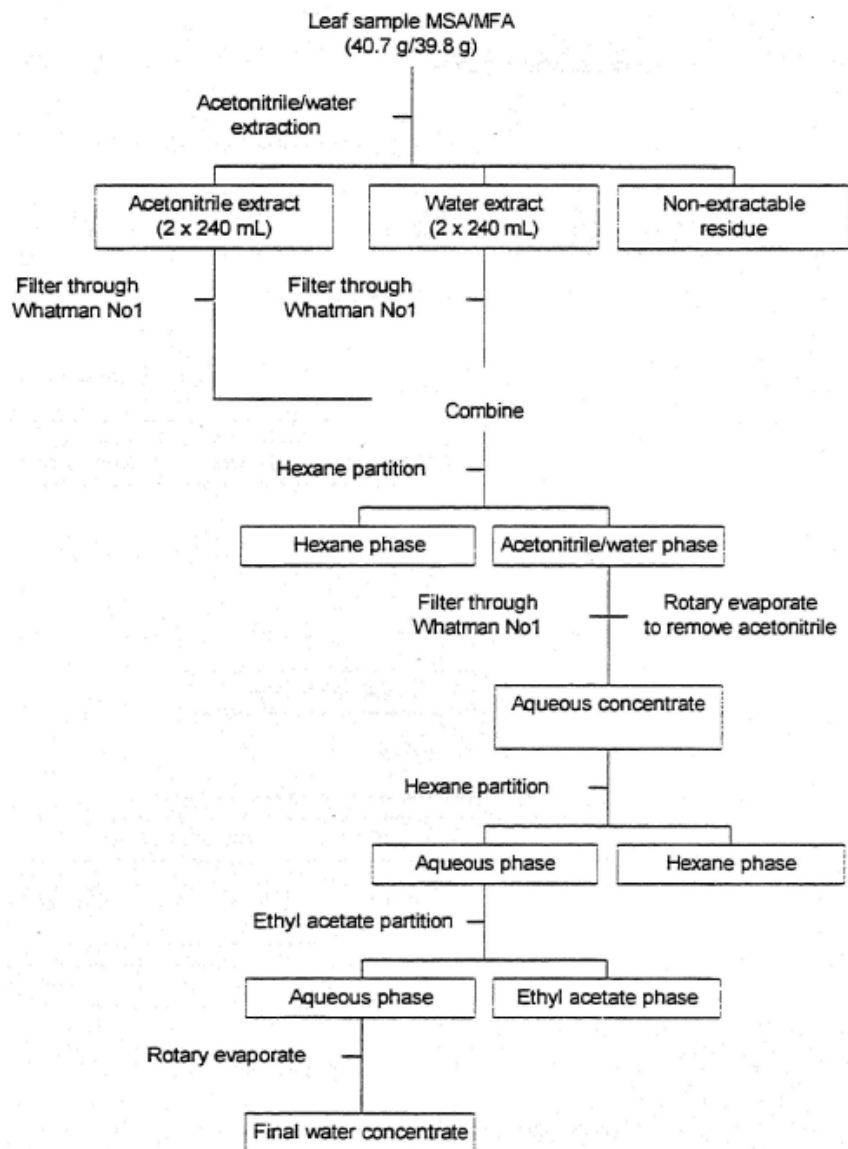


Figure B.7.1.3-2. Flowchart for Extraction and Hydrolysis of 8-day PHI Whole Immature Plant (Subsample 1).

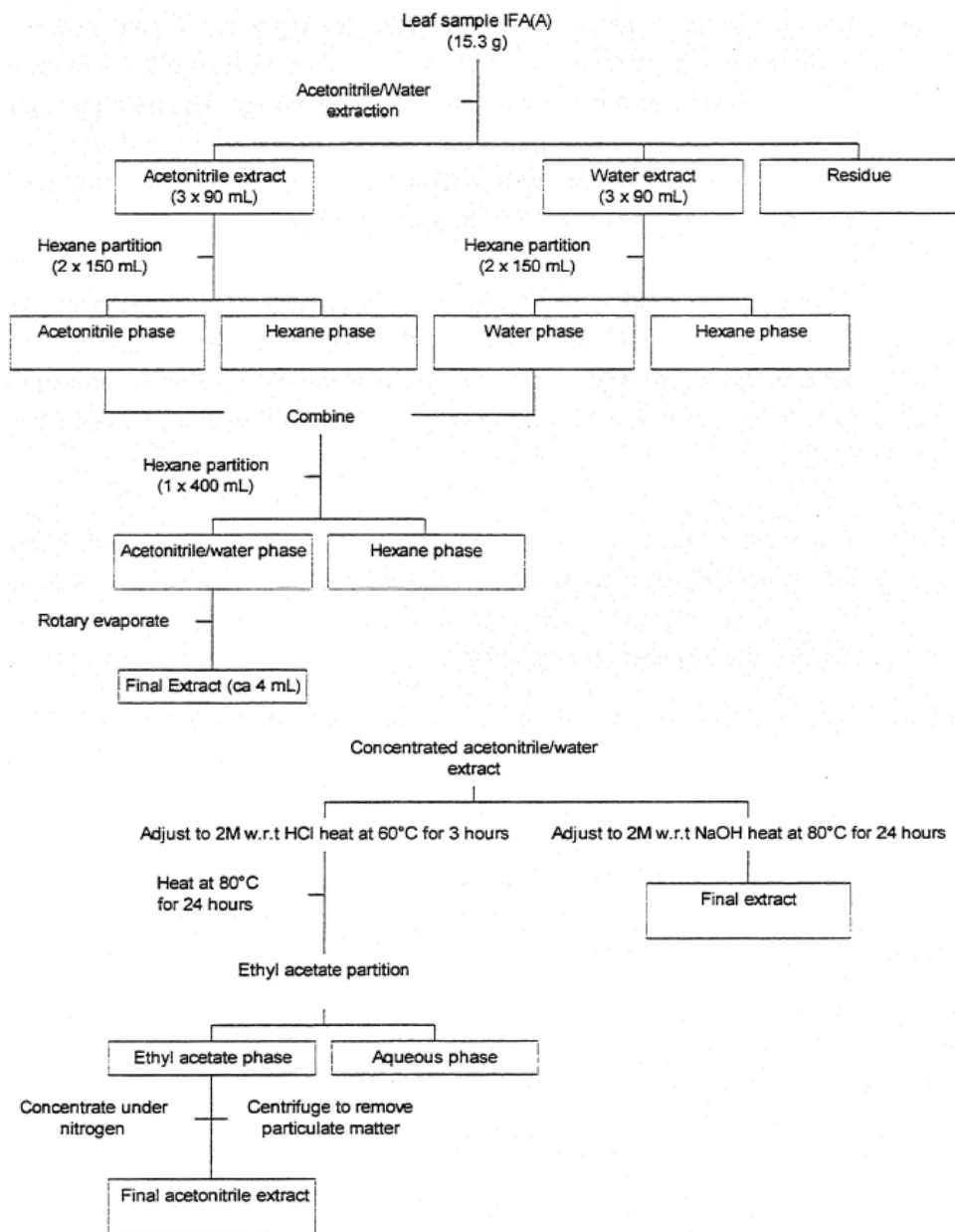
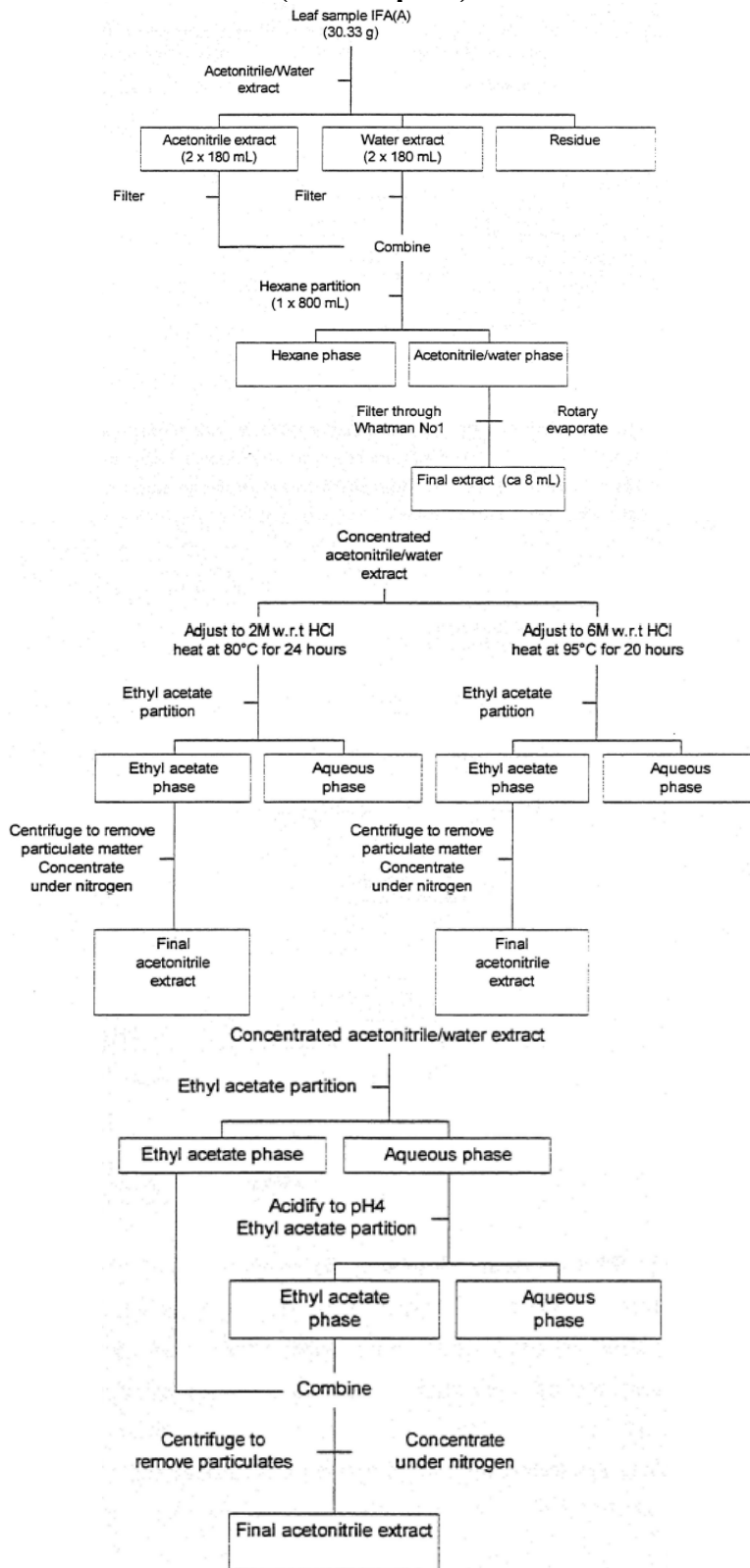


Figure B.7.1.3-3. Flowchart for Extraction and Hydrolysis of 8-day PHI Whole Immature Plant (Subsample 2).



Identification and Characterization

Extracts of 8-day PHI immature whole plants and 99- and 127-day PHI mature leaves were analyzed by HPLC using a system equipped with a Spherisorb ODS2 column, a UV detector (254 nm), a flow-through radiodetector, and a fraction collector, and utilizing mobile phase gradients of: water and ACN, each containing 1% acetic acid (Method 1); 0.01 M phosphate buffer (pH 2.5) and ACN (Method 3); or 0.01 M formate buffer (pH 3.0) and ACN (Method 4).

The combined ACN/water extract of 8-day PHI whole plants was also analyzed by TLC using silica gel plates (K6F) and solvent systems of hexane:ethyl acetate:triethylamine (10:10:1, v:v:v; Method 1); ethyl acetate:formic acid (100:1, v:v; Method 2); or ACN:water (17:3, v:v; Method 4). The 2 M NaOH hydrolysate was also analyzed by TLC using Method 4. Radioactivity was detected and quantitated using phosphorimaging and a linear analyzer.

Identification of TKC-94-cysteine (MET-30), and TKC-94-N-malonyl cysteine (MET-47) in 8-day PHI whole plants was confirmed by co-chromatography with reference standards using HPLC Methods 4 and 5 and/or TLC Method 5 (solvent system of ACN:water:acetic acid, 85:15:1, v:v:v) and freshly prepared extracts generated from a larger plant sample. Refer to Table B.7.1.3-13 for identified residue components and to Appendix I for the names and structures of reference standards used in the study.

The ethyl acetate and aqueous phases that were generated following partitioning of the combined ACN/water extract of 8-day PHI immature whole plants as well as the 2 and 6 M HCl hydrolysates of the extract were analyzed by LC/MS and LC/MS/MS to elucidate metabolite structures and confirm identification of parent and certain metabolites. LC/MS Method 1, which was used for analysis of intact metabolites in the ethyl acetate and aqueous phases, utilized the HPLC parameters of Method 4, with the addition of a Finnigan LCQ Ion Trap mass spectrometer operated in the positive ionization mode using electrospray ionization and full scan mass spectroscopy. LC/MS Method 2, which was used for analysis of the acid hydrolysates, utilized the HPLC parameters of Method 1, with the addition of a VG Quattro triple quadrupole mass spectrometer operated in the positive and negative ionization modes using electrospray ionization and full scan mass spectrometry for initial LC/MS analysis and multiple reaction monitoring for subsequent LC/MS/MS analyses.

II. RESULTS AND DISCUSSION

A. Total Radioactive Residues

The TRR in soybean matrices are summarized in Table B.7.1.3-5.

Table B.7.1.3-5. TRR in Soybean Matrices.						
Matrix	Application type ¹					
	Postemergence soil		Preemergence soil		Foliar	
	PHI (days)	Residues ² (ppm)	PHI (days)	Residues ² (ppm)	PHI (days)	Residues ² (ppm)
Immature: Whole plants	14	0.030	36	0.084	8	31.576
	27	0.016				
<i>Whole plants</i> ³			61	0.058	33	3.104
Leaves				0.096		5.674
Stems				0.015		0.135
Roots				0.196		0.112
Mature: <i>Whole plants</i> ³			125-134	0.183	97-106	0.034
Seed ⁴			125, 134	0.029	97, 106	0.011
Pods ⁴				0.107		0.028
Leaves			127	0.444	99	0.055
Stems			134	0.075	106	0.030
Roots				0.187		0.060

¹ Postemergence soil application was made 21 days after planting; preemergence application was made 1 day after planting; and foliar application was made 29 days after planting.

² Reported in parent equivalents. Calculated by summing extractable radioactivity (following solvent extraction and hydrolysis as applicable) and nonextractable radioactivity.

³ TRR for italicized fractions were calculated by the study author from the amount in each separated plant fraction (excluding roots).

⁴ Seed and pods were harvested from each treated plot on two separate occasions, and samples were combined for analysis.

B. Extraction, Characterization, and Distribution of Residues in Soybean

The distribution of radioactivity in soybean matrices is presented in Tables B.7.1.3-6 (immature matrices, postemergence soil application), B.7.1.3-7 (immature matrices, preemergence application), B.7.1.3-8 (mature matrices, preemergence application), B.7.1.3-9 (immature matrices, foliar application), and B.7.1.3-10 (mature matrices, foliar application). All samples of immature and mature soybean matrices were subjected to extraction and/or hydrolysis procedures. Following initial extraction and hydrolysis procedures, only three matrices were selected for further investigation of residues: mature leaves collected following preemergence and foliar applications and 8-day PHI immature whole plants collected following foliar application. No further investigations were conducted on seed or pods due to both low radioactivity and very limited extractability of residues into organic solvents, and chromatography was not attempted on the stem or root extracts of mature samples due to low residues and difficulties that were encountered in analyzing the residues in leaves (see below).

We note that raw data supporting chromatographic analyses were not provided in the submission; therefore, the study reviewer could not verify any of the reported quantitative data for HPLC or TLC analyses.

Soil Application

Table B.7.1.3-6. Distribution of Radioactive Residues in Immature Soybean Matrices Following Postemergence Soil Application of [¹⁴C]Pethoxamid at 1.17 lb ai/A (1.2 kg ai/ha).¹				
Metabolite Fraction	Whole plants (14-day PHI)		Whole plants (27-day PHI)	
	TRR = 0.030 ppm		TRR = 0.016 ppm	
	%TRR	ppm	%TRR	ppm
ACN	57.1	0.017	45.5	0.007
PES	42.9	0.013	54.5	0.008

¹ Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

Table B.7.1.3-7. Distribution of Radioactive Residues in Immature Soybean Matrices Following Preemergence Soil Application of [¹⁴C]Pethoxamid at 1.1 lb ai/A (1.2 kg ai/ha).¹								
Metabolite Fraction	Whole plants (36-day PHI)		Leaves (61-day PHI)		Stems (61-day PHI)		Roots (61-day PHI)	
	TRR = 0.084 ppm		TRR = 0.096 ppm		TRR = 0.015 ppm		TRR = 0.196 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN	60.7	0.051	55.3	0.053	42.9	0.006	37.9	0.074
PES	39.3	0.033	44.7	0.043	57.1	0.008	62.1	0.122

¹ Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

Table B.7.1.3-8. Distribution of Radioactive Residues in Mature Soybean Matrices Following Preemergence Soil Application of [¹⁴ C]Pethoxamid at 1.1 lb ai/A (1.2 kg ai/ha). ¹						
Metabolite Fraction	Seed (125- & 134-day PHIs)		Pods (125- & 134-day PHI)		Leaves (127-day PHI)	
	TRR = 0.029 ppm		TRR =0.107 ppm		TRR = 0.444 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN	ND	--	6.0	0.006	60.3	0.268
Acetone	ND	--	ND	--		
Water	49.3	0.014	48.0	0.051	30.3	0.135
Combined ACN/water					90.6	0.403
Unknown (Rt ~15 min)					5.4	0.024
Unknown (Rt ~16 min)					5.6	0.025
Unknown (Rt ~27 min)					6.1	0.027
Unknown (Rt ~33 min)					9.3	0.041
Unknown (Rt ~35 min)					5.3	0.024
Unknown (Rt ~36 min)					4.9	0.022
Unknown (Rt ~40 min)					5.8	0.026
Unknown (Rt ~46 min)					5.0	0.022
Unknown (Rt ~48 min)					4.6	0.020
Multiple minor unknowns ²					38.6	0.172
2 M HCl	14.1	0.004	14.0	0.015	3.9	0.017
PES	36.6	0.011	32.0	0.034	5.5	0.024
Metabolite Fraction	Stems (134-day PHI)		Roots (134-day PHI)			
	TRR = 0.075 ppm		TRR = 0.187 ppm			
	%TRR	ppm	%TRR	ppm		
ACN	58.3	0.044	39.3	0.074		
Acetone	ND	--				
Water	22.2	0.017				
2 M HCl	5.6	0.004				
PES	14.0	0.010	60.7	0.113		

¹ ND = Nondetectable. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable radioactivity (following solvent extraction and hydrolysis as applicable) and nonextractable residues.

² Remaining radioactive regions, each accounting for ≤4.5% TRR (≤0.020 ppm); total calculated by the study reviewer by subtraction.

Foliar Application

Table B.7.1.3-9. Distribution of Radioactive Residues in Immature Soybean Matrices Following Foliar Application of [¹⁴C]Pethoxamid at 1.1 lb ai/A (1.2 kg ai/ha).¹								
Metabolite Fraction	Whole plants ² (8-day PHI)		Leaves (33-day PHI)		Stems (33-day PHI)		Roots (33-day PHI)	
	TRR = 31.576 ppm		TRR = 5.674 ppm		TRR = 0.135 ppm		TRR = 0.112 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN	70.5	22.262	40.3	2.287	54.7	0.074	35.8	0.040
Water	26.1	8.247						
Combined ACN/water	96.6	30.509						
Ethyl acetate	26.7	8.420						
Pethoxamid	1.1	0.363						
TKC-94-N-malonyl cysteine (MET-47)	5.2	1.655						
TKC-94-thiol (MET-43)	2.8	0.896						
TKC-94-SO-thiol	1.6	0.498						
Unknown (Rt ~45 min)	2.6	0.807						
Unknown (Rt ~47 min)	1.5	0.461						
Unknown (Rt ~48 min)	1.4	0.438						
Unknown (Rt ~61 min)	3.0	0.947						
Multiple minor unks. ³	7.5	2.226						
Water	69.9	22.089						
TKC-94-SO-cysteine	6.4	2.034						
TKC-94-cysteine (MET-30)	20.3	6.403						
TKC-94-N-malonyl cysteine (MET-47)	12.2	3.850						
Unknown (Rt ~33 min)	5.7	1.800						
Unknown (Rt ~42 min)	3.9	1.221						
Multiple minor unks. ³	21.4	6.781						
0.1 M HCl	0.8	0.244						
0.1 M NaOH	1.0	0.320						
PES	1.6	0.502	59.7	3.387	45.3	0.061	64.2	0.072

¹ Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable radioactivity (following solvent extraction and hydrolysis as applicable) and nonextractable residues.

² In addition to the compounds listed below, which were identified and quantitated following partitioning of the combined ACN/water extract with ethyl acetate, the following compounds were tentatively identified but not quantitated following acid hydrolysis of the combined ACN/water extract: benzoic acid and N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)oxamic acid (MET-31). Putative structures were provided for two additional metabolites.

³ Remaining radioactive regions; total calculated by the study reviewer by subtraction.

Table B.7.1.3-10. Distribution of Radioactive Residues in Mature Soybean Matrices Following Foliar Application of [¹⁴ C]Pethoxamid at 1.1 lb ai/A (1.2 kg ai/ha). ¹						
Metabolite Fraction	Seed (97- & 106-day PHIs)		Pods (97- & 106-day PHI)		Leaves (99-day PHI)	
	TRR = 0.011 ppm		TRR =0.028 ppm		TRR = 0.055 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN	ND	--	ND	--	59.2	0.033
Acetone	ND	--	ND	--		
Water	58.1	0.006	38.1	0.011	28.1	0.015
2 M HCl	19.4	0.002	30.5	0.009	4.0	0.002
PES	22.5	0.002	31.4	0.009	8.7	0.005
Metabolite Fraction	Stems (106-day PHI)		Roots (106-day PHI)			
	TRR = 0.030 ppm		TRR = 0.060 ppm			
	%TRR	ppm	%TRR	ppm		
ACN	47.3	0.014	31.0	0.019		
Acetone	ND	--				
Water	13.5	0.004				
2 M HCl	13.5	0.004				
PES	25.6	0.008	69.0	0.042		

¹ ND = Nondetectable. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable radioactivity (following solvent extraction and hydrolysis as applicable) and nonextractable residues.

C. Storage Stability of Residues

Samples were stored frozen (*ca.* -20 °C) between harvest and extraction for analysis. Only limited study dates were provided in the submission. Sample harvest dates were calculated by the study reviewer from reported application dates and PHIs provided by the study author. No information was provided concerning when the metabolite profiles were established for leaves and immature whole plants. Based on dated chromatograms reflecting LC/MS analysis of the ethyl acetate and aqueous phases of 8-day PHI immature plant, samples were stored for up to 10.5 months prior to these analyses for characterization and identification of residues. In addition, the study author reported the date of final TLC analysis for an isolated metabolite which reflected a maximum potential storage interval of 22.9 months for the experimental phase of the study. Sample storage conditions and intervals are presented in Table B.7.1.3-11.

The study author noted that, based on the co-chromatography results generated using fresh sample extract of 8-day PHI immature whole plants prepared at some point after metabolite profiling, residues of TKC-94-N-malonyl-cysteine may have degraded to TKC-94-cysteine during storage. Storage stability data were submitted indicating that residues of pethoxamid are stable during frozen storage for up to 8 months in soybean forage and straw, and up to 12 months in soybean seed (refer to the DERs for MRIDs 49813540, 49813541, 49813544, and; B.7.6.2).

Table B.7.1.3-11. Summary of Storage Conditions.			
Matrix	Storage Temperature (°C)	Actual Storage Duration	Interval of Demonstrated Storage Stability
Soybean immature whole plants	-20	LC/MS analysis ¹ 319 days (10.5 months) TLC analysis of isolated metabolite ² 698 days (22.9 months)	Residues of pethoxamid are stable during frozen storage for up to 8 months in soybean forage and straw, and up to 12 months in soybean ³
Soybean leaves		≤698 days (22.9 months) ²	

¹ Calculated by the study reviewer from date of harvest and dated chromatograms.

² Calculated by the study reviewer from date of harvest and the end date provided by the study author for the practical phase of the study.

³ Refer to the DERs for MRIDs 49813540, 49813541, 49813544 -49813545; B.7.6.2

D. Identity of Residues in Soybean

The characterization and identification of radioactivity in soybean is summarized in Table B.7.1.3-12. HPLC analysis of the combined ACN/water extracts of mature leaves following preemergence application indicated that the residue was composed of a large number of unknowns, with the largest accounting for 9.3% TRR (0.041 ppm). Eight additional discrete unknowns were resolved, individually present at 4.6-6.1% TRR, with multiple minor unknowns, present individually at ≤4.5% TRR, together accounting for 38.6% TRR. The study author stated that the low levels of radioactivity and large amounts of co-extracted plant material in the sample made mass spectral identification of the unknowns impractical. HPLC analysis of the combined ACN/water extract of 99-day PHI mature leaves following foliar application (comprising 87.3% TRR, 0.048 ppm) was attempted using Method 3. The study author stated that evaluation of the resulting chromatogram was impossible due to the small amounts of radioactivity, but noted that the chromatogram suggested that the sample contained at least as many components as that of the 127-day PHI mature leaves and that it was unlikely that any single metabolite would exceed 10% TRR or 0.01 ppm.

The majority of characterization and identification efforts were focused on the 8-day PHI immature whole plants collected following foliar application because this matrix bore the highest residues of any matrix in the study, and >95% of the residues were extractable. Initial analysis of the combined ACN/water extract using HPLC Method 1 characterized the radioactivity in the sample as either polar material eluting with the load solvent or as a series of metabolites with retention times between 4 and 13 minutes. Attempts to further characterize the polar radioactivity via TLC using Methods 1, 2, and 4 were unsuccessful in resolving the compounds present in the sample. A small quantity of parent was identified using HPLC Method 3 and suggested that the metabolites in the immature plant extract were less polar than those observed in the corresponding mature extracts. The extract was subsequently partitioned with ethyl acetate, and the organic and aqueous phases were analyzed using HPLC Method 4, which was successful in more fully resolving the metabolites in the sample and was utilized in LC/MS and LC/MS/MS analysis to investigate the structures of the residue components.

Further characterization of the residues was conducted following strong acid hydrolysis (2 M and 6 M HCl at 80 and 95 °C, respectively) and base hydrolysis (2 M NaOH at 80 °C) of the

combined ACN/water extract. HPLC (Method 1) analysis of the acid hydrolysates indicated that polar radioactivity was converted to a number of less polar species (*ca.* six components between Rts 6-20 min) in the 2 M HCl hydrolysate, with a further simplified profile, consisting of a single major polar component (56% TRR, Rt ~7 min.), in the 6 M HCl hydrolysate. TLC Method 4 analysis of the acid and base hydrolysates suggested a greater degree of change in the metabolite profile resulting from acid hydrolysis than base hydrolysis; thus, the base hydrolysate was not further investigated.

Intact metabolites in the ethyl acetate and aqueous phases as well as hydrolysis products in the acid hydrolysates of the ACN/water extract of 8-day PHI immature whole plants were investigated by LC/MS and LC/MS/MS. Identification of pethoxamid was confirmed by LC/MS in the ethyl acetate phase, and the following metabolites were tentatively identified based on retention times, mass spectra, and molecular weights: TKC-94-N-malonyl cysteine (MET-47), TKC-94-thiol (MET-43), and TKC-94-SO-thiol. In the aqueous phase, TKC-94-cysteine (MET-30) and TKC-94-N-malonyl cysteine (MET-47) eluted together, with the malonyl-cysteine conjugate eluting as a shoulder on the peak, and a third metabolite, formed by oxidation of the sulfur atom, was tentatively identified as TKC-94-SO-cysteine. Identification of TKC-94-cysteine and TKC-94-N-malonyl cysteine was confirmed by HPLC and TLC co-chromatography using fresh sample extract prepared for this purpose. The study author noted that based on the co-chromatography results, it appeared that residues of TKC-94-N-malonyl-cysteine may have degraded to TKC-94-cysteine during storage.

On LC/MS and LC/MS/MS of the acid hydrolyzed extracts, benzoic acid and an oxamic acid metabolite (MET-31) were tentatively identified; the study author stated that benzoic acid was the single major component in the 6 M HCl hydrolyzed extract. In addition, possible structures were provided for two additional regions (designated as Region 9 and Region 5; refer to Table B.7.1.3-13). No quantitative data were provided for these analyses. The study author stated that the identification of MET-31 and benzoic acid following acid hydrolysis demonstrated that cleavage of conjugates could be achieved, and that the pethoxamid structure could be completely metabolized under these conditions.

Table B.7.1.3-12. Summary of Characterization and Identification of Radioactive Residues in Soybean Matrices Following Application of [¹⁴C]Pethoxamid at 1.1 lb ai/A (1.2 kg ai/ha).¹				
Compound ²	Preemergence application		Foliar application	
	Mature leaves (127-day PHI)		Immature whole plants (8-day PHI) ²	
	TRR = 0.444 ppm		TRR = 31.576 ppm	
	% TRR	ppm	% TRR	ppm
Pethoxamid	ND	--	1.1	0.363
TKC-94-SO-cysteine	ND	--	6.4	2.034
TKC-94-cysteine (MET-30)	ND	--	20.3	6.403
TKC-94-N-malonyl cysteine (MET-47)	ND	--	17.4	5.505
TKC-94-thiol (MET-43)	ND	--	2.8	0.896
TKC-94-SO-thiol	ND	--	1.6	0.498
Discrete unknowns	52.0	0.231	18.1	5.763
Multiple minor unknowns	38.6	0.172	28.9	9.007
HCl/NaOH hydrolysate(s)	3.9	0.017	1.8	0.564
Total extractable	94.5	0.420	98.4	31.073
Total identified	--	--	49.6	15.699
Total unidentified	90.6	0.403	47.0	14.770
Total bound residues (PES)	5.5	0.024	1.6	0.502
% Accountability ³	100		100	

¹ ND =Not detected. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable radioactivity (following solvent extraction and hydrolysis as applicable) and nonextractable radioactivity.

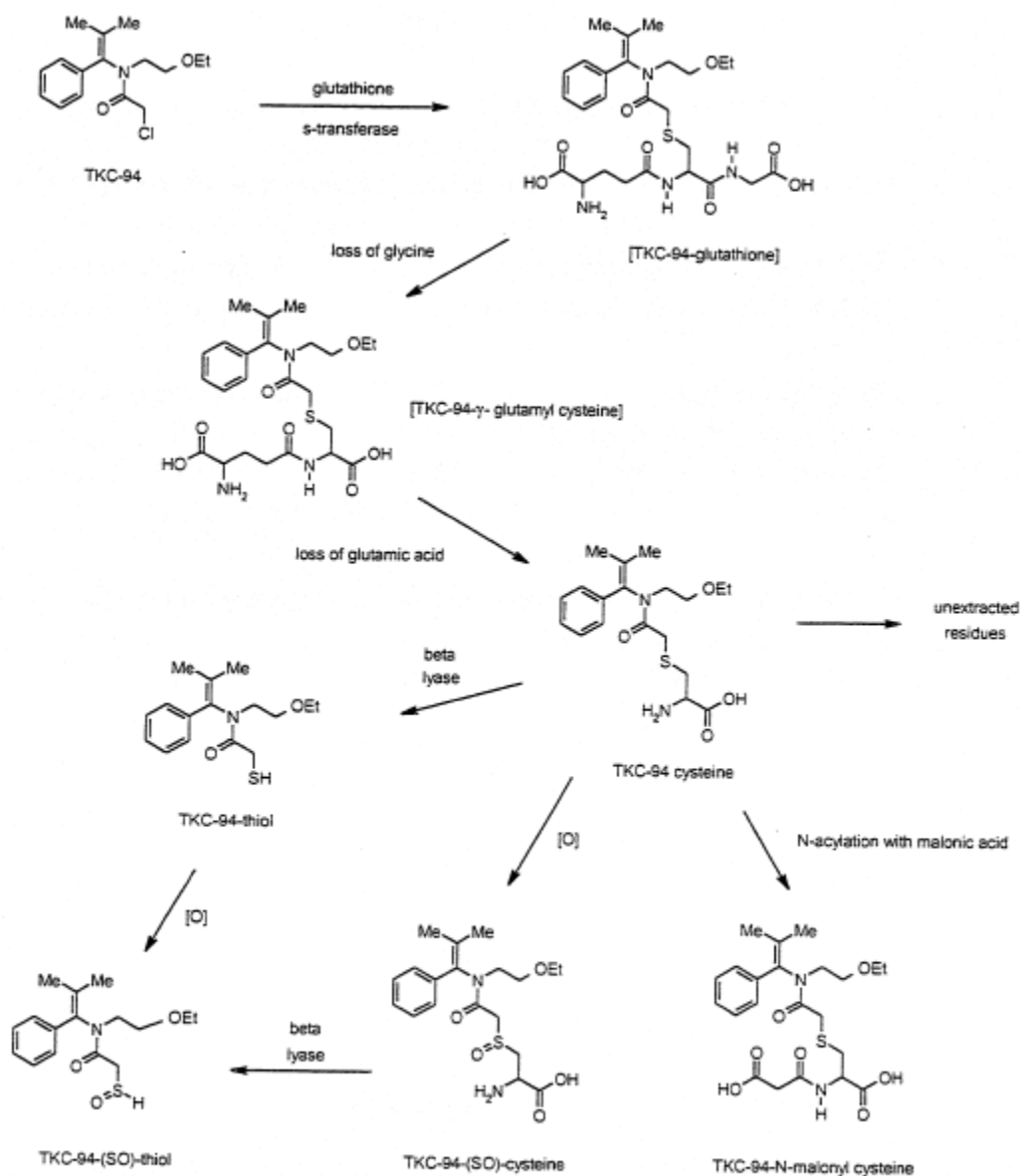
² For 8-day PHI whole plants, in addition to the listed compounds, which were identified and quantitated following partitioning of the combined ACN/water extract with ethyl acetate, the following compounds were tentatively identified but not quantitated following acid hydrolysis of the combined ACN/water extract: benzoic acid and N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)oxamic acid (MET-31), and putative structures were provided for two additional metabolites (designated as Region 9 and Region 5; refer to Table B.7.1.3-13).

³ Total (ppm)/TRR (ppm)×100

E. Proposed Metabolic Pathway

Based on the results of the soybean metabolism study, the study author concluded that pethoxamid was readily absorbed into the soybean plant and rapidly metabolized; refer to Figure B.7.1.3-4 for the proposed metabolic pathway. Due to the predominance of sulfur-containing metabolites, the study author proposed that pethoxamid was metabolized primarily via glutathione conjugation, with initial displacement of the chlorine atom by the sulfhydryl group of glutathione in a reaction catalyzed by glutathione-S-transferase. The glutathione conjugate (not found in the study) was metabolized initially by removal of glycine to yield TKC-94-γ-glutamyl cysteine (also not found), and then by removal of glutamic acid to yield TKC-94-cysteine (MET-30), which was the major metabolite observed in the immature plant. The presence of TKC-94-thiol (MET-43) and TKC-94-N-malonyl cysteine (MET-47) suggested extensive further metabolism of TKC-94-cysteine, possibly via β-lyase to form TKC-94-thiol and by malonyl transferase to form the N-malonyl conjugate. In addition, sulfur oxidation was a common route of metabolism, with SO derivatives observed for both TKC-94-cysteine and TKC-94-thiol. The appearance of metabolite MET-31 (not shown in the metabolic pathway) in the acid-hydrolyzed extracts suggests that this metabolite may be present in a conjugated form in the plant.

Figure B.7.1.3-4. Proposed Metabolic Profile of Pethoxamid in Soybean.



Compounds in brackets [] were not detected in this study

Table B.7.1.3-13. Identification of Compounds Found in the Metabolism Study.¹

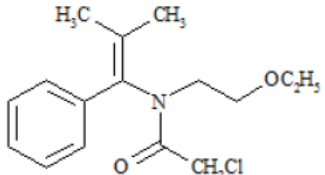
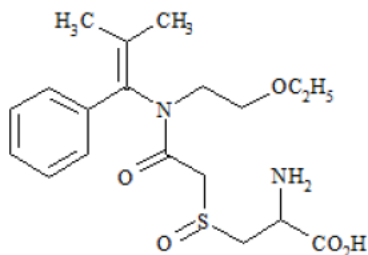
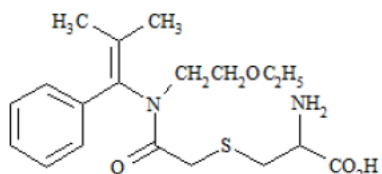
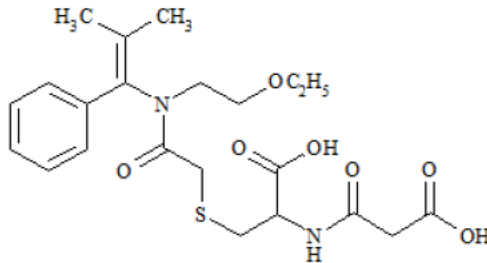
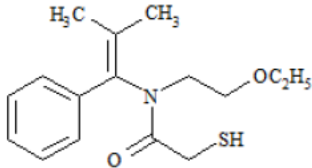
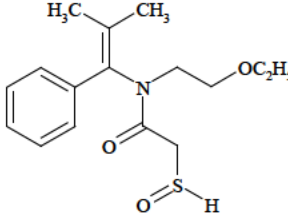
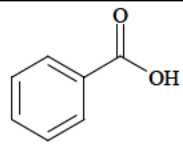
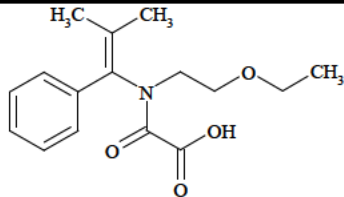
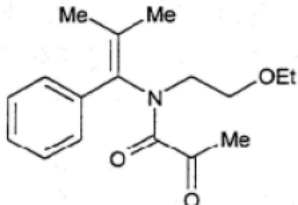
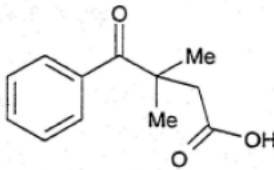
Common Name/Code ²	Chemical Name	Chemical Structure
Pethoxamid	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide	
TKC-94-SO-cysteine	None provided	
TKC-94-cysteine (MET-30)	S-[<i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]-D,L-cysteine	
TKC-94-N-malonyl cysteine (MET-47)	<i>N</i> -(2-carboxyacetyl)-S-[<i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]-D,L-cysteine	
TKC-94-thiol (MET-43)	<i>N</i> -(2-ethoxyethyl)-2-mercapto- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
TKC-94-SO-thiol	None provided	
Benzoic acid (following strong acid hydrolysis only)	Benzoic acid	

Table B.7.1.3-13. Identification of Compounds Found in the Metabolism Study.¹		
Common Name/Code ²	Chemical Name	Chemical Structure
MET-31 (following strong acid hydrolysis only)	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)oxamic acid	
Region 9 (following strong acid hydrolysis only; putative structure)	None provided	
Region 5 (following strong acid hydrolysis only; putative structure)	None provided	

¹ Refer to Appendix I for names and structures of all reference standards used in the study.

² Identification of pethoxamid, TKC-94-cysteine, and TKC-94-N-malonyl cysteine was confirmed; all other metabolites were tentatively identified.

III. CONCLUSIONS

The soybean metabolism study is considered scientifically acceptable. Samples of immature and mature soybean matrices were harvested following application of [phenyl-U-¹⁴C]pethoxamid at 1.1 lb ai/A (1.2 kg ai/ha) to soybean as an early postemergence application made to the soil 21 days after planting; a preemergence application made 1 day after planting; or a foliar application to soybean plants at BBCH 14-15 made 29 days after planting. The highest TRR were observed in immature whole plants collected 8 days following the foliar application, and the lowest TRR were found in seed harvested 97-134 days following soil and foliar applications. TRR in soybean matrices were generally lower in immature matrices harvested following soil application than following foliar application but were higher in mature matrices harvested following soil application, despite longer PHIs. TRR in immature whole plants were 0.030 and 0.016 ppm in samples harvested 14 and 27 days, respectively, following postemergence soil application; 0.084 ppm in samples harvested 36 days following preemergence application; and 31.576 ppm in samples harvested 8 days following foliar application. TRR in immature leaves, stems, and roots, respectively, were 0.096, 0.015, and 0.196 ppm in samples harvested 61 days following preemergence application, and 5.674, 0.135, and 0.112 ppm in samples harvested 33 days following foliar application. In mature soybean matrices harvested 125-134 days following preemergence application and 97-106 days following foliar application, TRR in seed and pod, respectively, were 0.029 and 0.107 ppm following preemergence application and 0.011 and 0.028 ppm following foliar application. TRR in leaves, stems, and roots respectively, were 0.444, 0.075, and 0.187 ppm following preemergence application and 0.055, 0.030, and 0.060 ppm following foliar application.

Following initial analysis and hydrolysis procedures, only three matrices were selected for further investigation of residues: mature leaves following both soil and foliar applications and 8-day PHI immature whole plants following foliar application.

A total of 49.6% TRR was identified in 8-day PHI immature whole plants. Pethoxamid was a minor residue component, accounting for 1.1% TRR. The major residue components were TKC-94-cysteine (MET-30) and TKC-94-N-malonyl cysteine (MET-47), accounting for 20.3% TRR and 17.4% TRR, respectively. Three other metabolites were identified and quantitated: TKC-94-SO-cysteine at 6.4% TRR, TKC-94-thiol (MET-43) at 2.8% TRR, and TKC-94-SO-thiol at 1.6% TRR. In addition, benzoic acid and oxamic acid metabolite MET-31 were tentatively identified but not quantitated following strong acid hydrolysis of the plant extract. Remaining radioactivity was comprised of six discrete unknowns, each present at 1.4-5.7% TRR, that together accounted for 18.1% TRR, and multiple minor unknowns that were not further resolved but together accounted for 28.9% TRR.

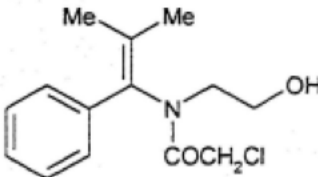
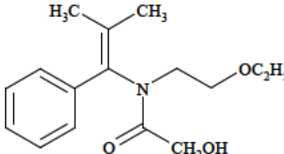
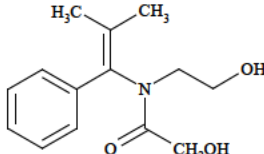
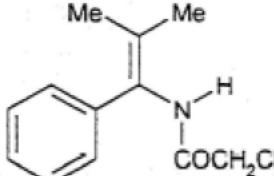
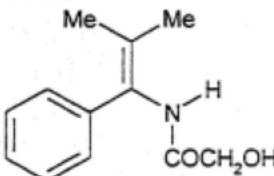
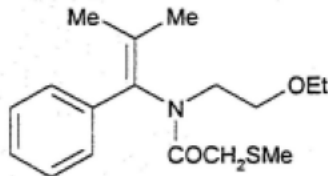
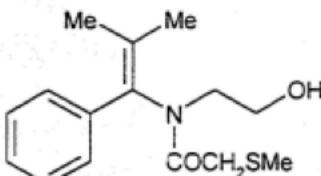
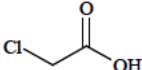
No residue components were identified in mature leaves. In 127-day PHI mature leaves collected following preemergence application, the residue was composed of a large number of unknowns, with the largest accounting for 9.3% TRR. Eight additional discrete unknowns were resolved, individually present at 4.6-6.1% TRR and together accounting for 52.0% TRR, with multiple minor unknowns, present individually at $\leq 4.5\%$ TRR, together accounting for 38.6% TRR. The study author stated that the low levels of radioactivity and large amounts of co-extracted plant material in the sample made mass spectral identification of the unknowns impractical. HPLC analysis of the combined ACN/water extract of 99-day PHI mature leaves was also conducted. The study author stated that evaluation of the resulting chromatogram was impossible due to the small amounts of radioactivity, but noted that the chromatogram suggested that the sample contained at least as many components as that of the 127-day PHI mature leaves and that it was unlikely that any single metabolite would exceed 10% TRR or 0.01 ppm.

Based on the results of the soybean metabolism study, the study author concluded that pethoxamid was readily absorbed into the soybean plant and rapidly metabolized. Due to the predominance of sulfur-containing metabolites, the study author proposed that pethoxamid was metabolized primarily via glutathione conjugation, with initial displacement of the chlorine atom by the sulfhydryl group of glutathione in a reaction catalyzed by glutathione-S-transferase. The glutathione conjugate (not found in the study) was metabolized initially by removal of glycine to yield TKC-94- γ -glutamyl cysteine (also not found), and then by removal of glutamic acid to yield TKC-94-cysteine (MET-30), which was the major metabolite observed in the immature plant. The presence of TKC-94-thiol (MET-43) and TKC-94-N-malonyl cysteine (MET-47) suggested extensive further metabolism of TKC-94-cysteine, possibly via β -lyase to form TKC-94-thiol and by malonyl transferase to form the N-malonyl conjugate. In addition, sulfur oxidation was a common route of metabolism, with SO derivatives observed for both TKC-94-cysteine and TKC-94-thiol. The appearance of MET-31 in the acid-hydrolyzed extracts suggests that this metabolite may be present in a conjugated form in the plant.

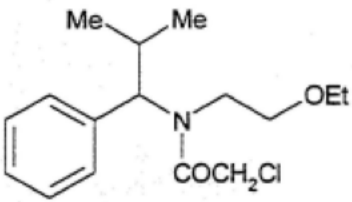
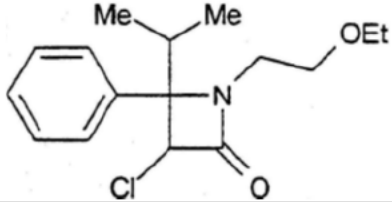
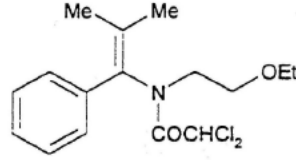
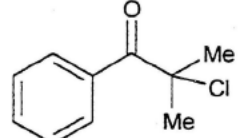
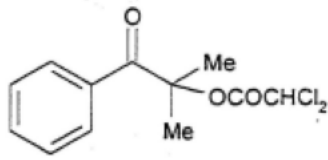
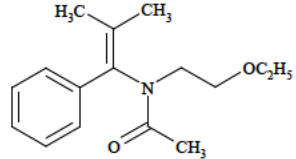
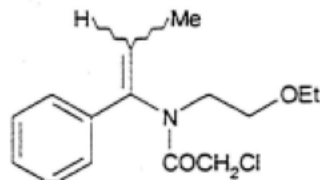
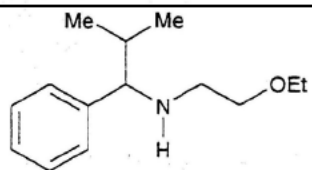
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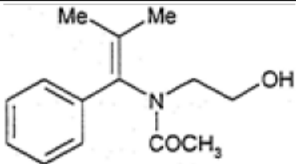
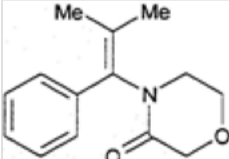
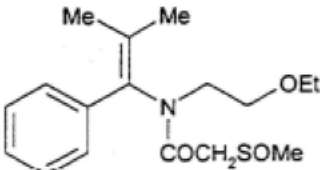
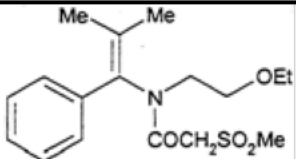
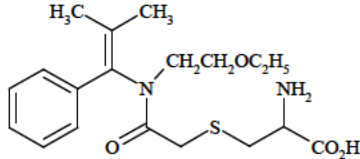
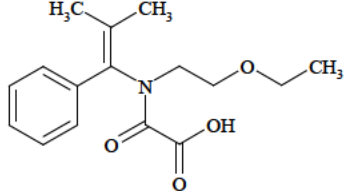
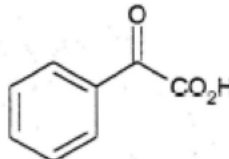
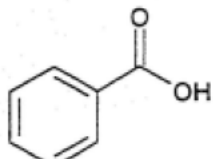
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Appendix I. Reference Standards Used in the Study.

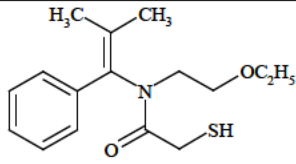
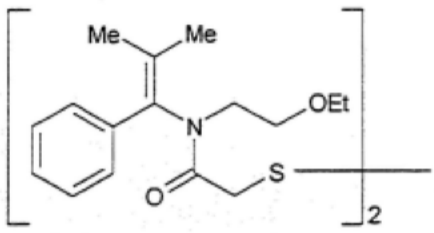
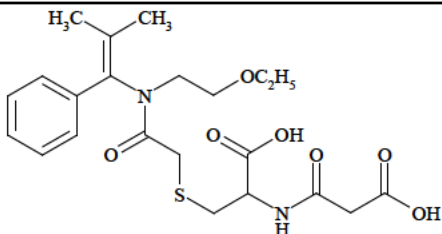
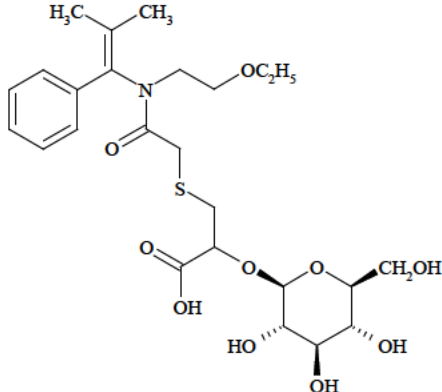
Name	Chemical name	Structure
MET-1	2-chloro-N-(2-hydroxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-2	N-(2-ethoxyethyl)-2-hydroxy-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-3	2-hydroxy-N-(2-hydroxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-4	2-chloro-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-5	2-hydroxy-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-6	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-(methylthio)acetamide	
MET-7	N-(2-hydroxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-(methylthio)acetamide	
MET-8	chloroacetic acid	

Name	Chemical name	Structure
MET-9	2-chloro-N-(2-ethoxyethyl)acetamide	
MET-10	N-(2-ethoxyethyl)benzamide	
MET-11	2-hydroxy-2-methylpropiophenone	
MET-12	propiophenone	
MET-13	2-(2-chloroacetoxy)-2-methylpropiophenone	
MET-14	isobutyrophenone	
MET-15	2-chloro-N-[2-(2-chloroacetoxy)ethyl]-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-16	2-chloro-N-(2-chloroethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide	

Name	Chemical name	Structure
MET-17	2-chloro-N-(2-ethoxyethyl)-N-[(2-methyl-1-phenyl)propyl]acetamide	
MET-18	3-chloro-N-(2-ethoxyethyl)-4-isopropyl-4-phenyl-2-azetidinone	
MET-19	2,2-dichloro-N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-20	2-chloro-2-methylpropiophenone	
MET-21	2-(2,2-dichloroacetoxy)-2-methylpropiophenone	
MET-22	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-23	(E,Z)-2-chloro-N-(2-ethoxyethyl)-N-(1-phenyl-1-propenyl)acetamide	
MET-24	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl)propylamine	
MET-25	2-ethoxyethylamine	

Name	Chemical name	Structure
MET-26	N-(2-hydroxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-27	N-(2-methyl-1-phenyl-1-propenyl)morpholine-3-one	
MET-28	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-(methylsulfinyl)acetamide	
MET-29	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-(methylsulfonyl)acetamide	
MET-30	S-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]-D,L-cysteine	
MET-31	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)oxamic acid	
MET-32	phenylglyoxylic acid	
MET-33	benzoic acid	

Name	Chemical name	Structure
MET-34	4-hydroxybenzoic acid	
MET-35	N-(2-hydroxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-(methylsulfinyl)acetamide	
MET-36	2-carboxy-3-hydroxycinnamic acid	
MET-37	3-hydroxy-2-methylpropiophenone	
MET-38	2-acetoxy-2-methylpropiophenone	
MET-40	(E,Z)-2-chloro-N-(2-hydroxyethyl)-N-(3-hydroxy-2-methyl-1-phenyl-1-propenyl)acetamide	
MET-41	3-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoyl methylthio]-D,L-lactic acid	
MET-42	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-sulfoacetamide	

Name	Chemical name	Structure
MET-43	N-(2-ethoxyethyl)-2-mercapto-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-44	bis S-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]	
MET-47	N-(2-carboxyacetyl)-S-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]-D,L-cysteine	
MET-48	3-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethylthio]-2-(α-D-glucopyranosyloxy)propionic acid	

B.7.2 Metabolism, Distribution, and Expression of Residues in Livestock (Annex IIA 6.2; Annex IIIA, 8.2)

B.7.2.1 Poultry

Document ID: MRID No. 49813530
PMRA No. Not available

Report: LaMar, J.E. (2015) The Metabolism of [¹⁴C]-Pethoxamid (1 Radiolabel) in Laying Hens. Study Numbers: 1460 PXA; 2503W. Unpublished study prepared by PTRL West, sponsored by Cheminova A/S, and submitted by FMC Corporation. 214 p.

Guidelines: EPA OCSPP Harmonized Test Guideline 860.1300 Nature of the Residue - Plants, Livestock (August 1996)
PMRA Regulatory Directive Dir98-02 – Residue Chemistry Guidelines, Section 2 -Nature of the Residue - Plants, Livestock
OECD Guideline 503 Metabolism in Livestock (January 2007)

GLP Compliance: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Acceptability: The study is considered scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP#452109.

Evaluator: Sheila Piper, Chemist, HED, RAB6 *Sheila Piper*

Secondary Evaluator: Robert Lanthier, PMRA

Note: This Data Evaluation Record (DER) was prepared under contract by CDM/CSS-Dynamac Joint Venture (submitted 2/21/18). The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies. The DER has been reviewed by HED and revised as necessary to reflect current Office of Pesticide Programs (OPP) policies.

EXECUTIVE SUMMARY

The metabolism of [phenyl-U-¹⁴C]pethoxamid (specific activity 55.27 µCi/mg) in laying hens was administered orally via gelatin capsule once daily for 7 consecutive days to 10 laying hens at a dose equivalent to 16.6 ppm in the feed (dry weight). Eggs were collected twice daily and pooled by day and time of collection. Excreta were collected daily and pooled by study day. Cage wash was collected following the final excreta collection and pooled. The hens were sacrificed approximately 20 hours after the final dose, and the following samples were collected from each animal and pooled by matrix: the entire liver, breast muscle, thigh muscle, abdominal fat, subcutaneous fat, and the gastrointestinal (GI) tract with contents. The in-life phase of the study was conducted by Wildlife International (Easton, MD), and the analytical phase of the study was conducted PTRL West (Hercules, CA).

The majority of the administered dose (AD) was recovered in the excreta (77.9% AD), with minor amounts recovered in the cage wash (0.1% AD) and GI tract (1.9% AD). Eggs accounted for 0.1% AD, and liver, muscle, and fat together accounted for ~0.1% AD. The overall recovery of the administered dose was 80.1%. TRR levels in eggs plateaued on Day 6 at a maximum of 0.059 ppm. TRR levels in tissues, determined by summing extractable and nonextractable

radioactivity were highest in liver (0.268 ppm) and were lower and similar in muscle (0.020-0.027 ppm) and fat (0.032-0.053 ppm).

Samples of eggs (Day 8), liver, muscle, fat, and excreta (composited from Days 2-8) were subjected to extraction and analysis. Samples of egg, liver, and muscle were extracted sequentially with aqueous acetonitrile (ACN) and ACN, and fat samples were extracted sequentially with acetone/hexane and acetone, with the extract fractions being pooled by matrix. Extraction with ACN/water released 47% TRR from liver, 67% TRR from egg, 33-40% TRR from muscle, and 79% TRR from excreta, and extraction with acetone/hexane released 38-47% TRR from fat. The initial post-extraction solids (PES) were subjected to mild acid (0.1 M HCl) and base (0.2 M KOH) hydrolyses, which together released an additional 19% TRR from liver, 9% TRR from eggs, 26-30% TRR from muscle, and 38-42% TRR from fat. Following the acid/base hydrolyses, the remaining PES fractions from liver, egg, thigh muscle, and subcutaneous fat were also subjected to protease digestion (pronase) in phosphate buffer, which released an additional 21% TRR from liver, 19% TRR from egg, 30% TRR from thigh muscle, and 17% TRR from subcutaneous fat. The overall extractability of radioactivity was 70-99% TRR from eggs and tissues, with levels of radioactivity ≤ 0.006 ppm in each of the final PES fractions.

Pethoxamid and six metabolites, MET-2, MET-3, MET-30, MET-42, MET-47, and MET-103, were quantified and initially identified in the solvent-extractable residues of hen matrices by co-chromatography with reference standards using high performance liquid chromatography (HPLC) with UV detection and radiodetection. Identifications were confirmed by one- and/or two-dimensional thin layer chromatography (1D- and 2D-TLC). TLC analysis of the solvent extracts confirmed the identification of pethoxamid and metabolites MET-2, MET-3, and MET-42 in liver and eggs, identification of MET-2 in fats, and identification of pethoxamid, MET-2, MET-3, MET-30, MET-42, and MET-103 in excreta. No confirmatory TLC analyses were conducted on muscle extracts due to the low levels of radioactivity (≤ 0.009 ppm). The structure of one unknown (RT17e) isolated from excreta was elucidated as a sulfate conjugate of des-ethyl pethoxamid by HPLC with mass spectrometry (LC/MS) detection. None of the radioactivity released by the acid/base or enzymatic hydrolyses was analyzed chromatographically due to low levels of radioactivity in these fractions. Samples were extracted and initially analyzed by HPLC within 1.3 months of sacrifice. Freezer storage stability was investigated using liver extracts, which were reanalyzed after 13.8 months of frozen storage. The metabolite profiles in the chromatograms obtained from the first and second HPLC analyses were similar for liver samples, demonstrating the stability of the ^{14}C -residue profile in poultry commodities for up to 13.8 months of frozen storage. No additional storage stability data are required to support this study.

Pethoxamid was extensively metabolized in hens, with only minor amounts of parent detected in liver (0.4% TRR, 0.001 ppm), eggs (1.7% TRR, 0.001 ppm), and fat (2.7-7.3% TRR, 0.001-0.004 ppm), and the six metabolites identified in eggs and tissues individually accounted for $\leq 8.2\%$ TRR (≤ 0.009 ppm) in any matrix. All six metabolites were detected in liver; however, MET-3 and MET-103 were not resolved. Metabolite levels in liver were as follows: MET-42 (3.4% TRR, 0.009 ppm), MET-3/MET-103 (2.7% TRR, 0.007 ppm), MET-30 (0.4% TRR, 0.001 ppm), MET-47 (3.4% TRR, 0.009 ppm), and MET-2 (0.5% TRR, 0.001 ppm). All six

metabolites were detected in eggs at the following levels: MET-42 (8.2% TRR, 0.004 ppm), MET-3 (2.8% TRR, 0.001 ppm), MET-103 (6.9% TRR, 0.003 ppm), MET-30 (3.3% TRR, 0.001 ppm), MET-47 (3.9% TRR, 0.002 ppm), and MET-2 (1.4% TRR, 0.001 ppm). In muscle, only metabolites MET-42 (2.7-3.7% TRR) and MET-3 (3.8% TRR) were detected, each at concentrations of 0.001 ppm. In fat, metabolite MET-2 (3.1-3.4% TRR) was detected, at ≤ 0.002 ppm. The overall level of identification was relatively low: 10.8% TRR in liver, 28.2% TRR in eggs, 3.7-6.5% TRR in muscle, and 5.8-10.7% TRR in fat.

The majority of solvent-extractable radioactivity in eggs and tissues (15.6-39.1% TRR) was comprised of single and multicomponent unknowns, each present at $\leq 5.6\%$ TRR (≤ 0.015 ppm) in liver, $\leq 8.6\%$ TRR (≤ 0.004 ppm) in eggs, $\leq 14.0\%$ TRR (≤ 0.003 ppm) in muscle and $\leq 21.1\%$ TRR (≤ 0.007 ppm) in fat. Radioactivity released by mild acid and base hydrolyses also accounted for substantial portions of the TRR in liver, muscle, and fat. Levels of radioactivity in the individual acid and base hydrolysates from eggs (≤ 0.003 ppm), muscle (≤ 0.005 ppm), and fat (≤ 0.013 ppm) were too low for additional analysis. Following partitioning of the combined acid/base hydrolysate of liver, organosoluble residues accounted for only 1.1% TRR (0.003 ppm), while a precipitate (15.3% TRR) was characterized as being associated with denatured proteins. Other major portions of the TRR in liver (17.2% TRR), eggs (18.6% TRR), and muscle (29.6% TRR) were characterized as being associated with proteinaceous fractions, and 10.1% TRR in liver was characterized as being associated with fatty acid-related components.

Pethoxamid and five of the metabolites (all but MET-3) detected in eggs and tissues were also detected in excreta, along with metabolite RT17e, which was unique to excreta. Radioactive residues identified in excreta included: pethoxamid (0.3% TRR), MET-42 (6.4% TRR), MET-103 (3.9% TRR), MET-30 (4.5% TRR), MET-47 (1.7% TRR), MET-2 (0.5% TRR), and RT17e (8.2% TRR). Other minor unknowns in excreta isolated by HPLC each accounted for $\leq 13.6\%$ TRR.

The study author proposed that pethoxamid is extensively metabolized in laying hens, primarily via glutathione-S-transferase to form the cysteine conjugates (MET-103 and MET-30), which are oxidized to yield the sulfonic acid (MET-42). Metabolite MET-2 may result from the direct hydrolysis of the chlorine atom of pethoxamid or from hydrolysis of MET-103 and/or MET-30. The ether bond of MET-2 is also cleaved to form MET-3. A sulfate conjugate of des-ethyl pethoxamid (RT17e) was also observed in excreta. A variety of bound residues were characterized as being associated with natural products, primarily proteins.

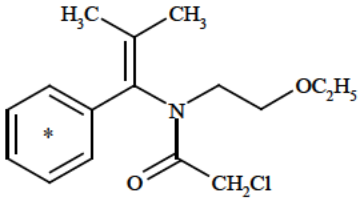
I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

The radiolabeled test substance, which was dissolved in toluene, was isotopically diluted with non-labeled pethoxamid. The diluted test substance was evaporated under nitrogen, redissolved in acetone, and dispensed into gelatin capsules containing powdered cellulose. The solvent was allowed to evaporate, and the capsules were stored frozen prior to administration. Spare capsules

were prepared to evaluate radiochemical purity pre- and post-dosing. Stability analyses indicated that the test substance was stable in the dosing capsules stored frozen throughout dosing (99.7% radiochemical purity, post-dose).

Table B.7.2.1-1. Pethoxamid Nomenclature.	
Common name	Pethoxamid
Identity	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide
CAS registry number	106700-29-2
Molecular weight	Non-labeled: 295.80 g/mol Radiolabeled: 296.7 g/mol
Company experimental name	TKC-94
Radiolabeled compound	[phenyl-U- ¹⁴ C]pethoxamid
Lot/Batch #	CFQ41685
Radiochemical purity	99.5% (pre-dose), 99.7% (post-dose)
Specific activity as received	29 mCi/mmol (97.74 µCi/mg; 217, 000 dpm/µg)
Specific activity of dosed	16.4 mCi/mmol (55.27 µCi/mg; 123,000 dpm/µg)
Position of radiolabel	

2. Animals

Table B.7.2.1-2. General Test Animal Information.					
Species	Breed	Age	Weight at Study Initiation (kg)	Health Status	Description of Housing/Holding Area
Hen	Not specified	32 weeks	1.571-2.056	Healthy	Climate-controlled room with individual pens (75 cm × 90 cm × 45 cm)

B. STUDY DESIGN

Dose Regime

The in-life phase of the study was conducted by Wildlife International (Easton, MD). Hens were fed a standard poultry diet throughout acclimation and dosing, and provided water *ad libitum*. Hens were housed in individual pens in a climate-controlled room; the temperature averaged 23.1 ± 0.8 °C during dosing with a relative humidity of $71 \pm 7\%$ and a 17-hour/7-hour light/dark cycle. Hens were weighed on Study Days 1 (prior to initial dose administration) and 8 (prior to sacrifice), and were monitored daily for any adverse health effects. Feed consumption was monitored daily and was steady for each hen throughout the dosing period. Ten hens were dosed orally once a day via gelatin capsule with [¹⁴C]pethoxamid for 7 consecutive days; dosing occurred daily following the morning egg collection. Daily feed consumption averaged 112-125 g/hen/day on a fresh-weight basis for Study Days 1 through 7, or 99-110 g/hen/day on a dry-weight basis. The hens remained healthy throughout the study; there were no clinical signs of toxicity or treatment-related effects on egg production, feed consumption, or body weight. We note that initial dose calculations were based on average feed consumption of 0.160 kg fresh

wt/hen/day, observed during the 7 days prior to dosing. Actual feed consumption and dose levels attained during dosing are presented in Tables B.7.2.1-3 and B.7.2.1-4.

Table B.7.2.1-3. Test Animal Dietary Regime.				
Composition of Diet	Average Feed Consumption ¹ (kg fresh wt/hen/day)	Water	Acclimation Period	Predosing
Study Game Bird Diet ²	0.112-0.125	<i>Ad libitum</i>	Approximately 5 weeks	No

¹ Averages for Study Days 1-7; overall feed consumption averaged 0.120 kg fresh wt/hen/day, or 0.1056 kg dry wt/hen/day.

² Wildlife International Game Bird Ration plus 5% limestone; 11.9% moisture content; 88.1% dry weight.

Table B.7.2.1-4. Test Animal Dosing Regime.				
Treatment Type	Treatment Level (ppm in diet) ¹	Vehicle	Dose (mg ai/kg bw/day)	Timing/Duration
Oral	16.6	Capsule	0.978	Once daily in the morning following egg collection for 7 consecutive days

¹ Dietary burden on a dry-weight basis; dietary concentration of pethoxamid was 14.7 ppm on a fresh-weight basis.

Sampling

Eggs were collected twice daily (Table B.7.2.1-5). Eggs were washed to remove any possible contamination from excreta, cracked (discarding the shells), pooled by study day and time of collection (AM or PM), homogenized, and stored frozen (temperature unspecified) until shipment to the analytical laboratory. Excreta were collected daily prior to dosing, pooled by study day, and frozen. Cage wash/rinse samples were collected at the end of the exposure period (Study Day 8) following the final excreta collection. Each cage pan was rinsed with at least 200 mL of ACN, and the washes were pooled and stored frozen.

Hens were sacrificed ~20 hours after the final dose, and the following samples were collected from each animal: the entire liver, breast muscle, thigh muscle, abdominal fat, subcutaneous fat, and the entire GI tract with contents. Tissue and GI tract samples were pooled by matrix, frozen and homogenized with dry ice, and stored frozen until shipment to the analytical laboratory. All samples were shipped frozen on dry ice by overnight courier and received within 19 days of sacrifice by the analytical laboratory (PTRL West, Hercules, CA), where samples were stored frozen (temperature unspecified).

Table B.7.2.1-5. Sample Collection Information.			
Eggs Collected	Excreta and Cage Wash Collected	Interval from Last Dose to Sacrifice	Tissues Harvested and Analyzed
Twice daily	Excreta – once daily Cage wash – sacrifice	~20 hours	Liver (entire), breast muscle, thigh muscle, abdominal fat, subcutaneous fat, and GI tract with contents

TRR Determination

TRR were determined directly by LSC for the cage wash sample and by combustion/LSC for excreta and the GI tract. Liver, muscle, fat, and egg samples were initially solubilized in Soluene tissue solubilizer at 50 °C prior to analysis by LSC. TRR in samples were also determined by summing radioactivity in extract and PES fractions. Radioactivity in extract fractions and

hydrolysates was determined by direct LSC, and radioactivity remaining in PES was determined by combustion/LSC or solubilization/LSC. The limits of detection and quantitation for TRR determinations were not reported.

Extraction and Analysis

Samples of liver, muscle (breast and thigh), fat (abdominal and subcutaneous), eggs (Day 8), and excreta (composited) were subjected to extraction and analysis. For excreta, equal portions of excreta homogenate from Study Days 2 through 8 were pooled for analysis. Generalized schematics of the extraction procedures are presented in Figures B.7.2.1-1 (liver, muscle, and egg) and B.7.2.1-2 (fat).

Liver, muscle, egg, and excreta samples were extracted twice with ACN:water (1:1, v:v) by shaking for 30 minutes and then extracted once with ACN. Extracts were isolated by centrifugation, combined, concentrated, and analyzed by HPLC and TLC (all matrices except muscle). To further investigate residues in liver, the combined ACN/water extract was subjected to acid hydrolysis with 1 N HCl (at 100 °C overnight), and the hydrolysate was analyzed by HPLC. The PES fractions from liver, muscle, and eggs were subjected to mild acid hydrolysis (0.1 M HCl at room temperature for 1 hour) and then mild basis hydrolysis (0.2 M KOH at room temperature for 1 hour), with the hydrolysates being isolated by centrifugation. The PES hydrolysates from liver were combined, acidified, and partitioned with ethyl acetate (EtOAc), then filtered to remove precipitate. Following the acid/base hydrolyses, subsamples of PES from liver, egg, thigh muscle, and subcutaneous fat were also treated with and without protease enzyme (pronase) in CaCl₂/phosphate buffer (10 mM CaCl₂ in 0.1 M NaH₂PO₄ buffer (pH 7.5) at 37 °C for 48 hours). The enzyme hydrolysate was separated by centrifugation and the amounts solubilized by protease and by buffer were determined by difference. For liver, the final PES fraction was solubilized under alkaline conditions (no further details provided), concentrated, redissolved in water, acidified, and partitioned with EtOAc.

Fat samples were extracted twice with acetone:hexane (1:1, v:v) by shaking for 30 minutes and then extracted once with acetone. Extracts were isolated by centrifugation, combined, concentrated, redissolved in hexane, then partitioned with ACN/hexane. The ACN fraction was analyzed by HPLC and TLC. The PES fractions were further subjected to mild acid and base hydrolyses as above, and a subsample of PES from subcutaneous fat was also subjected to protease digestion as described above.

Figure B.7.2.1-1. General Flowchart for the Extraction and Analysis of Liver, Muscle, and Egg.

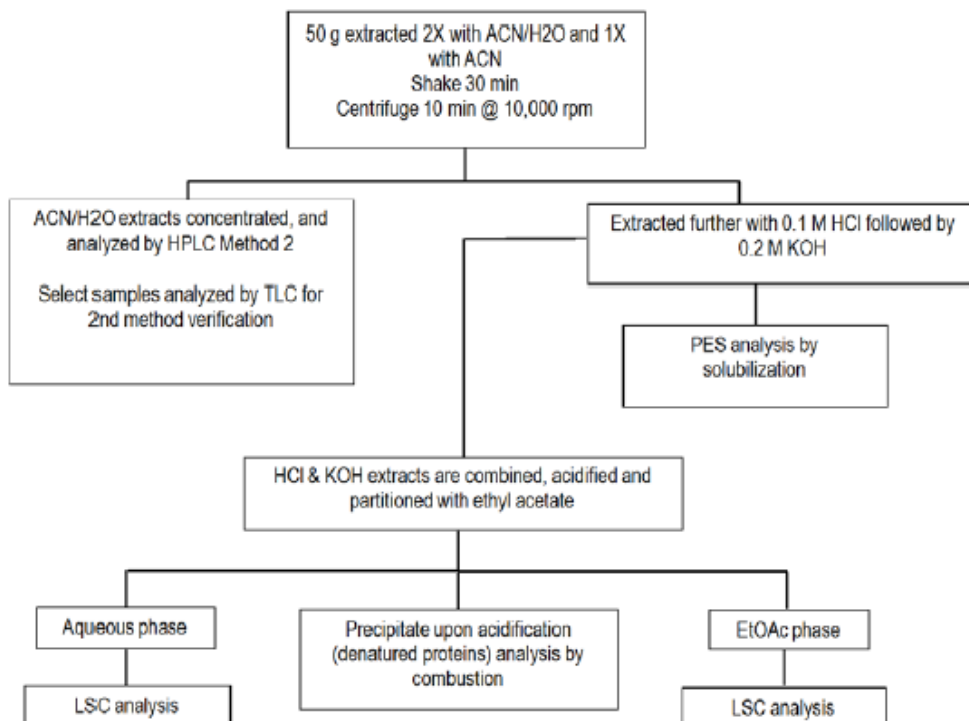
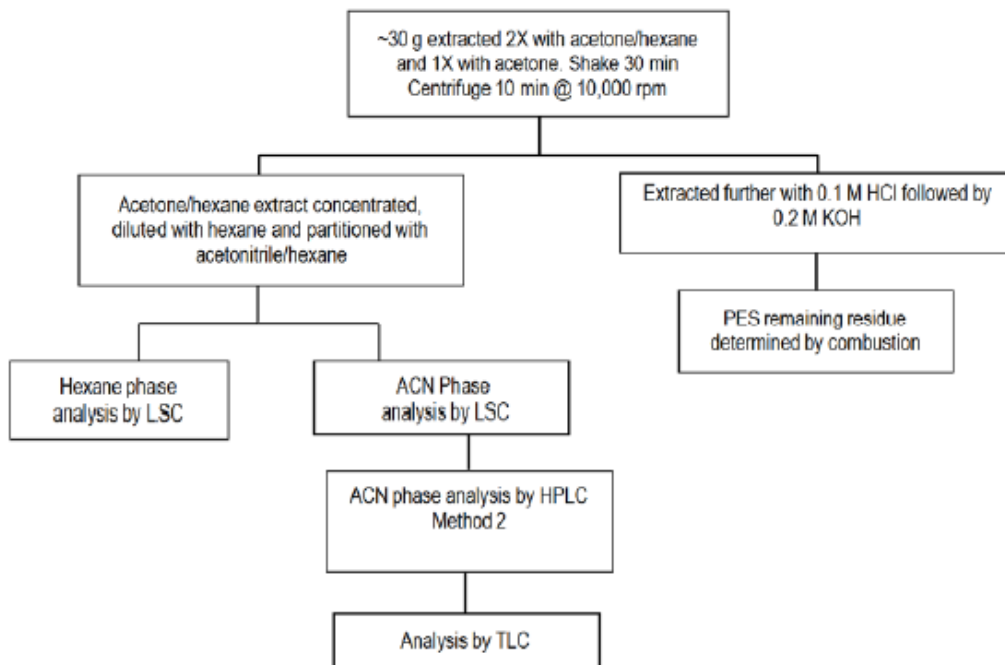


Figure B.7.2.1-2. General Flowchart for the Extraction and Analysis of Fat.



Identification and Characterization

Reverse-phase HPLC was used for the initial analysis and quantitation of ^{14}C -residues in sample extracts. The system (Method 2) consisted of a reverse-phase column (Capcell Pak C18 MGII) using a mobile phase gradient of water to ACN, each containing 0.1% trifluoroacetic acid, and was equipped with a UV detector (254 nm) and a flow-through radioactivity detector (RAD). Residues were initially identified by co-chromatography with nine reference standards (refer Table B.7.2.1-12). Additional HPLC systems were utilized for further characterization of extract fractions from liver or for isolation and characterization of specific components. These systems utilized UV and radiodetection and included the following:

- Method 3: similar to Method 2 but with a different mobile phase gradient
- Method 4: Inertsil Phenyl column with a mobile phase gradient of water to methanol (MeOH), each containing 0.1% trifluoroacetic acid
- Method 5: Luna Phenyl-hexyl column with a mobile phase gradient of water to ACN, each containing 0.1% formic acid
- Method 6: Luna Cyano column with a mobile phase gradient of water to ACN, each containing 0.1% formic acid
- Methods 7 & 8: Capcell CR (1:20) column with a mobile phase gradient of 10 mM ammonium acetate buffer (pH 6.0) to ACN containing 0.1% formic acid

Metabolite identities were confirmed by co-chromatography using 1D- or 2D-TLC analysis of solvent extracts from liver, eggs, fat, and excreta using one or more of the solvent systems below on silica gel 60 F₂₅₄ plates. Reference standards were detected by UV absorption, and ^{14}C -residues were detected using a phosphorimager. No quantitative data were provided with the TLC analyses.

- Method 1: 1D-TLC with 1% acetic acid in hexane:EtOAc (1:2, v:v)
- Method 2: 1D-TLC with chloroform:MeOH:acetic acid (7:3:0.3, v:v:v), and then redeveloped with 1% acetic acid in hexane:EtOAc (1:2, v:v)
- Method 3: 2D-TLC with chloroform:MeOH:acetic acid (7:3:0.3, v:v:v) in one dimension, then 1% acetic acid in hexane:EtOAc (1:2, v:v) in the second dimension, followed by butanol:acetic acid:water (3:2:1, v:v:v).

II. RESULTS AND DISCUSSION

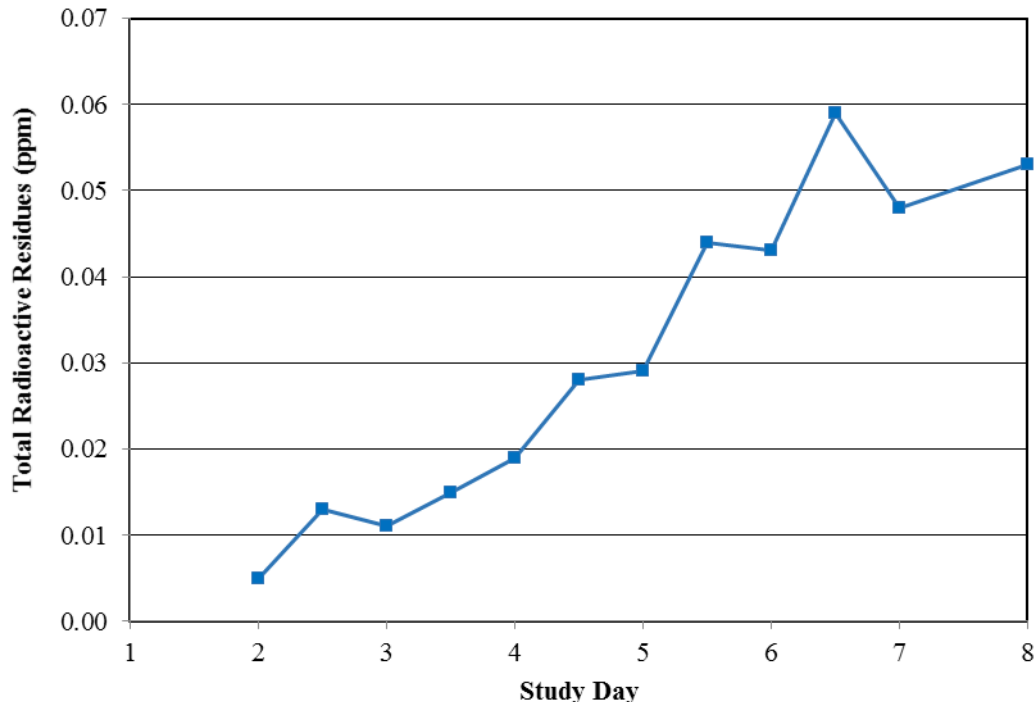
A. Total Radioactive Residues

The TRR in poultry matrices are presented in Table B.7.2.1-6. The majority of the AD was recovered in the excreta (77.9% AD), with minor amounts recovered in the cage wash (0.1% AD) and GI tract (1.9% AD). Eggs accounted for 0.1% AD, and liver, muscle, and fat together accounted for ~0.1% AD. The overall recovery of the administered dose was 80.1%. TRR levels in eggs plateaued on Day 6 at 0.059 ppm (Figure B.7.2.1-3). TRR levels in tissues were highest in liver (0.268 ppm) and were similar in muscle (0.020-0.027 ppm) and fat (0.032-0.053 ppm).

Table B.7.2.1-6. TRR in Eggs, Tissue, and Excreta.¹			
Matrix	Collection timing	¹⁴ C]Pethoxamid	
		ppm	% of dose
Eggs	Day 1 – PM	No sample ²	--
	Day 2 – AM	0.005	<0.01
	Day 2 – PM	0.013	<0.01
	Day 3 – AM	0.011	<0.01
	Day 3 – PM	0.015	<0.01
	Day 4 – AM	0.019	0.01
	Day 4 – PM	0.028	<0.01
	Day 5 – AM	0.029	0.01
	Day 5 – PM	0.044	<0.01
	Day 6 – AM	0.043	0.02
	Day 6 – PM	0.059	<0.01
	Day 7 – AM	0.048	0.02
	Day 7 – PM	No sample ²	--
	Day 8 – AM	0.053 (0.043)	0.03
	Duration	--	0.1
Liver	Sacrifice (Day 8)	0.341 (0.268)	0.1
Breast muscle	Sacrifice (Day 8)	0.035 (0.020)	<0.01
Thigh muscle	Sacrifice (Day 8)	0.037 (0.027)	<0.01
Abdominal fat	Sacrifice (Day 8)	0.030 (0.032)	<0.01
Subcutaneous fat	Sacrifice (Day 8)	0.051 (0.053)	<0.01
GI tract, with contents	Sacrifice (Day 8)	1.415	1.9
Cage wash	Sacrifice (Day 8)	0.083	0.1
Excreta	Day 1	ND	--
	Day 2	7.227	10.4
	Day 3	7.603	9.9
	Day 4	9.089	12.4
	Day 5	7.547	10.5
	Day 6	8.394	11.9
	Day 7	6.736	9.1
	Day 8	12.606	13.7
	Duration		77.9
Recovery of administered ¹⁴C-dose			80.1

¹ Determined by combustion/LSC (excreta and GI tract), direct LSC (cage wash), or solubilization/LSC (eggs and tissues). Ppm values in parentheses were calculated by summing extractable and nonextractable radioactivity. These values were used for all further determinations.

² No eggs were available for collection during the afternoon of Study Days 1 and 7.

Figure B.7.2.1-3. TRR in Eggs as a Function of Time.**B. Extraction, Characterization, and Distribution of Residues in Poultry**

The distribution of radioactivity in poultry matrices is presented in Tables B.7.2.1-7 (liver, eggs, and excreta) and B.7.2.1-8 (muscle and fat).

Aqueous ACN and ACN extractions released 47% TRR from liver, 67% TRR from eggs (Day 8), 33-40% TRR from muscle, and 79% TRR from composited excreta (Days 2-8); these solvent fractions were concentrated and analyzed by HPLC. For fat, acetone/hexane and acetone extractions released 38-47% TRR. Following concentration and partitioning with ACN and hexane, the ACN fraction from fat accounted for 26-34% TRR and was analyzed by HPLC. Subsequent mild acid and base hydrolyses released an additional 19% TRR from liver, 9% TRR from eggs, 26-30% TRR from muscle, and 38-42% TRR from fat. With the exception of liver, these hydrolysates contained low levels of radioactivity (≤ 0.013 ppm) and were not further analyzed. For liver, the combined acid/base hydrolysates were acidified and partitioned between water (2.2% TRR) and EtOAc (1.1% TRR), with most of the radioactivity remaining in a precipitate (15.3% TRR). Treatment of the PES fractions remaining after acid/base hydrolyses with protease in phosphate buffer released an additional 21% TRR from liver, 19% TRR from eggs, 30% TRR from thigh muscle, and 17% TRR from subcutaneous fat. None of the radioactivity released by acid/base or enzymatic hydrolysis was analyzed chromatographically.

The overall extractability of radioactivity was 70-99% TRR from eggs and tissues, and 79% TRR from excreta. The final PES fractions accounted for 0.7% TRR in liver, 4.7% TRR in eggs, 11.1-30.0% in muscle, and 3.8-15.6% TRR in fat, with levels of radioactivity being ≤ 0.006 ppm in each final PES fraction.

Note to EPA Reviewer: The % TRR values for residue components in all matrices were recalculated by the study reviewer. The % TRR reported by the study author in Tables 8 and 13-18 of the submission were calculated incorrectly: a number of minor residue components that appeared in the chromatograms beginning with Figure 8 for liver and Figure 31 for eggs and remaining matrices were excluded from the calculations.

Table B.7.2.1-7. Distribution of Radioactive Residues in Poultry Excreta, Liver, and Eggs Following Dosing with [¹⁴C]Pethoxamid at 16.6 ppm.¹						
Metabolite Fraction	Liver		Eggs (Day 8)		Excreta (Days 2-8)	
	TRR = 0.268 ppm		TRR = 0.043 ppm		%AD = 77.9	
	%TRR	ppm	%TRR	ppm	%TRR	%AD
Combined ACN/water	46.6	0.125	67.4	0.029	78.6	61.2
Pethoxamid	0.4	0.001	1.7	0.001	0.3	0.2
MET-42	3.4	0.009	8.2	0.004	6.4	5.0
MET-3	2.7	0.007	2.8	0.001	ND	--
MET-103			6.9	0.003	3.9	3.0
MET-30	0.4	0.001	3.3	0.001	4.5	3.5
MET-47	3.4	0.009	3.9	0.002	1.7	1.3
MET-2	0.5	0.001	1.4	0.001	0.5	0.4
RT17e	ND	--	ND	--	8.2	6.4
Minor unknowns ²	35.9	0.095	39.1	0.017	53.3	43.6
0.1 M HCl hydrolysate	4.5	0.012	2.3	0.001		
0.2 M KOH hydrolysate	14.2	0.038	7.0	0.003		
Combined hydrolysates	18.7	0.050				
Aqueous	2.2	0.006				
EtOAc	1.1	0.003				
Precipitate ³	15.3	0.041				
PES	34.7	0.093	23.3	0.101	21.4	16.7
Protease soluble ⁴	17.2	0.046	18.6	0.008		
Buffer soluble	3.7	0.010	<0.1	<0.001		
PES ⁵	13.8	0.037	4.7	0.002		
Aqueous	3.0	0.008				
EtOAc ⁵	10.1	0.027				
Precipitate	0.7	0.002				

¹ ND = Not detected. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Single or multicomponent unknowns with each single component present at ≤5.6% TRR (≤0.015 ppm) in liver, ≤8.6% TRR (≤0.004 ppm) in eggs, and ≤13.6% TRR in excreta.

³ Characterized as likely associated with denatured proteins.

⁴ Characterized as being associated with protein.

⁵ Characterized as being associated with fatty acid related components.

Table B.7.2.1-8. Distribution of Radioactive Residues in Poultry Muscle and Fat Following Dosing with [¹⁴C]Pethoxamid at 16.6 ppm.¹								
Metabolite Fraction	Breast muscle		Thigh muscle		Abdominal fat		Subcutaneous fat	
	TRR = 0.020 ppm		TRR = 0.027 ppm		TRR = 0.032 ppm		TRR = 0.053 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Combined ACN/water (muscle) or acetone/hexane (fat)	40.0	0.008	33.3	0.009	46.9	0.015	37.7	0.020
ACN					34.4	0.011	26.4	0.014
Pethoxamid	ND	--	ND	--	2.7	0.001	7.3	0.004
MET-42	2.7	0.001	3.7	0.001	ND	--	ND	--
MET-3	3.8	0.001	ND	--	ND	--	ND	--
MET-2	ND	--	ND		3.1	0.001	3.4	0.002
Minor unknowns ²	33.5	0.006	30.3	0.008	28.6	0.010	15.6	0.008
Hexane					12.5	0.004	11.3	0.006
0.1 M HCl hydrolysate	5.0	0.001	3.7	0.001	18.8	0.006	17.0	0.009
0.2 M KOH hydrolysate	25.0	0.005	22.2	0.006	18.8	0.006	24.5	0.013
PES	30.0	0.006	40.7	0.011	15.6	0.005	20.8	0.011
Protease soluble ³			29.6	0.008			5.7	0.003
Buffer soluble			<0.1	<0.001			11.3	0.006
PES			11.1	0.003			3.8	0.002

¹ ND = Not detected. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Single or multicomponent unknowns with each single component present at ≤14.0% TRR (≤0.003 ppm) in breast muscle, ≤7.4% TRR (≤0.002 ppm) in thigh muscle, ≤21.1% TRR (≤0.007 ppm) in abdominal fat, and ≤5.3% TRR (≤0.003 ppm) in subcutaneous fat.

³ Characterized as being associated with protein.

C. Storage Stability of Residues

Sample storage conditions and intervals are presented in Table B.7.2.1-9. All samples were reportedly stored frozen (<0 °C) as per the study protocol, although exact storage temperatures were not reported. Based on dated spreadsheets and chromatograms in the raw data, egg and tissue samples were solvent extracted, and the extracts were analyzed by HPLC within 1.3 months of sacrifice. The composite excreta sample was extracted and profiled within 1.6 months of sacrifice. The acid/base hydrolyses of the initial PES samples occurred within 1.4 months, and protease treatment of selected PES fractions occurred within 1.8 months. The final HPLC analysis of the solvent extract from liver was conducted 16.1 months after sacrifice.

To demonstrate the stability of ¹⁴C-residues during frozen storage, the solvent extract from liver was reanalyzed after 13.8 months of storage using the same HPLC method (Method 2) that was used for the initial analysis. Comparison of the radiochromatograms from the first and second analyses of the liver extract showed that the distribution of ¹⁴C-residues was generally similar between the two analyses, although comparison of specific metabolites was not possible due to the diffuse and multicomponent nature of the residue profile. As the initial ¹⁴C-residue profiles for all matrices were completed within 1.6 months of sacrifice, and ¹⁴C-residues in the initial solvent extract were shown to be generally stable under frozen storage, no additional storage stability data are required to support this study.

Table B.7.2.1-9. Summary of Storage Conditions.			
Matrix	Storage Temperature (°C)	Actual Storage Duration ¹	Interval of Demonstrated Storage Stability
Eggs and tissues	Frozen (temperature unspecified)	41 days (1.3 months)	The residue profile in liver extracts was stable under frozen conditions for up to 13.8 months.
Excreta		49 days (1.6 months)	

¹ Duration from sample collection to initial HPLC analysis for metabolite profiling.

D. Identity of Residues in Poultry

The characterization and identification of residues in poultry matrices is summarized in Tables B.7.2.1-10 (tissues and eggs) and B.7.2.1-11 (excreta). Pethoxamid and six metabolites, MET-2, MET-3, MET-30, MET-42, MET-47, and MET-103, were identified and quantitated in tissues and excreta by HPLC Method 2. TLC analysis of the solvent extracts confirmed the identification of pethoxamid and metabolites MET-2, MET-3, and MET-42 in liver and eggs, identification of MET-2 in fats, and identification of pethoxamid, MET-2, MET-3, MET-30, MET-42, and MET-103 in excreta. No confirmatory TLC analyses were conducted on muscle extracts due to the low levels of radioactivity (≤ 0.009 ppm).

Pethoxamid and all six metabolites were detected as minor residue components ($\leq 3.4\%$ TRR) in the combined ACN/water extract of liver, although metabolites MET-3 and MET-103 were not resolved. The majority of solvent-extractable radioactivity in liver (35.7% TRR) was composed of single and/or multicomponent unknowns, each present at $\leq 5.6\%$ TRR (≤ 0.015 ppm). When the combined acid/base hydrolysates were acidified and partitioned with EtOAc, the majority of the radioactivity (15.3% TRR, 0.041 ppm) was associated with a precipitate that was believed to be associated with denatured proteins. Following protease hydrolysis of the remaining PES, 17.2% TRR was characterized as being associated with protein, and following alkaline solubilization of the final PES and partitioning with EtOAc, 10.1% TRR was associated with fatty acid or saponifiable biomolecules.

Pethoxamid and six metabolites were initially detected in eggs at the following levels: pethoxamid (1.7% TRR) MET-42 (8.2% TRR), MET-3 (2.8% TRR), MET-103 (6.9% TRR), MET-30 (3.3% TRR), MET-47 (3.9% TRR), and MET-2 (1.4% TRR). As in liver, the majority of solvent-extractable radioactivity in egg (39.1% TRR) was composed of single or multicomponent unknowns, each present at $\leq 8.6\%$ TRR (≤ 0.004 ppm). No additional analyses were conducted on solvent-extractable ¹⁴C-residues from egg, due to the low levels of radioactivity (≤ 0.004 ppm) associated with HPLC peaks. Protease digestion of the remaining PES released 18.6% TRR which was characterized as being associated with protein.

In breast and thigh muscle, only metabolites MET-42 (2.7-3.7% TRR) and MET-3 (3.8% TRR) were identified, each at concentrations of 0.001 ppm; pethoxamid was not detected. The majority of solvent-extractable radioactivity in muscle (30.3-33.5% TRR) was composed of single or multicomponent unknowns, each present at $\leq 14.0\%$ TRR (≤ 0.003 ppm). Following protease digestion of the remaining PES, 29.6% TRR (0.008 ppm) was characterized as being associated with protein.

For abdominal and subcutaneous fat, HPLC analyses of the ACN fractions (26.4-34.4% TRR) following partitioning of the combined acetone/hexane extract with ACN/hexane identified only

parent (2.7-7.3% TRR) and MET-2 (3.1-3.4% TRR) at concentrations of ≤ 0.004 and ≤ 0.002 ppm, respectively. The majority of radioactivity in the ACN fractions (15.6-28.6% TRR) was composed of single or multicomponent unknowns, each present at $\leq 21.1\%$ TRR (≤ 0.007 ppm). Protease digestion of the remaining PES fraction from subcutaneous fat released 5.7% TRR (0.003 ppm), which was characterized as being associated with protein.

Pethoxamid and all of the metabolites detected in eggs and tissues except MET-3 were also detected in excreta, along with one metabolite unique to excreta (RT17e) that was tentatively identified as a sulfate conjugate of des-ethyl pethoxamid following isolation and LC/MS analysis. Radioactive residues identified in excreta by HPLC Method 2 included: pethoxamid (0.3% TRR), MET-42 (6.4% TRR), MET-103 (3.9% TRR), MET-30 (4.5% TRR), MET-47 (1.7% TRR), MET-2 (0.5% TRR), and RT17e (8.2% TRR). Other minor unknowns in excreta isolated by HPLC each accounted for $\leq 13.6\%$ TRR.

In an attempt to improve resolution of the ^{14}C -residue profile in liver following HPLC analysis using Method 2, the liver extract was also analyzed using Methods 5, 6, 7 and 8; however, none of these systems greatly improved resolution of the profile or identified a greater portion of the residue. In each case, the residue profile was shown to consist of multiple components with each component accounting for $\leq 7.8\%$ TRR, with the exception of one peak that was resolved using Method 7, which exceeded 10% of the TRR. This peak (11.6% TRR) was part of a broad multicomponent fraction and would likely account for $< 10\%$ TRR if isolated. Following acid hydrolysis of the ACN/water extract of liver, the resulting profile still showed a complex mixture of poorly resolved peaks, although a distinct peak was detected for MET-2 (3.2% TRR), indicating a conversion of some components to MET-2 under acidic conditions.

Liver unknowns RT16 (6.4% TRR, 0.017 ppm) and RT17 (7.5% TRR, 0.020 ppm) were further investigated following isolation using HPLC Method 3. Unknown RT16 was further separated into three minor non-acidic, weak acid, and strong acid components (RT16a, -b, and -c, each $\leq 3.4\%$ TRR) using a weak anion exchange solid phase extraction cartridge (Strata X-AW) that was eluted sequentially with sodium acetate buffer (0.1 M, pH 5.4), ACN, 2% formic acid in MeOH, and 5% ammonium hydroxide in MeOH. Unknown RT17 (7.5% TRR, 0.020 ppm) was isolated by preparative HPLC (method not specified) and resolved into two components (each $\leq 5.2\%$ TRR, 0.014 ppm) on further analysis using HPLC Method 4.

To further investigate unknown RT17, the corresponding residue in feces was isolated by HPLC Method 2 and analyzed by full scan LC/MS using electrospray ionization in both the positive and negative ion modes, both before and after treatment with sulfatase enzyme. No further details were provided concerning the method. On treatment with sulfatase, the isolated component, RT17e was cleaved to a less polar residue. Based on the results of LC/MS, a proposed structure was developed for RT17e as a sulfate conjugate of des-ethyl pethoxamid. The presence of RT17e in liver could not be confirmed.

To further characterize residues in the final EtOAc phase of liver (which solidified on cooling), the fraction was concentrated to remove solvent, redissolved in water, and adjusted to pH 10 with KOH. The majority of the residue formed a precipitate with a soap-like consistency. On partitioning with water, the majority of the ^{14}C -residues partitioned into the aqueous phase. The

study author stated that this result suggested possible radiolabel association with fatty acids or other saponifiable components.

Table B.7.2.1-10. Summary of Characterization and Identification of Radioactive Residues in Poultry Eggs and Tissues Following Dosing with [¹⁴C]Pethoxamid at 16.6 ppm.¹												
Compound/Fraction	Liver		Eggs (Day 8)		Breast Muscle		Thigh muscle		Abdominal fat		Subcutaneous fat	
	TRR = 0.268 ppm		TRR = 0.043 ppm		TRR = 0.020 ppm		TRR = 0.027 ppm		TRR = 0.032 ppm		TRR = 0.053 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	%TRR	ppm	%TRR	ppm
Pethoxamid	0.4	0.001	1.7	0.001	ND	--	ND	--	2.7	0.001	7.3	0.004
MET-42	3.4	0.009	8.2	0.004	2.7	0.001	3.7	0.001	ND	--	ND	--
MET-3	2.7	0.007	2.8	0.001	3.8	0.001	ND	--	ND	--	ND	--
MET-103			6.9	0.003	ND	--	ND	--	ND	--	ND	--
MET-30	0.4	0.001	3.3	0.001	ND	--	ND	--	ND	--	ND	--
MET-47	3.4	0.009	3.9	0.002	ND	--	ND	--	ND	--	ND	--
MET-2	0.5	0.001	1.4	0.001	ND	--	ND	--	3.1	0.001	3.4	0.002
Minor unknowns ²	35.9	0.095	39.1	0.017	33.5	0.006	30.3	0.008	28.6	0.010	15.6	0.008
0.1 M HCl hydrolysate	NA ³	--	2.3	0.001	5.0	0.001	3.7	0.001	18.8	0.006	17.0	0.009
0.2 M KOH hydrolysate			7.0	0.003	25.0	0.005	22.2	0.006	18.8	0.006	24.5	0.013
Precipitate ⁴	15.3	0.041	NA	--	NA	--	NA	--	NA	--	NA	--
Protease soluble ⁴	17.2	0.046	18.6	0.008	NA	--	29.6	0.008	NA	--	5.7	0.003
Buffer soluble	3.7	0.010	<0.1	<0.001	NA	--	<0.1	<0.001	NA	--	11.3	0.006
Aqueous-soluble	5.2	0.014	NA	--	NA	--	NA	--	NA	--	NA	--
Organosoluble (EtOAc/hexane) ⁵	11.2	0.030	NA	--	NA	--	NA	--	12.5	0.004	11.3	0.006
Total extractable	99.3	0.266	95.3	0.041	70.0	0.014	88.8	0.024	84.5	0.027	96.2	0.051
Total identified	10.8	0.028	28.2	0.013	6.5	0.002	3.7	0.001	5.8	0.002	10.7	0.006
Total characterized	42.6	0.114	18.6	0.008	NA	--	29.6	0.008	NA	--	5.7	0.003
Total unidentified	45.9	0.122	48.4	0.021	63.5	0.012	56.2	0.015	78.7	0.026	79.7	0.042
Total bound residues (PES)	0.7	0.002	4.7	0.002	30.0	0.006	11.1	0.003	15.6	0.005	3.8	0.002
% Accountability ⁶	100		100		100		100.6		100.1		100	

¹ ND = Not detected; NA = not applicable. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Comprised of minor single or multicomponent HPLC unknowns, with each single component present at: ≤5.6% TRR (≤0.015 ppm) in liver; ≤8.6% TRR (≤0.004 ppm) in eggs; ≤14.0% TRR (≤0.003 ppm) in breast muscle; ≤7.4% TRR (≤0.002 ppm) in thigh muscle; ≤21.1% TRR (≤0.007 ppm) in abdominal fat; and ≤5.3% TRR (≤0.003 ppm) in subcutaneous fat.

³ Combined and subjected to partitioning with ethyl acetate which yielded aqueous and organosoluble fractions and the precipitate below.

⁴ Characterized as associated with or likely associated with protein.

⁵ Including 10.1% TRR (0.027 ppm) in liver characterized as being associated with fatty acid-related components.

⁶ Total (ppm)/TRR (ppm)×100.

Table B.7.2.1-11. Summary of Characterization and Identification of Radioactive Residues in Poultry Excreta Following Dosing with [¹⁴C]Pethoxamid at 16.6 ppm.¹		
Compound/Fraction	Excreta, Compositing Days 2-8 (77.9% AD)	
	% TRR	% AD
Pethoxamid	0.3	0.2
RT17e	8.2	6.4
MET-42	6.4	5.0
MET-103	3.9	3.0
MET-30	4.5	3.5
MET-47	1.7	1.3
MET-2	0.5	0.4
Minor HPLC unknowns ²	53.3	43.6
Total extractable	78.6	61.2
Total identified	25.5	19.8
Total unidentified	53.3	43.6
Total bound residues (PES)	21.4	13.8
% Accountability ³	100.0	

¹ Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Comprised of minor single or multicomponent HPLC unknowns, with each single component present at ≤13.6% TRR.

³ Total (ppm)/TRR (ppm)×100.

E. Proposed Metabolic Pathway

The study author proposed that pethoxamid is extensively metabolized in laying hens (Figure B.7.2.1-4), primarily via glutathione-S-transferase to form the cysteine conjugates (MET-103 and MET-30), which are oxidized to yield the sulfonic acid (MET-42). Metabolite MET-2 may result from the direct hydrolysis of the chlorine atom of pethoxamid or from hydrolysis of MET-103 and/or MET-30. The ether bond of MET-2 is also cleaved to form MET-3. A sulfate conjugate of des-ethyl pethoxamid (RT17e) was also observed in excreta. A variety of bound residues were characterized as being associated with natural products, primarily proteins.

Figure B.7.2.1-4. Proposed Metabolic Profile of Pethoxamid in Poultry.

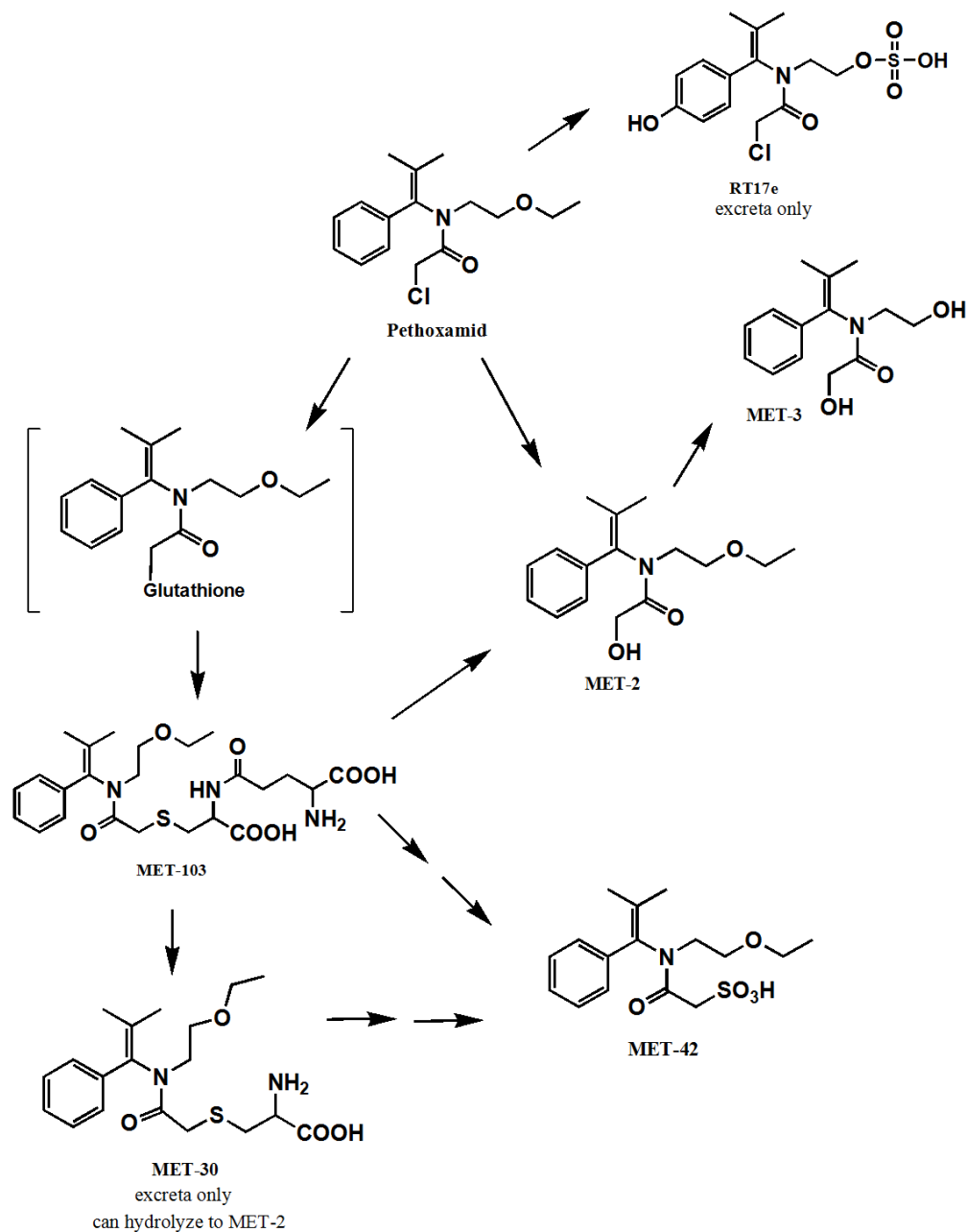


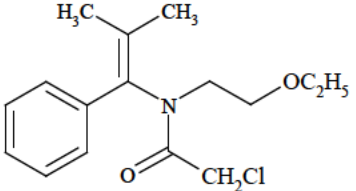
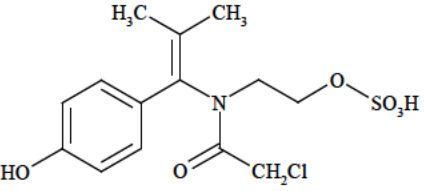
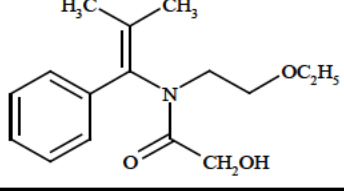
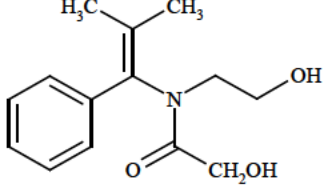
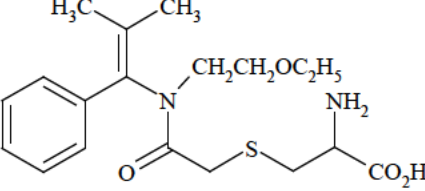
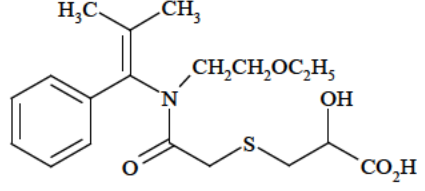
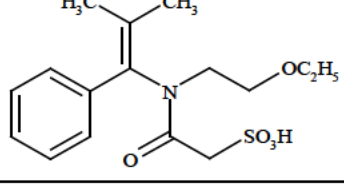
Table B.7.2.1-12. Identification of Compounds from Metabolism Study (both proposed and found).		
Common Name/Code	Chemical Name	Chemical Structure
Pethoxamid	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenylprop-1-en-1-yl)acetamide	
RTR17e	sulfuric acid mono-(2-{acetyl-[1-(4-hydroxy-phenyl)-2-methyl-propenyl]-amino}-ethyl) ester	
MET-2	<i>N</i> -(2-ethoxyethyl)-2-hydroxy- <i>N</i> -(2-methyl-1-phenylprop-1-en-1-yl)acetamide	
MET-3	2-hydroxy- <i>N</i> -(2-hydroxyethyl)- <i>N</i> -(2-methyl-1-phenylprop-1-en-1-yl)acetamide	
MET-30	<i>S</i> -(2-((2-ethoxyethyl)(2-methyl-1-phenylprop-1-en-1-yl)-amino)-2-oxoethyl)cysteine	
MET-41	3-((2-((2-ethoxyethyl)(2-methyl-1-phenylprop-1-en-1-yl)amino)-2-oxoethyl)thio)-2-hydroxypropanoic acid	
MET-42	2-[(2-ethoxyethyl)(2-methyl-1-phenyl-1-propen-1-yl)amino]-2-oxo-ethanesulfonic acid	

Table B.7.2.1-12. Identification of Compounds from Metabolism Study (both proposed and found).		
Common Name/Code	Chemical Name	Chemical Structure
MET-47	11-carboxy-6-(2-methyl-1-phenylprop-1-en-1-yl)-7,13-dioxo-3-oxa-9-thia-6,12-diazapentadecan-15-oic acid	
MET-103	16-amino-11-carboxy-6-(2-methyl-1-phenylprop-1-en-1-yl)-7,13-dioxo-3-oxa-9-thia-6,12-diazaheptadecan-17-oic acid	
Additional reference standard compounds used in the study but not identified in any matrix		
MET-50	3-((2-((2-ethoxyethyl)(2-methyl-1-phenylprop-1-en-1-yl)amino)-2-oxoethyl)sulfinyl)-2-hydroxypropanoic acid	

III. CONCLUSIONS

The poultry metabolism study is considered scientifically acceptable. Ten laying hens were dosed orally via capsules for 7 consecutive days with [^{14}C]pethoxamid at levels equivalent to 16.6 ppm in the feed (dry weight). The majority of the AD was recovered in the excreta (77.9% AD), with minor amounts recovered in the cage wash (0.1% AD) and GI tract (1.9% AD). Eggs accounted for 0.1% AD, and liver, muscle, and fat together accounted for ~0.1% AD. The overall recovery of the administered dose was 80.1%. TRR levels in eggs plateaued on Day 6 at a maximum of 0.059 ppm. TRR levels in tissues were highest in liver (0.268 ppm) and were lower and similar in muscle (0.020-0.027 ppm) and fat (0.032-0.053 ppm).

Pethoxamid was extensively metabolized in hens, with only minor amounts of parent detected in liver (0.4% TRR), eggs (0.8% TRR), and fat (2.7-7.3% TRR), and the six metabolites identified in eggs and tissues, MET-2, MET-3, MET-30, MET-42, MET-47, and MET-103, individually accounted for $\leq 8.2\%$ TRR in any matrix. All six metabolites were detected in liver; however, MET-3 and MET-103 were not resolved. Metabolite levels in liver were as follows: MET-42 (3.4% TRR), MET-3/MET-103 (2.7% TRR), MET-30 (0.4% TRR), MET-47 (3.4% TRR), and MET-2 (0.5% TRR). All six metabolites were detected in eggs at the following levels: MET-42 (8.2% TRR), MET-3 (2.8% TRR), MET-103 (6.9% TRR), MET-30 (3.3% TRR), MET-47 (3.9% TRR), and MET-2 (1.4% TRR). In muscle, only metabolites MET-42 (2.7-3.7% TRR) and MET-3 (3.8% TRR) were detected, and in fat, only metabolite MET-2 (3.1-3.4% TRR) was detected. The overall level of identification was relatively low: 10.8% TRR in liver, 28.2% TRR in eggs, 3.7-6.5% TRR in muscle, and 5.8-10.7% TRR in fat.

The majority of solvent-extractable radioactivity in eggs and tissues (15.6-39.1% TRR) was comprised of single and multicomponent unknowns, each present at $\leq 5.6\%$ TRR in liver, $\leq 8.6\%$ TRR in eggs, $\leq 14.0\%$ TRR in muscle and $\leq 21.1\%$ TRR in fat. Radioactivity released by mild acid and base hydrolyses also accounted for substantial portions of the TRR in liver (18.7% TRR), muscle (25.9-30.0% TRR), and fat (37.6-41.5% TRR). Levels of radioactivity in the acid and base hydrolysates from eggs (≤ 0.003 ppm), muscle (≤ 0.005 ppm), and fat (≤ 0.013 ppm) were too low for additional analysis. Following partitioning of the combined acid/base hydrolysate of liver, organosoluble residues accounted for only 1.1% TRR, while a precipitate (15.3% TRR) was characterized as being associated with denatured proteins. Other major portions of the TRR in liver (17.2% TRR), eggs (18.6% TRR), and muscle (29.6% TRR) were characterized as being associated with proteinaceous fractions, and 10.1% TRR in liver was characterized as being associated with fatty acid-related components.

Pethoxamid and five of the metabolites (all but MET-3) detected in eggs and tissues were also detected in excreta, along with metabolite RT17e, which was unique to excreta. Radioactive residues identified in excreta included: pethoxamid (0.3% TRR), RT17e (8.2% TRR), MET-42 (6.4% TRR), MET-103 (3.9% TRR), MET-30 (4.5% TRR), MET-47 (1.7% TRR), and MET-2 (0.5% TRR). Other minor unknowns in excreta isolated by HPLC each accounted for $\leq 13.6\%$ TRR.

The study author proposed that pethoxamid is extensively metabolized in laying hens, primarily via glutathione-S-transferase to form the cysteine conjugates (MET-103 and MET-30), which are oxidized to yield the sulfonic acid (MET-42). Metabolite MET-2 may result from the direct hydrolysis of the chlorine atom of pethoxamid or from hydrolysis of MET-103 and/or MET-30. The ether bond of MET-2 is also cleaved to form MET-3. A sulfate conjugate of des-ethyl pethoxamid (RT17e) was also observed in excreta. A variety of bound residues were characterized as being associated with natural products, primarily proteins.

REFERENCES

None.

**B.7.2 Metabolism, Distribution, and Expression of Residues in Livestock
(Annex IIA 6.2; Annex IIIA, 8.2)**

B.7.2.2 Ruminants

Document ID: MRID No. 49813531
PMRA No. Not available

Report: Corden, M.T. (2001) 14C-TKC-94 Metabolism in the Lactating Goat. Study Numbers: 101 PXA; TON 016/992809. Unpublished study prepared by Huntingdon Life Science, Ltd., sponsored by Cheminova, Inc., and submitted by FMC Corporation. 101 p.

Guidelines: EPA OCSPH Harmonized Test Guideline 860.1300 Nature of the Residue - Plants, Livestock (August 1996)
PMRA Regulatory Directive Dir98-02 – Residue Chemistry Guidelines, Section 2 -Nature of the Residue - Plants, Livestock
OECD Guideline 503 Metabolism in Livestock (January 2007)

GLP Compliance: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Acceptability: The study is considered scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP#452109.

Evaluator: Sheila Piper, Chemist, HED, RAB6 *Sheila Piper*

Secondary Evaluator: Robert Lanthier, PMRA

Note: This Data Evaluation Record (DER) was prepared under contract by CDM/CSS-Dynamac Joint Venture (submitted 2/21/18). The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies. The DER has been reviewed by HED and revised as necessary to reflect current Office of Pesticide Programs (OPP) policies.

EXECUTIVE SUMMARY

The metabolism of [phenyl-U-¹⁴C]pethoxamid (specific activity 81.1 µCi/mg) in a lactating goat was administered orally via gelatin capsule once daily for 5 consecutive days to a single lactating goat at a dose equivalent to 9.4 ppm in the feed (fresh-weight basis). Milk was collected twice daily and pooled by study day. Urine, feces, and cage wash samples were collected daily. The goat was sacrificed 23 hours after the final dose, and the following samples were collected: liver, kidney, muscle (foreleg and rump), fat (peritoneal and subcutaneous), bile, blood, and gastrointestinal (GI) tract with contents. The in-life and analytical phases of the study were conducted by Huntingdon Life Sciences, Ltd. (Huntingdon, UK).

The majority of the administered dose (AD) was recovered in the urine (70.15% AD) and feces (30.50% AD), with minor amounts recovered in the cage washes (0.29% AD). Another 0.53 and 0.02% of the AD were recovered in the liver and kidneys, respectively. The overall recovery of the AD was 101.5%. Total radioactive residues (TRR) in milk were low and remained steady throughout the study at 0.007-0.009 ppm. TRR levels in tissues were highest in liver (0.555 ppm) and kidney (0.147 ppm) and were lowest in muscle (0.003 ppm) and fat (0.002 ppm).

Samples of milk, muscle, and fat were not further analyzed as TRR were <0.01 ppm in these samples.

Samples of liver and kidney and composited samples of urine and feces (Days 1-5) were subjected to extraction and analysis. Treatment with protease and subsequent extraction with acetonitrile (ACN) released 94% TRR from liver and 93% TRR from kidney. Subsequent base hydrolysis (0.1 M NaOH) released an additional 1% TRR from liver and 3% TRR from kidney, which were not further analyzed, and the remaining post-extraction solid (PES) fractions accounted for 5.4% TRR (0.030 ppm) in liver and 4.5% TRR (0.007 ppm) in kidney. Solvent extraction of composited feces (Days 1-5) with aqueous ACN and ACN released 26% TRR, and the remaining PES accounted for 74% TRR. Composited samples of urine (Days 1-5) were analyzed directly.

Radioactive residues in the protease/ACN extracts from liver and kidney were profiled and initially identified by co-chromatography with reference standards using normal-phase one-dimensional thin layer chromatography (1D-TLC). The identities of the cysteine conjugate of pethoxamid (MET-30) and the glucuronide conjugate of metabolite MET-1 were confirmed by reverse-phase TLC and/or high performance liquid chromatography with mass spectrometry (LC/MS) and tandem mass spectrometry (LC/MS/MS) detection. Two minor metabolites, MI-E and MET-32, were tentatively identified by TLC co-chromatography but identification was not confirmed using a separate system. Samples were stored at <-15 °C and initially extracted for analysis within 2.3 months of sacrifice. Initial chromatographic profiling for ¹⁴C-residues occurred within 2.6 months of sacrifice, and all experimental procedures were completed within 12.3 months of sacrifice. Although no supporting storage stability data were included with the report, adequate storage stability data are available from the related poultry metabolism study (refer to the DER for MRID 49813530, B.7.2.1) indicating that ¹⁴C-residues in liver extracts are relatively stable for up to 13.8 months of frozen storage. No additional storage stability data are required to support this study.

Analyses of liver, kidney, and urine indicated that pethoxamid was extensively metabolized in the goat. Parent was not detected in any matrix, and the metabolite profile was relatively complex. In liver, identified metabolites accounted for 32.5% TRR and included MET-30 (21.6% TRR, 0.120 ppm) and MET-1 glucuronide (10.9% TRR, 0.060 ppm). Two major unknowns (Regions GL9 and GL10) that together accounted for 18.4% TRR (0.102 ppm) were found to be comprised of six components, each ≤4.8% TRR (≤0.027 ppm). Two components were found to co-chromatograph with metabolites MI-E (6.8% TRR, 0.038 ppm) and MET-32 (1.9% TRR, 0.011 ppm); however, identifications could not be confirmed using a second method. The remainder of the TRR consisted of minor unknowns that were largely polar in nature, with each accounting for ≤5.9% TRR (≤0.033 ppm).

No metabolites were conclusively identified in kidney; metabolite MET-32 was tentatively identified at 5.3% TRR (0.008 ppm) but the identification could not be confirmed using a second method. The majority of ¹⁴C-residues in kidney consisted of four relatively polar regions of radioactivity (GK1, 2, 3, and 5) and one less polar region (GK 9) that together accounted for 66.6% TRR (0.098 ppm), with each component present at ≤16.5% TRR (≤0.024 ppm). Acid hydrolysis largely converted these fractions to less polar components, with the two major

components, GK4 and GK9, accounting for 18.4% TRR (0.027 ppm) and 13.0% TRR (0.019 ppm), respectively. Given the generally polar nature of the major residue components, and their levels in the kidney (≤ 0.027 ppm), no further characterization is required.

Metabolites identified in urine included MET-30 (14.7% TRR, 10.3% AD) and MET-1 glucuronide (39.3% TRR, 27.6% AD), which together accounted for 54.0% TRR; the remainder of the TRR in urine consisted of multiple relatively polar unknowns that each accounted for $\leq 8.5\%$ TRR. Treatment with β -glucuronidase converted most of these polar ^{14}C -residues to less polar components, suggesting that a large fraction of the polar residues included glucuronide conjugates. Analysis of solvent-extractable ^{14}C -residues from feces detected 11 unknown components, each of which accounted for $\leq 4.2\%$ TRR; no further work was conducted on fecal extracts.

Pethoxamid was extensively metabolized in goat, with the metabolite profile in liver, kidney, and urine consisting of multiple components. Based on metabolites identified in liver and urine, the primary routes of metabolism in goat involve (i) cleavage of the ethoxy side chain and conjugation with glucuronic acid to yield MET-1 glucuronide and (ii) conjugation with glutathione and subsequent degradation of the glutathione moiety to yield the cysteine conjugate, MET-30. Further metabolism of the cysteine conjugate would yield MI-E, which was tentatively identified as a minor residue in liver and was previously identified in the rat metabolism study.

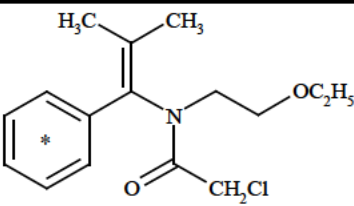
I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

The radiolabeled test substance dissolved in methanol (MeOH) was isotopically diluted with non-labeled pethoxamid. The diluted test substance was dispensed into gelatin capsules containing dextrose, and the solvent was allowed to evaporate. The capsules were stored frozen ($< -15^\circ\text{C}$) prior to administration. Spare capsules were prepared to evaluate radiochemical purity pre- and post-dosing. Stability analyses indicated that the test substance was stable in the dosing capsules stored frozen throughout dosing ($> 98\%$ radiochemical purity).

Table B.7.2.2-1. Pethoxamid Nomenclature.	
Common name	Pethoxamid
Identity	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide
CAS registry number	106700-29-2
Molecular weight	295.8 g/mol
Company experimental name	TKC-94
Radiolabeled compound	[phenyl- ^{14}C]pethoxamid
Lot/Batch #	CFQ10530
Radiochemical purity	$\geq 98.5\%$
Specific activity as received	3.96 MBq/mg; 107 $\mu\text{Ci}/\text{mg}$
Specific activity of dosed	3.00 MBq/mg; 81.1 $\mu\text{Ci}/\text{mg}$ (180,000 dpm/ μg)

Table B.7.2.2-1. Pethoxamid Nomenclature.	
Position of radiolabel	

2. Animals

Table B.7.2.2-2. General Test Animal Information.					
Species	Breed	Age	Weight at Study Initiation (kg)	Health Status	Description of Housing/Holding Area
Goat	British Saanen	2 years	46.0	Healthy	Stainless steel pen

B. STUDY DESIGN

Dose Regime

The in-life and analytical phases of the study were conducted by Huntingdon Life Sciences Ltd. (Huntingdon, UK). The goat was fed a standard dairy goat diet throughout acclimation and dosing, and provided water *ad libitum*. Beginning 3 days prior to dosing, the goat was housed under a 12-hour light/dark cycle in a stainless-steel cage suitable for the collection of urine and feces. Minimum and maximum daily temperatures were recorded, but not reported. The goat was weighed on Study Days 1 (prior to initial dose administration) and 6 (prior to sacrifice), and was monitored daily for any adverse health effects. Feed consumption was monitored daily and was relatively steady throughout the dosing period. The goat was dosed orally once a day via gelatin capsule with [¹⁴C]pethoxamid for 5 consecutive days; dosing occurred immediately following the morning milking. Daily feed consumption averaged 2.372 kg/day on an as-fed weight basis for Study Days 1 through 5. As the moisture content of the diet was not reported, the dietary concentration of the test substance on a dry-weight basis could not be calculated. Daily health evaluations of the goat during dosing were not reported, but feed consumption and milk production remained relatively steadily throughout dosing. Actual feed consumption and dose levels attained during dosing are presented in Tables B.7.2.2-3 and B.7.2.2-4.

Table B.7.2.2-3. Test Animal Dietary Regime.				
Composition of Diet	Average Feed Consumption ¹ (kg fresh wt/day)	Water	Acclimation Period	Predosing
Concentrate ration ² and fresh meadow hay or grass nuts	2.372	<i>Ad libitum</i>	27 days	No

¹ Average for Study Days 1-5; overall feed consumption was 11.86 kg during dosing. Dry weight of the feed was not reported.

² Cereal and vegetable protein concentrate (Spillers Meadow Fresh Original Goat Mix).

Table B.7.2.2-4. Test Animal Dosing Regime.				
Treatment Type	Treatment Level (ppm in diet) ¹	Vehicle	Dose (mg ai/kg bw/day)	Timing/Duration
Oral	9.4	Capsule	0.495	Once daily following the AM milking for 5 consecutive days

¹ Dietary burden on an as-fed basis. Calculated by the study reviewer.

Note to EPA Reviewer: The difference between the treatment level that we calculated and the 10.1-ppm treatment level calculated by the study author appears to result from their having excluded the Day 1 hay contribution to the diet from their calculations.

Sampling

Milk was collected twice daily in the morning prior to dosing and in the afternoon at least 6 hours after dosing. A subsample from each afternoon milking was stored at 4 °C and pooled with a similar subsample from the subsequent morning milking (Table B.7.2.2-5). A subsample of the pooled milk was radioassayed, and the remainder was frozen. Urine and feces were collected daily prior to dosing and stored frozen. Prior to dosing each day, the cage was rinsed with 1-2 L of water, and at sacrifice the cage was washed and rinsed with 5 L of water. Cage wash/rinse samples were stored frozen. Blood samples (10 mL) were also collected 1 hour before the first dose; 2 hours after each daily dose; at 1, 2, 3, 4, 6, 8, and 12 hours after the final dose; and immediately prior to sacrifice.

The goat was sacrificed 23 hours after the final dose, and the following samples were collected: liver, kidney, muscle (foreleg and rump), fat (peritoneal and subcutaneous), bile (from gall bladder), blood, and the entire GI tract with contents. Fecal samples were homogenized with dry ice, and tissue samples were homogenized under unspecified conditions. All samples were stored frozen (<-15 °C) until analysis.

Table B.7.2.2-5. Sample Collection Information.			
Milk Collected	Feces, Urine, and Cage Wash Collected	Interval from Last Dose to Sacrifice	Tissues Harvested and Analyzed
Twice daily	Daily	23 hours	Liver (entire), kidney (both), muscle (foreleg and rump), fat (peritoneal and subcutaneous), bile, blood, rumen and reticulum with contents, omasum and abomasum with contents, intestines with contents

TRR Determination

TRR in samples of urine, milk, cage wash, and bile were determined in duplicate directly by LSC, and TRR in samples of feces, tissues, and whole blood were determined in triplicate by combustion/LSC. Radioactivity in extract fractions and hydrolysates was determined by direct LSC, and radioactivity remaining in PES was determined by combustion/LSC. The limit of detection for TRR determinations was 2x background.

Extraction and Analysis

Samples of milk, muscle, and fat were not extracted for analysis as TRR levels were <0.01 ppm in these samples.

Subsamples of liver and kidney were initially extracted with ACN and ACN/water, then subjected to enzyme hydrolysis with protease. The aqueous ACN extractions released only minor amounts of the TRR from liver and kidney (3.4-11.8% TRR), while the protease treatment released the majority of the TRR (83.1-93.3% TRR); thus, the extraction procedure was revised. Subsamples of liver and kidney for analysis were first treated with protease (Type 1, crude from bovine pancreas) in phosphate buffer (pH 7.0) at 37 °C for ~72 hours. The resulting enzyme hydrolysate was separated by centrifugation, and the PES were extracted with ACN, which was pooled with the initial hydrolysate. The combined protease/ACN extract was radioassayed and reserved for TLC analysis. The remaining PES were base hydrolyzed with 0.1 M NaOH at 37 °C for 16-18 hours, centrifuged, and extracted with ACN; the resulting base hydrolysate and ACN fractions were combined and radioassayed but were not further analyzed.

To further investigate residues in liver and kidney, a subsample of the protease/ACN extract was concentrated to dryness then subjected to acid hydrolysis with 0.5 M HCl (at 37 °C for 18 hours) and reserved for TLC analysis. In addition, subsamples of the protease/ACN extract of liver and kidney were concentrated to an aqueous remainder and partitioned with ethyl acetate (EtOAc) under neutral and acidic (pH 1-2) conditions. The resulting EtOAc and aqueous fractions were radioassayed but were not further analyzed.

A composited sample of urine (Days 1-5) was analyzed directly by TLC and following treatment with: β -glucuronidase (from *Helix pomatia*, in acetate buffer (pH 5) at 37 °C for ~18 hours); protease (in phosphate buffer (pH 7) at 37 °C for ~18 hours); or acid (1.0 M HCl at 37 °C for ~18 hours).

A composited sample of feces (Days 1-5) was extracted sequentially with ACN (3x) and ACN:water (1:1, v:v; 3x), with the extracts being separated by centrifugation and pooled by solvent. Subsamples of the extracts were combined, concentrated, and analyzed by TLC.

No flow charts of the extraction procedures were provided.

Identification and Characterization

Normal- and reverse-phase 1D-TLC was used for the initial analysis and quantitation of ¹⁴C-residues in protease/ACN extracts from liver and kidney, composited urine (Days 1-5, direct analysis), and solvent extracts from composited feces (Days 1-5). Normal-phase TLC was conducted on silica gel 60 F₂₅₄ plates using the following solvent systems:

F: chloroform:MeOH:formic acid:water (70:25:3:3, v:v:v:v)

J: chloroform:MeOH:acetic acid (70:30:3, v:v:v)

K: hexane:acetone (1:1, v:v)

Reverse-phase TLC was conducted on octadecylsilane-coated plates (LCK18F) using the following solvent systems:

L: ACN:water:acetic acid (45:50:5, v:v:v)

L₂: MeOH:water:acetic acid (45:50:5, v:v:v)

Regions of radioactivity were identified by co-chromatography with reference standards and with urine and bile extracts from a rat metabolism study. Radioactivity on plates was detected and quantified using a Fujix BAS 2000 Bio Image Analyzer, and reference standards were detected by quenching of UV fluorescence. Refer to Table B.7.2.2-10 for identified residue components and to Appendix I for the names and structures of reference standards used in the study.

For reverse-phase TLC analysis of the liver protease/ACN extract using Solvent System L, the extract was first cleaned up by elution through a C-18 solid-phase extraction (SPE) cartridge eluted sequentially with water and MeOH. The resulting MeOH eluates, which contained 84% of the applied radioactivity, were combined and concentrated for TLC analysis.

Extracts were also reportedly analyzed by HPLC using a reverse-phase column (Zorbax RX-C8) with a mobile phase gradient of 0.015 M ammonium formate (pH 2) to ACN, and equipped with an in-line radioactivity monitor and a UV detector (254 nm). No results from HPLC analyses of sample fractions were included in the study report.

To further investigate three TLC regions in liver (GL10, GL12, and GL13), the corresponding regions were isolated from urine (GU11, GU13, and GU16). The composited urine sample was purified by SPE as described above, and the resulting MeOH fractions were combined, reduced in volume, fractionated by normal-phase TLC using solvent system F. The radioactive bands corresponding to GU11, GU13, and GU16 were removed by scraping, extracted with MeOH, and concentrated for further analysis by TLC. We note that the study author subsequently referred to the isolated regions in both figures and text primarily by the liver identifiers. In addition, the protease/ACN extract of liver was applied to preparative TLC plates, and band GL10 was collected, along with GL9 due to reduced resolution during the preparative TLC, for further TLC analysis.

LC/MS and LC/MS/MS analyses were used to identify two unknowns isolated from urine. The analyses were conducted using a Finnigan TSQ7000 mass spectrometer with electrospray ionization in the positive ion mode. HPLC separation was conducted using a reverse phase C18 column (Waters NovaPak) and a gradient mobile phase of 0.1% trifluoroacetic acid and ACN.

II. RESULTS AND DISCUSSION

A. Total Radioactive Residues

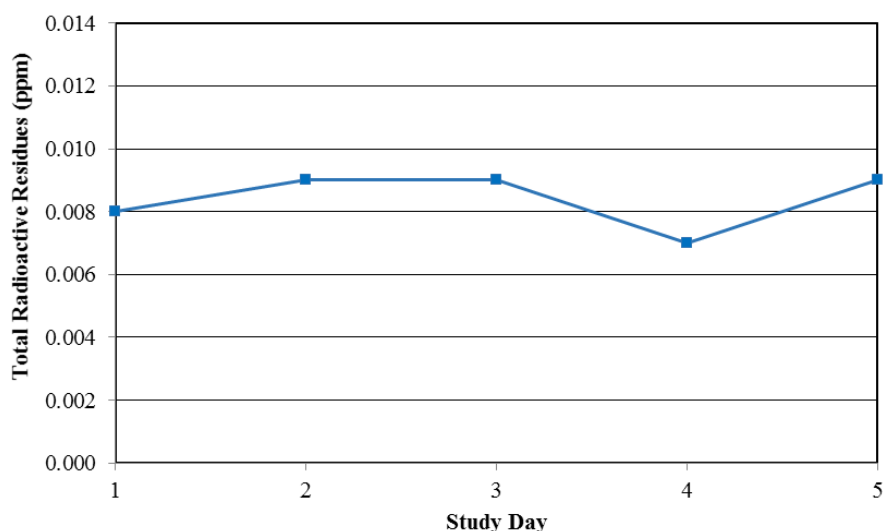
The TRR in goat matrices are presented in Table B.7.2.2-6. The majority of the AD was recovered in the urine (70.15% AD) and feces (30.50% AD), with minor amounts recovered in the cage washes (0.29% AD). Another 0.53 and 0.02% of the AD were recovered in the liver and kidneys, respectively. The overall recovery of the administered dose was 101.5%. TRR

levels in milk were low and remained steady throughout the study at 0.007-0.009 ppm (Figure B.7.2.2-1). TRR levels in tissues were highest in liver (0.555 ppm) and kidney (0.147 ppm) and were lowest in muscle (0.003 ppm) and fat (0.002 ppm).

Table B.7.2.2-6. TRR in Milk, Tissues, Blood, Bile, and Excreta.¹			
Matrix	Collection timing	[¹⁴ C]Pethoxamid	
		ppm	% of total dose
Milk	Day 1	0.008	NR
	Day 2	0.009	NR
	Day 3	0.009	NR
	Day 4	0.007	NR
	Day 5	0.009	NR
Liver	Day 5 (sacrifice)	0.555	0.53
Kidney	Day 5 (sacrifice)	0.147	0.02
Muscle (foreleg)	Day 5 (sacrifice)	0.003	NR
Muscle (rump)	Day 5 (sacrifice)	0.003	NR
Fat (peritoneal)	Day 5 (sacrifice)	0.002	NR
Fat (subcutaneous)	Day 5 (sacrifice)	0.002	NR
GI tract w/ contents	Day 5 (sacrifice)	NR	NR
Whole blood ²	NR	0.012	NR
Bile	Day 5 (sacrifice)	0.134	NR
Urine	Day 1	NR	13.16
	Day 2	NR	13.97
	Day 3	NR	14.95
	Day 4	NR	14.11
	Day 5	NR	13.97
	Duration	NR	70.15
Feces	Day 1	NR	5.03
	Day 2	NR	6.58
	Day 3	NR	5.95
	Day 4	NR	6.00
	Day 5	NR	6.93
	Duration	NR	30.50
Cage wash	Days 1-5	NR	0.29
Recovery of administered ¹⁴C-dose		NR	NR

¹ Determined directly by LSC for urine, milk, bile, and cage wash, and by combustion/LSC for feces, tissues, and blood. NR = not reported.

² Numerous blood samples were obtained at various intervals, but the sampling time for the blood sample used for radioassay was not reported. It is presumed to be the sample collected at sacrifice.

Figure B.7.2.2-1. TRR in Milk as a Function of Time.**B. Extraction, Characterization, and Distribution of Residues in Goat**

The distribution of radioactivity in goat liver, kidney, and urine is presented in Table B.7.2.2-7.

Treatment with protease and subsequent extraction with ACN released 94% TRR from liver and 93% TRR from kidney. Base hydrolysis released an additional 1% TRR from liver and 3% TRR from kidney, and the remaining PES fractions accounted for 5.4% TRR (0.030 ppm) in liver and 4.5% TRR (0.007 ppm) in kidney. Subsamples of the protease/ACN extracts from liver and kidney were concentrated and partitioned under neutral and acidic conditions with EtOAc, which indicated that the majority of the residues were aqueous soluble (69 and 82% for liver and kidney, respectively). The protease/ACN extracts from liver and kidney were analyzed by normal-phase TLC using two different solvent systems (F and J). Results from TLC analyses using solvent system F are presented in Table B.7.2.2-7.

The composited urine sample (Days 1-5), which accounted for 70.15% AD, was analyzed directly by TLC (Table B.7.2.2-7), and following enzyme hydrolysis with β -glucuronidase or protease, or acid hydrolysis with 1.0 M HCl.

Solvent extraction of the composited fecal sample (Days 1-5; 30.5% AD) with aqueous ACN released 26.2% TRR, and the remaining PES accounted for 73.8% TRR. TLC analysis of the fecal extract yielded 11 separate fractions, each of which accounted for $\leq 4.2\%$ of the TRR ($\leq 1.3\%$ AD). No further characterization was conducted on feces.

Table B.7.2.2-7. Distribution of Radioactive Residues in Goat Liver, Kidney, and Urine Following Dosing with [¹⁴C]Pethoxamid at 9.4 ppm.¹

Metabolite/Fraction ²	Liver		Kidney		Urine (Days 1-5)	
	TRR = 0.555 ppm		TRR = 0.147 ppm		70.15% AD	
	%TRR	ppm	%TRR	ppm	% TRR	%AD
Protease/ACN extract ³	93.5	0.519	92.7	0.136		
MET-30 (TKC-94-cysteine; GL13/GU16)	21.6	0.120	ND	--	14.7	10.3
MET-1 glucuronide conjugate (GL12/GU13&GU14)	10.9	0.060	ND	--	39.3	27.6
Region GL1/GK1/GU1 (TLC origin)	5.3	0.029	16.5	0.024	0.7	0.5
Region GL3/GK2/GU3 (polar)	3.4	0.019	10.7	0.016	2.0	1.4
Region GL4/GK3/GU4 (polar)	3.7	0.021	12.1	0.018	0.9	0.6
Region GL6/GK5/GU6	5.9	0.033	16.1	0.024	2.1	1.5
Region GL7/GK6/GU7	6.8 ⁴	0.038	1.7	0.002	1.0	0.7
Region GL9/GK9/GU10 ⁶	7.2	0.040	11.2	0.016	3.7	2.6
Region GL10/GU11 ⁶	11.2	0.062	ND	--	8.5	6.0
Region GL11/GK10/GU12	1.9 ⁵	0.011	5.3 ⁵	0.008	2.8	2.0
Minor unknowns ⁷	9.2	0.052	12.8	0.020	22.1	15.4
Non-specific radioactivity ⁸	6.4	0.036	6.3	0.009	2.2	1.5
EtOAc	24.9	0.138	10.8	0.016		
Aqueous	68.6	0.381	81.9	0.120		
0.1 M NaOH hydrolysate	1.2	0.007	2.7	0.004		
PES	5.4	0.030	4.5	0.007		

¹ ND = Not detected. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were normalized by the study author based on recovery. The TLC data for each matrix were obtained by normal-phase 1D-TLC using solvent system F.

² Corresponding TLC regions of radioactivity are presented for liver, kidney, and urine; TLC regions accounting for ≥5% TRR in any matrix are presented. Regions are specified by GL# for liver, GK# for kidney, and GU# for urine; not all regions were detected in each matrix.

³ In liver, protease hydrolysis released 84.2% TRR (0.467 ppm) and extraction with ACN released 9.3% TRR (0.052 ppm). These data were not provided for kidney.

⁴ Tentatively identified as metabolite MI-E by co-chromatography in liver, but was not confirmed by a second method

⁵ Tentatively identified as metabolite MET-32 by co-chromatography in liver and kidney, but was not confirmed by a second method.

⁶ Liver regions GL9 and GL10 and urine region GU11 were isolated, and further separated into six components, each accounting for ≤4.8% TRR (≤0.027 ppm) in liver; no quantitative data were provided for urine.

⁷ Minor TLC regions with each component present at ≤2.4% TRR (≤0.013 ppm) in liver, ≤4.5% TRR (≤0.007 ppm) in kidney, and ≤4.9% TRR (≤3.4% AD) in urine.

⁸ Non-specific radioactivity was comprised of low levels of radioactivity on the TLC plate not associated with a specific spot or region.

C. Storage Stability of Residues

Sample storage conditions and intervals are presented in Table B.7.2.2-8. All samples were stored at <-15 °C until analysis. Samples were initially extracted for analysis within 0.5 months of sacrifice for urine and feces, 1.0 month for liver, and 2.3 months for kidney. Initial chromatographic profiling for ¹⁴C-residues occurred within 0.8 months of sacrifice for urine and feces, 1.2 months for liver, and 2.6 months for kidney, and all experimental procedures were completed within 12.3 months of sacrifice. The study author stated that where extracts were reanalyzed, the resulting profiles were compared to the original analysis to check that no qualitative changes had occurred; however, no chromatograms reflecting this type of re-analysis were included in the submission. No additional storage stability data will be required as extracts were profiled within 2.6 months of sacrifice, all experimental procedures were completed within

12.3 months, and supporting storage stability data are available from the related poultry metabolism study (refer to the DER for MRID 49813530, B.7.2.1). In the poultry metabolism study, the residue profile in liver extracts was shown to be stable under frozen conditions for up to 13.8 months.

Table B.7.2.2-8. Summary of Storage Conditions.			
Matrix	Storage Temperature (°C)	Actual Storage Duration ¹	Interval of Demonstrated Storage Stability
Liver	<-15	36 days (1.2 months)	¹⁴ C-residues in liver extracts are relatively stable for up to 13.8 months of frozen storage (MRID 49813530)
Kidney		78 days (2.6 months)	
Excreta		23 days (0.8 months)	

¹ Duration from sample collection to initial TLC analysis for metabolite profiling.

D. Identity of Residues in Goat

The characterization and identification of residues in goat liver, kidney, and urine is summarized in Table B.7.2.2-9.

Following the initial TLC analyses of the liver protease/ACN extract, metabolite MET-30 (21.6% TRR) was identified as a major ¹⁴C-residue. The glucuronide conjugate of MET-1 (10.9% TRR) was also identified as a major ¹⁴C-residue following isolation and LC/MS and LC/MS/MS analysis of the corresponding fraction in urine. Pethoxamid was not detected. Two minor regions of radioactivity, GL7 and GL11, accounting for 6.8 and 1.9% TRR, respectively, were tentatively identified as metabolites MI-E and MET-32 based on TLC co-chromatography, but identification of these metabolites was not confirmed using a separate system. With the exception of TLC regions GL9 (7.2% TRR) and GL10 (11.2% TRR), each of the remaining TLC regions accounted for ≤5.9% TRR (0.033 ppm). Acid hydrolysis of the protease extract had no substantial impact on the metabolite profile. In conjunction with analysis of the corresponding fraction in urine, Regions GL9 and GL10 were isolated by preparative TLC and combined, and the combined fraction (18.4% TRR) was reanalyzed by reverse-phase TLC. The resulting chromatogram showed six regions of radioactivity, with each region accounting for ≤4.8% TRR. The identity of MET-30 in liver was confirmed by co-chromatography on TLC systems J, F, and L, and by LC/MS and LC/MS/MS of the corresponding fraction isolated from urine. The identity of MET-1 glucuronide was confirmed by TLC comparison of the corresponding fraction isolated from urine before and after treatment with β-glucuronidase, which released the MET-1 component.

Initial TLC analysis of the kidney protease extract did not detect pethoxamid or any of the available reference standards, except for MET-32 (5.3% TRR), which was tentatively identified by TLC co-chromatography but was not confirmed using a separate system. The majority of radioactivity consisted of six major unknown TLC regions (GK1, GK2, GK3, GK5, and GK9), with each region accounting for 10.7-16.5% TRR. Regions GK1, GK2, and GK3 were comprised of relatively polar components based on their retention factors. The remaining extractable radioactivity consisted of minor unknowns, each accounting for ≤5.3% TRR (≤0.008 ppm). Acid hydrolysis of the protease/ACN extract substantially altered the metabolite profile, decreasing the total level of radioactivity associated with GK1, GK2, GK3, and GK5 from 55.4 to 24.6% TRR, with concomitant increases in less polar components such as GK4 (18.4% TRR)

and GK9 (13.0% TRR). None of these regions were identified, and the remaining metabolites identified in liver and urine were not identified in kidney.

TLC analysis of composited urine (Days 1-5) detected 21 separate components, with four regions accounting for the majority of the TRR, including: GU11 (8.5% TRR), GU13 (30.3% TRR), GU14 (9.0% TRR), and GU16 (14.7% TRR). The remaining 17 regions each accounted for $\leq 4.9\%$ TRR in urine. Region GU16 was identified as metabolite MET-30, and regions GU13 and GU14 were identified as the MET-1 glucuronide conjugate following isolation and LC/MS and LC/MS/MS analysis. Urine region GU11 co-chromatographed with liver regions GL 9 and GL10 and was shown to consist of six components by reverse-phase TLC analysis. Treatment with acid (0.5 M HCl), β -glucuronidase, or protease had no substantial impact on the levels of GU11 and GU16; however, β -glucuronidase treatment eliminated regions GU13 and GU14, with a concomitant increase in region GU22 (42.6% TRR), which co-chromatographed with MET-1 in TLC analysis, confirming the identity of the glucuronide conjugate.

Compound/Fraction	Liver		Kidney		Urine (Days 1-5)	
	TRR = 0.555 ppm		TRR = 0.147 ppm		70.15% AD	
	% TRR	ppm	% TRR	ppm	%TRR	%AD
MET-30 (TKC-94-cysteine; GL13/GU16)	21.6	0.120	ND	--	14.7	10.3
MET-1 glucuronide conjugate (GL12/GU13&GU14)	10.9	0.060	ND	--	39.3	27.6
Region GL1/GK1/GU1 (TLC origin)	5.3	0.029	16.5 ²	0.024	0.7	0.5
Region GL3/GK2/GU3 (polar)	3.4	0.019	10.7 ²	0.016	2.0	1.4
Region GL4/GK3/GU4 (polar)	3.7	0.021	12.1 ²	0.018	0.9	0.6
Region GL6/GK5/GU6	5.9	0.033	16.1 ²	0.024	2.1	1.5
Region GL7/GK6/GU7	6.8 ³	0.038	1.7	0.002	1.0	0.7
Region GL9/GK9/GU10	18.4 ⁴	0.102	11.2 ²	0.016	3.7	2.6
Region GL10/GU11			ND	--	8.5	6.0
Region GL11/GK10/GU12	1.9 ⁵	0.011	5.3 ⁵	0.008	2.8	2.0
Minor unknowns ⁶	9.2	0.052	12.8	0.020	22.1	15.4
Non-specific radioactivity	6.4	0.036	6.3	0.009	2.2	1.5
0.1 M NaOH hydrolysate	1.2	0.007	2.7	0.004	NA	--
Total extractable	94.7	0.526	95.4	0.140	NA	--
Total identified	32.5	0.180	NA	--	54.0	37.9
Total unidentified	61.0	0.271	90.1	0.133	46.0	32.2
Total bound residues (PES)	5.4	0.030	4.5	0.007	NA	--
% Accountability ⁷	100.1		99.9		100.0	

¹ ND = Not detected; NA = not applicable. Ppm are reported in parent equivalents, and TRR values were normalized by the study author based on recovery.

² Acid hydrolysis (0.5 M HCl, 37 °C, 18 hr) of the kidney protease/ACN extract substantially decreased the levels of GK1 (5.2% TRR), GK2 (8.6% TRR), GK3 (2.1% TRR), and GK5 (8.7% TRR), and increased the levels of less polar components, GK4 (18.4% TRR, 0.027 ppm) and GK9 (13.0% TRR, 0.019 ppm).

³ Tentatively identified as MI-E in liver, but identification could not be confirmed.

⁴ Found to be comprised of six components, each accounting for $\leq 4.8\%$ TRR (≤ 0.027 ppm) in liver; no quantitative data were provided for urine.

⁵ Tentatively identified as MET-32 in liver and kidney, but identification could not be confirmed.

⁶ Comprised of minor single or multicomponent unknowns each $\leq 2.4\%$ TRR in liver, $\leq 4.5\%$ TRR in kidney, and $\leq 4.9\%$ TRR in urine.

⁷ Total (ppm)/TRR (ppm) $\times 100$.

E. Proposed Metabolic Pathway

Pethoxamid was extensively metabolized in goat (Figure B.7.2.2-2), with the metabolite profile in liver, kidney, and urine consisting of multiple components. Based on metabolites identified in liver and urine, the primary routes of metabolism in goat involve (i) cleavage of the ethoxy side chain and conjugation with glucuronic acid to yield MET-1 glucuronide and (ii) conjugation with glutathione and subsequent degradation of the glutathione moiety to yield the cysteine conjugate, MET-30. Further metabolism of the cysteine conjugate would yield MI-E, which was tentatively identified as a minor residue in liver and was previously identified in the rat metabolism study.

Figure B.7.2.2-2. Proposed Metabolic Profile of Pethoxamid in Goat.¹

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¹ The study author indicated that compounds in brackets were not identified in the study; however, metabolite MET-30 was actually identified in the study, and metabolite MI-E was not conclusively identified.

Table B.7.2.2-10. Identification of Compounds from Goat Metabolism Study. ¹		
Common Name/Code	Chemical Name	Chemical Structure
TKC-94 cysteine conjugate (MET-30)	S-(2-((2-ethoxyethyl)(2-methyl-1-phenylprop-1-en-1-yl)-amino)-2-oxoethyl)cysteine	
MET-1 glucuronide conjugate (MI-B11 glucuronide)		
MI-E ²	2-methanesulfinyl-N-(2-methyl-1-phenyl-propenyl)-N-vinyl-acetamide	
MET-32 ²	phenylglyoxylic acid	

¹ Reference standards utilized in the goat metabolism study are presented in Appendix I.

² The identification of MI-E and MET-32 (in liver and/or kidney) was tentative and not supported by a confirmatory analysis.

III. CONCLUSIONS

The goat metabolism study is considered scientifically acceptable. Following dosing of a single goat with [^{14}C]pethoxamid via capsule for 5 consecutive days at doses equivalent to 9.4 ppm in the feed (as fed basis), the majority of the AD was recovered in the urine (70.15% AD) and feces (30.50% AD), with minor amounts recovered in the cage washes (0.29% AD). Another 0.53 and 0.02% of the AD were recovered in the liver and kidneys, respectively. The overall recovery of the administered dose was 101.5%. TRR levels in milk were low and remained steady throughout the study at 0.007-0.009 ppm. TRR levels in tissues were highest in liver (0.555 ppm) and kidney (0.147 ppm) and were lowest in muscle (0.003 ppm) and fat (0.002 ppm). Based on the high levels of urinary excretion and low levels of radioactivity in milk, muscle, and fat, pethoxamid was rapidly absorbed and excreted by the goat.

Analyses of liver, kidney, and urine indicate that pethoxamid was extensively metabolized in the goat as parent was not detected in any matrix, and the metabolite profile was relatively complex. In liver, identified metabolites accounted for 32.5% TRR and included MET-30 (21.6% TRR) and MET-1 glucuronide (10.9% TRR). Two major unknowns (Regions GL9 and GL10) that together accounted for 18.4% TRR were found to be comprised of six components, each $\leq 4.8\%$ TRR. Two components were found to co-chromatograph with metabolites MI-E (6.8% TRR) and MET-32 (1.9% TRR); however, identifications could not be confirmed using a second method. The remainder of the TRR consisted of minor unknowns that were largely polar in nature, with each accounting for $\leq 5.9\%$ TRR.

No metabolites were conclusively identified in kidney; metabolite MET-32 was tentatively identified at 5.3% TRR but the identification could not be confirmed using a second method. The majority of ^{14}C -residues in kidney consisted of four relatively polar regions of radioactivity (GK1, 2, 3, and 5) and one less polar region (GK 9) that together accounted for 66.6% TRR, with each component present at $\leq 16.5\%$ TRR). Acid hydrolysis largely converted these fractions to less polar components, with the two major components, GK4 and GK9, accounting for 18.4% TRR and 13.0% TRR, respectively. Given the generally polar nature of the major residue components, and their levels in the kidney (≤ 0.027 ppm), no further characterization is required.

Metabolites identified in urine included MET-30 (14.7% TRR) and MET-1 glucuronide (39.3% TRR), which together accounted for 54.0% TRR; the remainder of the TRR in urine consisted of multiple relatively polar unknowns that each accounted for $\leq 8.5\%$ TRR. Treatment with β -glucuronidase converted most of these polar ^{14}C -residues to less polar components, suggesting that a large fraction of the polar residues included glucuronide conjugates. Analysis of solvent-extractable ^{14}C -residues from feces detected 11 unknown components, each of which accounted for $\leq 4.2\%$ TRR; no further work was conducted on fecal extracts.

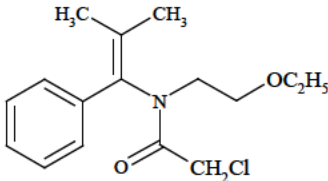
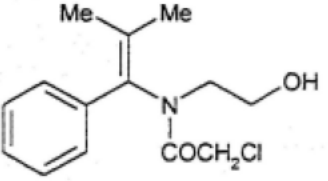
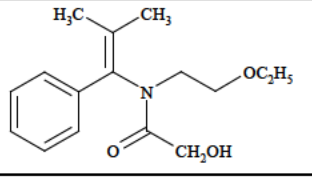
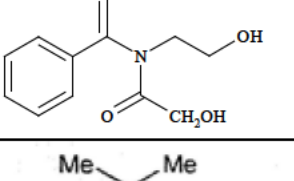
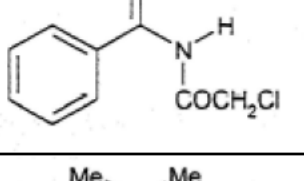
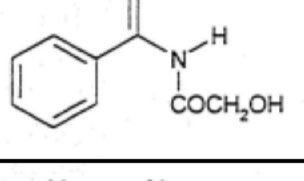
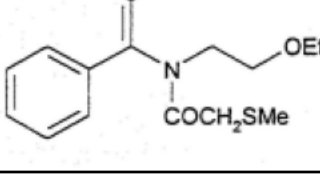
Pethoxamid was extensively metabolized in goat, with the metabolite profile in liver, kidney, and urine consisting of multiple components. Based on metabolites identified in liver and urine, the primary routes of metabolism in goat involve (i) cleavage of the ethoxy side chain and conjugation with glucuronic acid to yield MET-1 glucuronide and (ii) conjugation with glutathione and subsequent degradation of the glutathione moiety to yield the cysteine conjugate,

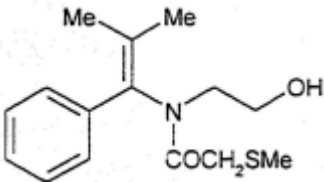
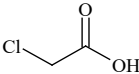
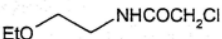
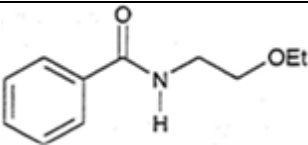
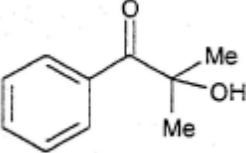
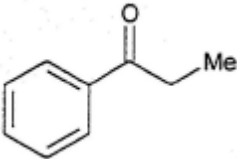
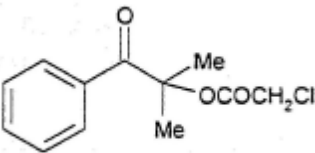
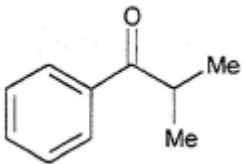
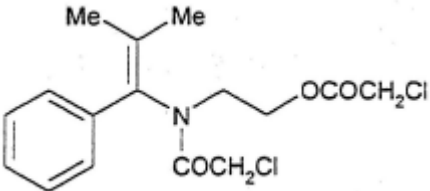
MET-30. Further metabolism of the cysteine conjugate would yield MI-E, which was tentatively identified as a minor residue in liver and was previously identified in the rat metabolism study

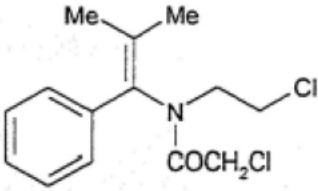
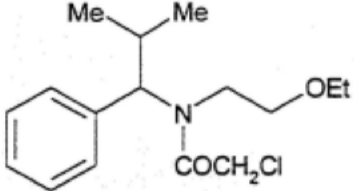
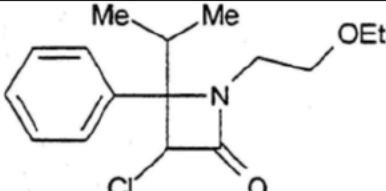
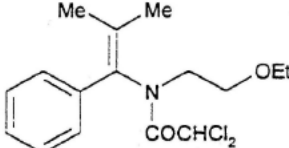
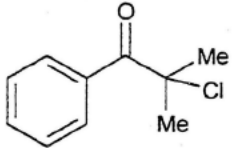
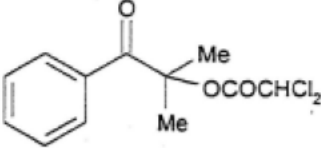
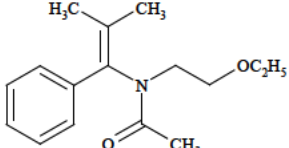
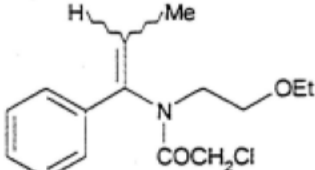
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None.

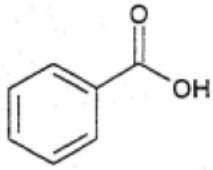
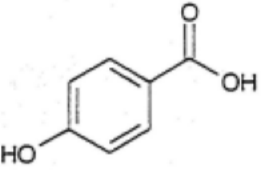
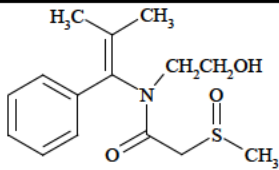
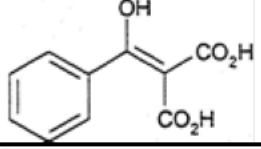
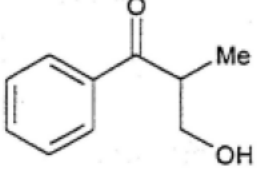
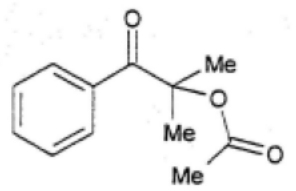
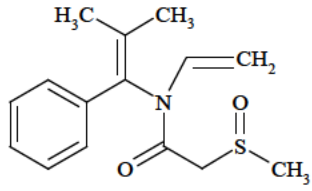
Appendix I. Reference Standards Used in the Study.

Name	Chemical name	Structure
Pethoxamid	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide	
MET-1	2-chloro- <i>N</i> -(2-hydroxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-2	<i>N</i> -(2-ethoxyethyl)-2-hydroxy- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-3	2-hydroxy- <i>N</i> -(2-hydroxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-4	2-chloro- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-5	2-hydroxy- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-6	<i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)-2-(methylthio)acetamide	

Name	Chemical name	Structure
MET-7	N-(2-hydroxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-(methylthio)acetamide	
MET-8	chloroacetic acid	
MET-9	2-chloro-N-(2-ethoxyethyl)acetamide	
MET-10	N-(2-ethoxyethyl)benzamide	
MET-11	2-hydroxy-2-methylpropiophenone	
MET-12	propiophenone	
MET-13	2-(2-chloroacetoxy)-2-methylpropiophenone	
MET-14	isobutyrophenone	
MET-15	2-chloro-N-[2-(2-chloroacetoxy)ethyl]-N-(2-methyl-1-phenyl-1-propenyl)acetamide	

Name	Chemical name	Structure
MET-16	2-chloro-N-(2-chloroethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-17	2-chloro-N-(2-ethoxyethyl)-N-[(2-methyl-1-phenyl)propyl]acetamide	
MET-18	3-chloro-N-(2-ethoxyethyl)-4-isopropyl-4-phenyl-2-azetidinone	
MET-19	2,2-dichloro-N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-20	2-chloro-2-methylpropiophenone	
MET-21	2-(2,2-dichloroacetoxy)-2-methylpropiophenone	
MET-22	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-23	(E,Z)-2-chloro-N-(2-ethoxyethyl)-N-(1-phenyl-1-propenyl)acetamide	

Name	Chemical name	Structure
MET-24	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl)propylamine	
MET-25	2-ethoxyethylamine	
MET-26	N-(2-hydroxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-27	N-(2-methyl-1-phenyl-1-propenyl)morpholine-3-one	
MET-28	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-(methylsulfinyl)acetamide	
MET-29	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-(methylsulfonyl)acetamide	
MET-30	S-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]-D,L-cysteine	
MET-31	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)oxamic acid	
MET-32	phenylglyoxylic acid	

Name	Chemical name	Structure
MET-33	benzoic acid	
MET-34	4-hydroxybenzoic acid	
MET-35	N-(2-hydroxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-(methylsulfinyl)acetamide	
MET-36	2-carboxy-3-hydroxycinnamic acid	
MET-37	3-hydroxy-2-methylpropiophenone	
MET-38	2-acetoxy-2-methylpropiophenone	
MI-E	2-methanesulfinyl-N-(2-methyl-1-phenyl-propenyl)-N-vinyl-acetamide	

**B.7.6 Residues Resulting from Supervised Trials
(Annex IIA 6.3; Annex IIIA 8.3)**

B.7.6.1 Residues in Target Crops

B.7.6.1.1 Soybean

Document ID: MRID No. 49813547
PMRA No. Not Available

Report: Carringer, S.J. (2015) Magnitude and Decline of Pethoxamid Residues in/on Soybean Raw Agricultural and Processed Commodities Following One Pre-plant or Pre-emergence Application and One Post-emergence Application of Pethoxamid 600 g/L EC (2014). Study Numbers: 1504 PXA; TCI-14-400; 81559. Unpublished study prepared by The Carringers, Inc., sponsored by Cheminova A/S, and submitted by FMC Corporation. 454 p.

Guidelines: EPA OCSPP Harmonized Test Guideline 860.1500 Crop Field Trials (August 1996)
PMRA Regulatory Directive DIR98-02 – Residue Chemistry Guidelines, Section 9 – Crop Field Trials
PMRA Regulatory Directive DIR2010-05 – Revisions to the Residue Chemistry Crop Field Trial Requirements
OECD Guideline 509 Crop Field Trial (September 2009)

GLP Compliance: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Acceptability: The study is considered scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP#452109.

Evaluator: Sheila Piper, Chemist, HED, RAB6 *Sheila Piper*

Secondary Evaluator: Robert Lanthier, PMRA

Note: This Data Evaluation Record (DER) was prepared under contract by CDM/CSS-Dynamac Joint Venture (submitted 2/16/18). The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies. The DER has been reviewed by HED and revised as necessary to reflect current Office of Pesticide Programs (OPP) policies.

EXECUTIVE SUMMARY

Eighteen field trials for pethoxamid on soybeans were conducted in Canada and/or the United States encompassing North American Free Trade Agreement (NAFTA) Growing Zones 2 (NC and SC; 2 trials, 4 (AR, LA, and MO; 3 trials), and 5 (IA, IL, MB, MI, MN, MO, NE, and WI; 13 trials) during the 2014 growing season. At three pairs of test sites (in IA, IL, and MN), trials were determined to be replicates; therefore, residues were averaged, and each pair of sites was taken to be one independent trial, which is reflected in the above trial count.

At each site consisted of one untreated plot and one treated plot reflecting a preplant or preemergence application to the soil followed by a foliar broadcast application targeting the R1

growth stage (first flowers open; ~BBCH 60). Applications were made with a 5.0 lb ai/gal (600 g ai/L) emulsifiable concentrate (EC) formulation of pethoxamid at 1.96-2.10 lb ai/A/application (2.20-2.35 kg ai/ha/application), with 39- to 70-day retreatment intervals, for total seasonal rates of 3.96-4.12 lb ai/A (4.44-4.62 kg ai/ha). Applications were made using ground equipment in spray volumes of 5-20 gal/A (45-187 L/ha). At one site, an additional plot was treated at an exaggerated rate (~5x the field trial rates; TRT 4) for a total application rate of 20.0 lb ai/A (22.4 kg ai/ha) for the collection of soybean seed for possible processing. An adjuvant (nonionic surfactant or crop oil concentrate) was added to the spray mixture for all foliar applications. Duplicate treated samples of soybean forage and hay were harvested at BBCH 60-70 at a preharvest interval (PHI) of 6-7 days; hay was dried for 1-10 days prior to collection. Duplicate treated samples of soybean seed were harvested at maturity at a PHI of 86-112 days. At two trials, additional forage and hay samples were harvested at PHIs of 0, 14, and 21 days, and additional seed samples were collected 6-7 days before, and 7 and 14 days after nominal harvest to assess residue decline.

Samples were maintained frozen at the field sites, during shipping, and at the laboratory prior to analysis. The maximum storage intervals for samples between harvest/collection and extraction for analysis were 7.4 months for forage, 8.9 months for hay, and 5.2 months for seed. Samples were analyzed within 30 days of extraction. Acceptable storage stability data were submitted indicating that residues of pethoxamid are stable during frozen storage for up to 8 months in soybean forage and straw, and up to 12 months in soybean seed (refer to the DERs for MRIDs 49813540-49813541, 49813544- 49813545). These data are adequate to support the sample storage conditions and durations from the submitted study.

Samples were analyzed for residues of pethoxamid using a high performance liquid chromatography method with tandem mass spectrometry detection (LC/MS/MS), adapted from Eurofins Agrosience Services (EAS) Method No. AGR/MOA/PTX-8, the proposed enforcement method for crop commodities (refer to the DER for MRID 49813534, B.5.2.1.1). The limit of quantitation (LOQ; determined as the lowest level of method validation, LLMV) was 0.01 ppm for pethoxamid in soybean matrices. Acceptable method validation and concurrent recoveries were obtained from samples of soybean forage and hay fortified with pethoxamid at 0.01-200 ppm, and soybean seed fortified at 0.01-0.5 ppm. The fortification levels were adequate to bracket measured residue levels.

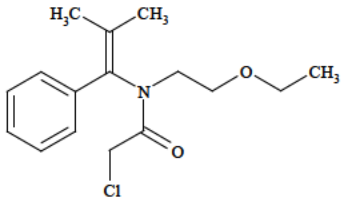
Following a preplant or preemergence application and subsequent foliar application at a total rate of 3.96-4.12 lb ai/A (4.44-4.62 kg ai/ha), individual sample (and per-trial average) residues of pethoxamid were 0.154-23.3 (0.245-21.1) ppm in/on forage and 0.083-21.4 (0.295-16.1) ppm in/on hay harvested at a PHI of 6-7 days. Residues of pethoxamid were below the LOQ in/on all soybean seed samples harvested at a PHI of 86-112 days. Residues were also below the LOQ (<0.01 ppm) in/on soybean seed samples from the exaggerated-rate (5x) plot; therefore, the processing study was not conducted.

In the residue decline studies, residues of pethoxamid were below the LOQ (<0.01 ppm) in/on soybean seed samples from all sampling intervals; therefore, residue decline could not be evaluated. Residues in/on forage and hay declined with increasing PHIs. Average residues in/on forage declined from 141-158 ppm at the 0-day PHI to 0.032-0.100 ppm at the 21-day PHI;

average residues in/on hay declined from 81.1-161 ppm to 0.210-0.222 ppm over the same interval.

I. MATERIALS AND METHODS

A. MATERIALS

Table B.7.6.1.1-1. Nomenclature for Pethoxamid.	
Common name	Pethoxamid
Identity	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide
CAS registry number	106700-29-2
Company experimental name	TKC-94
	

B. Study Design

1. Test Procedure

A total of eighteen field trials on soybean were conducted with a 5.0 lb ai/gal (600 g ai/L) EC formulation of pethoxamid during the 2014 growing season. Field trial locations by NAFTA growing zone are summarized in Table B.7.6.1.1-2.

All trials, except for those listed in the table below, were separated by ≥ 20 miles and are therefore considered independent (568_Criteria for Independence of Trials 4/23/13 (EPA and PMRA)). The trials separated by < 20 miles have been assessed for independence as detailed in the table below. HED has determined that three pairs of trials conducted in IL, IA, and MN should each be considered a single trial with replicate samples for purposes of 860.1500 data requirements.

Table B.7.6.1.1-1. Independent Trial Determination ¹		
Trial Nos.	Differences	Decision
TCI-14-400-		
10 and 11 (IL)	<u>Location:</u> Separated by 7 miles <u>Variety:</u> HS 42A12 vs. HS 39A22 <u>Timing:</u> 1 st applications separated by 13 days; 2 nd applications separated by 10 days <u>Spray volume:</u> 16-19 vs. 16-17 gal/A	Replicates
13 and 14 (IA)	<u>Location:</u> Separated by 16.3 miles <u>Variety:</u> 92Y75 vs. XB33AH14R2 <u>Timing:</u> 1 st applications separated by 8 days; 2 nd applications separated by 7 days. <u>Spray volume:</u> 15-18 vs. 18-19 gal/A	Replicates

Table B.7.6.1.1-1. Independent Trial Determination¹		
Trial Nos.	Differences	Decision
TCI-14-400-		
20 and 21 (MN)	<u>Location:</u> Separated by 4 miles <u>Variety:</u> Viking 1707R2N vs. Pioneer 91Y41 <u>Timing:</u> 1 st applications separated by 10 days; 2 nd applications separated by 8 days <u>Spray volume:</u> 14-20 vs. 15-17 gal/A	Replicates

¹ All assessments are based on the replicate trial guidance presented in draft memo 568_Criteria for Independence of Trials 4/23/13 (EPA and PMRA).

Table B.7.6.1.1-2. Trial Numbers and Geographical Locations.															
Crop	No. Trials	NAFTA Growing Zone												Total	
		1	2	3	4	5	5B	6	7	8	9	10	11		12
Soybean	Sub.		2		3	13 ¹									18
	U.S. Req. ²		2/2		3/2	15/11									20/15
	Can. Req. ³					13	3								16

¹ HED has determined that three pairs of trials conducted in Zone 5 are each replicates.

² As per Table 5 of 860.1500 for soybean; the second number reflects a 25% reduction in the number of field trials allowed for the crop as a representative commodity in support of a crop group/subgroup tolerance as applicable (refer to Tables 2 and 3) or when application results in no quantifiable residues.

³ As per Table 1.2 of PMRA Regulatory Directive DIR2010-05 for soybean.

Locations and detailed use patterns for the trials are provided in Table B.7.6.1.1-3. Preplant and preemergence applications were made to a variety of soil types: loamy sand (Trials 01, 02, and 19), loam (Trials 03 and 07), silt loam (Trials 04, 05, 08, 09, 10, 11, 12, 15, 16, 17, and 18), sandy clay loam (Trials 06 and 20), silty clay loam (Trials 13 and 14), and clay loam (Trial 21).

Table B.7.6.1.1-3. Study Use Pattern.									
Location: City, State/Province; Year (Trial ID TCI-14-400-)	End-use Product ¹	TRT No. ²	Method of Application; Timing of Application	Volume (gal/A) [L/ha]	Rate per Application (lb ai/A) [kg ai/ha]	Retreatment Interval (days)	Total Rate (lb ai/A) [kg ai/ha]	Surfactant/ Adjuvant ³	
Seven Springs, NC; 2014 (01)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	17 [159]	2.00 [2.24]	--	3.96 [4.44]	None	
			2. Foliar broadcast; first flowers open, BBCH 60 (R1)	17 [159]	1.96 [2.20]	70		NIS @ 0.25% v/v	
Elko, SC; 2014 (02)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, on day of planting	15 [140]	2.00 [2.24]	--	4.03 [4.52]	None	
			2. Foliar broadcast; first flowers open, BBCH 60 (R1)	15 [140]	2.03 [2.28]	54		COC @ 1.0% v/v	
Proctor, AR; 2014 (03)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, day after planting	5.0 [47]	2.02 [2.26]	--	4.03 [4.52]	None	
			2. Foliar broadcast; first flowers open, BBCH 60 (early bloom R1)	5.1 [48]	2.01 [2.25]	43		NIS @ 0.25% v/v	
Fisk, MO; 2014 (04)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	20 [187]	1.99 [2.23]	--	3.97 [4.45]	None	
			2. Foliar broadcast; first flowers open, BBCH 60	20 [187]	1.98 [2.22]	47		COC @ 1.0% v/v	

Table B.7.6.1.1-3. Study Use Pattern.								
Location: City, State/Province; Year (Trial ID TCI-14-400-)	End-use Product ¹	TRT No. ²	Method of Application; Timing of Application	Volume (gal/A) [L/ha]	Rate per Application (lb ai/A) [kg ai/ha]	Retreatment Interval (days)	Total Rate (lb ai/A) [kg ai/ha]	Surfactant/ Adjuvant ³
Cheneyville, LA; 2014 (05)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, day after planting	16 [150]	2.09 [2.34]	--	4.12 [4.62]	None
			2. Foliar broadcast; first flowers open, BBCH 60-61, flowers bottom/mid plant	14 [131]	2.03 [2.28]	44		NIS @ 0.26% v/v
Portage la Prairie, MB; 2014 (06)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	11 [103]	2.07 [2.32]	--	4.08 [4.57]	None
			2. Foliar broadcast; first flowers open, BBCH 50-60 (majority at ~BBCH 60)	11 [103]	2.01 [2.25]	53		COC @ 1.0% v/v
Conklin, MI; 2014 (07)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	4.9 [46]	2.01 [2.25]	--	4.02 [4.51]	None
			2. Foliar broadcast; first flowers open, BBCH 60 (80% at R1)	5.1 [48]	2.01 [2.25]	54		NIS @ 0.25% v/v
Delavan, WI; 2014 (08)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, day after planting	19 [178]	2.01 [2.25]	--	4.01 [4.49]	None
			2. Foliar broadcast; first flowers open, BBCH 60 (R1)	15 [140]	2.00 [2.24]	50		COC @ 1.0% v/v
Wyoming, IL; 2014 (09)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, day after planting	16 [150]	2.06 [2.31]	--	4.09 [4.58]	None
			2. Foliar broadcast; first flowers open, BBCH 60	15 [140]	2.03 [2.28]	44		NIS @ 0.25% v/v
Carlyle, IL; 2014 (10)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	19 [178]	1.99 [2.23]	--	3.99 [4.47]	None
			2. Foliar broadcast; first flowers open, BBCH 60	16 [150]	2.00 [2.24]	39		COC @ 1.0% v/v
Shattuc, IL; 2014 (11)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on the day of planting	17 [159]	2.03 [2.28]	--	4.07 [4.56]	None
			2. Foliar broadcast; first flowers open, BBCH 60 (R1)	16 [150]	2.04 [2.29]	42		NIS @ 0.25% v/v
Grantfork, IL; 2014 (12)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, on day of planting	14 [131]	1.99 [2.23]	--	4.05 [4.54]	None
			2. Foliar broadcast; first flowers open, BBCH 60 (R1, flowering)	16 [150]	2.06 [2.31]	48		COC @ 1.0% v/v
Richland, IA; 2014 (13)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, on day of planting	15 [140]	2.00 [2.24]	--	3.98 [4.46]	None
			2. Foliar broadcast; first flowers open, BBCH 60 (R1, V6-V7)	18 [168]	1.98 [2.22]	39		NIS @ 0.25% v/v
Hedrick, IA; 2014 (14)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	19 [178]	2.00 [2.24]	--	4.00 [4.48]	None
			2. Foliar broadcast; beginning of flowering, BBCH 61	18 [168]	2.00 [2.24]	40		COC @ 1.0% v/v
		4	1. Broadcast to soil; preplant, on day of planting	19 [178]	10.0 [11.2]	--	20.0 [22.4]	None
			2. Foliar broadcast; beginning of flowering, BBCH 61	18 [168]	10.0 [11.2]	40		COC @ 1.0% v/v

Table B.7.6.1.1-3. Study Use Pattern.								
Location: City, State/Province; Year (Trial ID TCI-14-400-)	End-use Product ¹	TRT No. ²	Method of Application; Timing of Application	Volume (gal/A) [L/ha]	Rate per Application (lb ai/A) [kg ai/ha]	Retreatment Interval (days)	Total Rate (lb ai/A) [kg ai/ha]	Surfactant/ Adjuvant ³
Kirkville, MO; 2014 (15)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, on day of planting	4.8 [45]	2.01 [2.25]	--	4.01 [4.49]	None
			2. Foliar broadcast; beginning of flowering, BBCH 61 (R1)	4.9 [46]	2.00 [2.24]	42		NIS @ 0.25% v/v
Grandview, IA; 2014 (16)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	16 [150]	2.00 [2.24]	--	4.10 [4.60]	None
			2. Foliar broadcast; first flowers open, BBCH 60 (R1)	16 [150]	2.10 [2.35]	40		COC @ 1.0% v/v
Donnellson, IA; 2014 (17)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, 5 days after planting	17 [159]	1.99 [2.23]	--	4.04 [4.53]	None
			2. Foliar broadcast; first flowers open, BBCH 60 (R1)	15 [140]	2.05 [2.30]	47		NIS @ 0.25% v/v
York, NE; 2014 (18)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, on day of planting	4.9 [46]	1.99 [2.23]	--	4.00 [4.48]	None
			2. Foliar broadcast; first flowers open, BBCH 60	5.0 [47]	2.01 [2.25]	41		COC @ 1.0% v/v
Osceola, NE; 2014 (19)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	19 [178]	1.97 [2.21]	--	3.97 [4.45]	None
			2. Foliar broadcast; first flowers open, BBCH 60	18 [168]	2.00 [2.24]	54		NIS @ 0.25% v/v
Geneva, MN; 2014 (20)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, on day of planting	14 [131]	1.99 [2.23]	--	4.01 [4.49]	None
			2. Foliar broadcast; first flowers open, BBCH 60	20 [187]	2.02 [2.26]	50		COC @ 1.0% v/v
Ellendale, MN; 2014 (21)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on the day of planting	15 [140]	1.99 [2.23]	--	3.99 [4.47]	None
			2. Foliar broadcast; first flowers open, BBCH 60 (R1)	17 [159]	2.00 [2.24]	48		NIS @ 0.25% v/v

¹ A 5.0 lb ai/gal (600 g ai/L) EC formulation of pethoxamid.

² Treated plots received a preplant/preemergence soil application followed by a foliar application; TRT 2 plots were treated at total rates of 3.96-4.12 lb ai/A (4.44-4.62 kg ai/ha) and the TRT 4 plot was treated at exaggerated rates totaling 20.0 lb ai/A (22.4 kg ai/ha).

³ NIS = Nonionic surfactant; COC = Crop oil concentrate.

Soybeans were grown and maintained using typical agricultural practices. Irrigation was used at trial sites 01, 02, 03, 04, 18, and 19. Despite variations in weather conditions reported at some trial sites, no unusual weather conditions were reported to have adversely affected crop production or yield during the study, except at Trial 07, which was restarted due to heavy rains after the first application, and experienced yields slightly lower than normal due to the later planting date. Phytotoxicity (speckling of leaves, leaf damage, or leaf puckering) was observed after the first application at Trial 05, and after the foliar application at Trials 02, 03, 06, and 11, but yields at these trials were normal.

Sample Handling and Preparation

Single control and duplicate treated samples of soybean forage and hay were harvested at BBCH 60-70 at a PHI of 6-7 days; hay was dried for 1-10 days prior to collection to a moisture content of 8.5-63.7% (drying conditions were not reported). Single untreated and duplicate treated samples of soybean seed were harvested at maturity at a PHI of 86-112 days; bulk samples of untreated (60.5 kg) and treated (77.0 kg) seed were collected from the control and exaggerated-rate plots, respectively, at Trial 14 for possible processing. At two trials, additional forage and hay samples were harvested at PHIs of 0, 14, and 21 days, and additional seed samples were collected 6-7 days before, and 7 and 14 days after nominal harvest to assess residue decline. Samples were placed into frozen storage within 3.5 hours of collection and were stored frozen (temperatures not reported) at the field sites prior to shipment by ACDS freezer truck to the analytical laboratory, ABC Laboratories, Inc. (Columbia, MO). At the laboratory, samples were homogenized in the presence of dry ice and stored frozen (*ca.* -20 °C) until extraction for analysis.

2. Description of Analytical Procedures

Samples of soybeans were analyzed for residues of pethoxamid using an LC/MS/MS method adapted from EAS Method No. AGR/MOA/PTX-8, as described in “Validation of an Analytical Method for the Determination of Pethoxamid Residues in Various Crops.” The method is the proposed enforcement method for crop commodities (refer to the DER for MRID 49813534, B.5.2.1.1).

Briefly, samples were extracted with acetonitrile (ACN). QuEChERS extraction salts (magnesium sulfate:sodium chloride:sodium citrate tribasic dihydrate:sodium citrate dibasic sesquihydrate; 4:1:1:0.5, w:w:w:w) were added, and samples were shaken and centrifuged. A portion of the supernatant was purified by the addition of cleanup salts (primary secondary amine:C18EC:magnesium sulfate; 1:1:6, w:w:w). Samples were shaken and centrifuged, and the supernatant was diluted with ACN:water (1:9, v:v) for analysis by LC/MS/MS. The method monitors the following ion transitions for pethoxamid: m/z 296 $\rightarrow$ 131 (quantitation) and m/z 296 $\rightarrow$ 250 (confirmation). The LOQ (determined as the LLMV) was 0.01 ppm for pethoxamid, and the estimated limit of detection (LOD) was 0.003 ppm (1/3 LOQ).

II. RESULTS AND DISCUSSION

Method performance was evaluated by use of method validation and concurrent recovery samples of soybean forage and hay fortified with pethoxamid at 0.01-200 ppm, and soybean seed fortified at 0.01-0.5 ppm. Recoveries were within the acceptable range of 70-120%; therefore, the method was considered valid for the determination of pethoxamid residues in soybean matrices (Table B.7.6.1.1-4). It is noted that acceptable method validation recoveries were also obtained for soybean matrices using the confirmatory ion transition; these data are not presented herein. The fortification levels bracketed the measured residues. Concurrent recoveries were corrected for any apparent residues in controls.

The detector response was linear (coefficient of determination, $r^2 \geq 0.9970$) within the range of 0.01-2 ng/mL. Representative chromatograms of control samples, fortified samples, and treated samples were provided. The control chromatograms generally had no peaks of interest above the chromatographic background. The fortified sample chromatograms contained only the analyte of interest, and peaks were symmetrical and well defined. Apparent residues in/on controls were nondetectable (<0.003 ppm), except in/on five untreated hay samples which bore detectable residues of pethoxamid below the LOQ (0.003-0.006 ppm). Quantifiable residues were found in/on one sample each of untreated forage and hay (both from Trial 14), at 0.0335 and 0.0145 ppm, respectively; residues in/on the respective treated samples were much higher than those in the controls (0.454 and 1.77 ppm in/on forage, and 0.776 and 1.08 ppm in/on hay). Residues in treated samples were not corrected for apparent residues in controls.

Table B.7.6.1.1-4. Summary of Method Validation and Concurrent Recoveries of Pethoxamid from Soybean Matrices.				
Matrix	Fortification Level (ppm)	Sample Size (n)	Recoveries ¹ (%)	Mean $\pm$ Std. Dev. (%)
Method Validation				
Soybean forage	0.01, 0.5	6	91-108	102 $\pm$ 6
Soybean hay	0.01, 0.5	6	80-99	95 $\pm$ 8
Soybean seed	0.01, 0.5	6	75-101	91 $\pm$ 12
Concurrent Recoveries				
Soybean forage	0.01-200	14	86-100	94 $\pm$ 5
Soybean hay	0.01-200	16	73-103	87 $\pm$ 9
Soybean seed	0.01, 0.10	14	76-97	90 $\pm$ 6

¹ Concurrent recoveries were corrected for any apparent residues in controls.

The maximum storage intervals for samples between harvest/collection and extraction for analysis were 7.4 months for forage, 8.9 months for hay, and 5.2 months for seed (Table B.7.6.1.1-5). Samples were analyzed within 30 days of extraction. Acceptable storage stability data were submitted indicating that residues of pethoxamid are stable during frozen storage for up to 8 months in soybean forage and straw, and up to 12 months in soybean seed (refer to the DERs for MRIDs 49813540, 49813541, and 49813544, and; B.7.6.2). These data are adequate to support the sample storage conditions and durations from the submitted study.

Table B.7.6.1.1-5. Summary of Storage Conditions.			
Matrix	Storage Temperature (°C)	Actual Storage Duration ¹	Interval of Demonstrated Storage Stability
Soybean forage	≤ -20 °C	133-226 days (4.4-7.4 months)	Residues of pethoxamid are stable during frozen storage for up to 8 months in soybean forage and straw, and up to 12 months in soybean seed ²
Soybean hay		132-224 days (4.3-7.4 months); 271 days (8.9 months) reanalysis	
Soybean seed		58-159 days (1.9-5.2 months)	

¹ Interval from harvest/collection to extraction. Samples were analyzed within 0-30 days of extraction.

² Refer to the DERs for MRIDs 49813540, 49813541, 49813544, and 49813545 B.7.6.2.

The results from the submitted field trials are presented in Table B.7.6.1.1-6 and summarized in Table B.7.6.1.1-7. Following a preplant or preemergence application and subsequent foliar application at a total rate of 3.96-4.12 lb ai/A (4.44-4.62 kg ai/ha), residues of pethoxamid were highest in/on forage (0.154-23.3 ppm) and hay (0.083-21.4 ppm) harvested at a PHI of 6-7 days. Residues of pethoxamid were nondetectable (<0.003 ppm) in/on all soybean seed samples harvested at a PHI of 86-112 days, including samples from the exaggerated-rate (5x) plot.

In the residue decline studies, residues of pethoxamid were below the LOQ (<0.01 ppm) in/on soybean seed samples from all sampling intervals; therefore, residue decline could not be evaluated. Residues in/on forage and hay declined with increasing PHIs (Figures B.7.6.1.1-1 and B.7.6.1.1-2). Average residues in/on forage declined from 141-158 ppm at the 0-day PHI to 0.032-0.100 ppm at the 21-day PHI; average residues in/on hay declined from 81.1-161 ppm to 0.210-0.222 ppm over the same interval.

The study author noted that analyses of soybean hay residue decline samples from Trial 10 indicated that one of the 7-day PHI samples may have been switched with one of the 21-day PHI samples. Reanalysis of these samples in duplicate, conducted 8.9 months after sample collection, confirmed that the samples had been switched. The results reported in Table B.7.6.1.1-6 reflect the correct PHI.

Table B.7.6.1.1-6. Residue Data from Soybean Field Trials with Pethoxamid.¹							
Location: City, State/Province; Year (Trial ID TCI-14-400-)	Zone	Crop Variety	TRT No. ²	Rate (lb ai/A) [kg ai/ha]	Matrix	PHI ³ (days)	Residues ⁴ (ppm) [Average]
Seven Springs, NC; 2014 (01)	2	AG6931	2	3.96 [4.44]	Forage	7	0.244, 0.371 ⁵ [0.308]
					Hay	7 (6)	0.490, 0.382 [0.436]
					Seed	112	ND, ND [<0.01]
Elko, SC; 2014 (02)	2	Asgrow 7231	2	4.03 [4.52]	Forage	7	1.43, 0.639 [1.03]
					Hay	7 (2)	2.14, 2.22 [2.18]
					Seed	97	ND, ND [<0.01]
Proctor, AR; 2014 (03)	4	AG4934	2	4.03 [4.52]	Forage	7	0.386, 0.631 [0.509]
					Hay	7 (1)	1.27, 3.82 [2.55]
					Seed	89	ND, ND [<0.01]
Fisk, MO; 2014 (04)	4	P50T64R	2	3.97 [4.45]	Forage	7	0.438, 0.333 [0.386]
					Hay	7 (2)	0.757, 0.564 [0.661]
					Seed	104	ND, ND [<0.01]
Cheneyville, LA; 2014 (05)	4	AFG4531	2	4.12 [4.62]	Forage	7	0.364, 0.462 [0.413]
					Hay	7 (7)	0.622, 0.708 [0.665]
					Seed	105	ND, ND [<0.01]
Portage la Prairie, MB; 2014 (06)	5	Tilston	2	4.08 [4.57]	Forage	6	13.6, 14.3 [14.0]
					Hay	6 (10)	21.4, 10.7 [16.1]
					Seed	87	ND, ND [<0.01]
Conklin, MI; 2014 (07)	5	BN1012650	2	4.02 [4.51]	Forage	6	1.03, 0.978 [1.00]
					Hay	6 (2)	2.24, 2.05 [2.15]
					Seed	94	ND, ND [<0.01]
Delavan, WI; 2014 (08)	5	AG2134	2	4.01 [4.49]	Forage	7	2.22, 2.35 [2.29]
					Hay	7 (6)	4.31, 3.75 [4.03]
					Seed	91	ND, ND [<0.01]
Wyoming, IL; 2014 (09)	5	AG 3030/ A1016053	2	4.09 [4.58]	Forage	7	3.28, 4.55 [3.92]
					Hay	7 (7)	5.87, 6.85 [6.36]
					Seed	97	ND, ND [<0.01]

Table B.7.6.1.1-6. Residue Data from Soybean Field Trials with Pethoxamid.¹							
Location: City, State/Province; Year (Trial ID TCI-14-400-)	Zone	Crop Variety	TRT No. ²	Rate (lb ai/A) [kg ai/ha]	Matrix	PHI ³ (days)	Residues ⁴ (ppm) [Average]
Carlyle, IL; 2014 ⁶ (10) and Shattuc, IL; 2014 (11)	5	HS 42A12; HS 39A22	2	3.99 [4.47]; 4.07 [4.56]	Forage	0	155, 160 [158]
						7; 6	6.18, 4.16, 6.17, 4.66 [5.29]
						14	0.240, 0.119 [0.180]
						21	0.037, 0.027 [0.032]
					Hay	0 (2)	69.0, 93.2 [81.1]
						7 (3); 6 (5)	3.45, 2.44 ⁷ , 5.62, 5.52 [4.26]
						14 (3)	0.386, 0.640 [0.513]
						21 (3)	0.173, 0.248 ⁸ [0.210]
					Seed	81	ND, ND [<0.01]
						88; 97	ND, ND, ND, ND [<0.01]
						95	ND, ND [<0.01]
						102	ND, ND [<0.01]
Grantfork, IL; 2014 (12)	5	5N431R2	2	4.05 [4.54]	Forage	7	2.85, 2.60 [2.73]
					Hay	7 (2)	5.84, 3.98 [4.91]
					Seed	98	ND, ND [<0.01]
Richland, IA; 2014 ⁹ (13) and Hedrick, IA; 2014 (14)	5	92Y75; XB33AH14R2	2	3.98 [4.46]; 4.00 [4.48]	Forage	0	141, 141 [141]
						7; 7	2.78, 2.43, 0.454, 1.77 [1.86]
						14	0.360, 0.380 [0.370]
						21	0.087, 0.113 [0.100]
					Hay	0 (5)	170, 151 [161]
						7 (2); 7 (5)	5.58, 6.29, 0.776, 1.08 [3.43]
						14 (3)	0.391, 0.653 [0.522]
						21 (5)	0.280, 0.163 [0.222]
					Seed	90	ND, ND [<0.01]
						96; 111	ND, ND, ND, ND [<0.01]
						103	ND, ND [<0.01]
						110	ND, ND [<0.01]
			4 ¹⁰	20.0 [22.4]	Seed	111	ND, ND [<0.01]
Kirkville, MO; 2014 (15)	5	92Y75	2	4.01 [4.49]	Forage	7	0.154, 0.336 [0.245]
					Hay	7 (4)	0.083, 0.508 [0.295]
					Seed	111	ND, ND [<0.01]
Grandview, IA; 2014 (16)	5	92Y75	2	4.10 [4.60]	Forage	7	18.9, 23.3 [21.1]
					Hay	7 (2)	14.8, 15.8 [15.3]
					Seed	109	ND, ND [<0.01]
Donnellson, IA; 2014 (17)	5	92Y75	2	4.04 [4.53]	Forage	7	0.920, 0.859 [0.890]
					Hay	7 (2)	1.65, 1.87 [1.76]
					Seed	97	ND, ND [<0.01]
York, NE; 2014 (18)	5	27120191	2	4.00 [4.48]	Forage	7	4.06, 3.94 [4.00]
					Hay	7 (6)	4.94, 5.87 [5.41]
					Seed	86	ND, ND [<0.01]
Osceola, NE; 2014 (19)	5	S28-A2	2	3.97 [4.45]	Forage	7	0.296, 0.270 [0.283]
					Hay	7 (5)	0.536, 0.526 [0.531]
					Seed	110	ND, ND [<0.01]

Table B.7.6.1.1-6. Residue Data from Soybean Field Trials with Pethoxamid.¹							
Location: City, State/Province; Year (Trial ID TCI-14-400-)	Zone	Crop Variety	TRT No. ²	Rate (lb ai/A) [kg ai/ha]	Matrix	PHI ³ (days)	Residues ⁴ (ppm) [Average]
Geneva, MN; 2014 (20) and Ellendale, MN; 2014 (21)	5	Viking 1707R2N; Pioneer 91Y41	2	4.01 [4.49]; 3.99 [4.47]	Forage	7; 7	6.81, 4.94, 5.51, 8.31 [6.39]
					Hay	7 (4); 7 (3)	9.46, 7.68, 10.4, 11.5 [9.76]
					Seed	98; 88	ND, ND, ND, ND [<0.01]

¹ A 5.0 lb ai/gal (600 g ai/L) EC formulation of pethoxamid was used.

² Treated plots received a preplant/preemergence soil application followed by a foliar application; TRT 2 plots were treated at total rates of 3.96-4.12 lb ai/A (4.44-4.62 kg ai/ha) and the TRT 4 plot was treated at exaggerated rates totaling 20.0 lb ai/A (22.4 kg ai/ha).

³ The number of days hay was dried prior to collection is reported in parentheses. The target PHI is **bolded** for the decline studies.

⁴ ND = Not detected (<LOD). The LOD was 0.003 ppm and the LOQ was 0.01 ppm. Per-trial averages were calculated by the study reviewer using the LOQ for all residues reported as <LOQ.

⁵ Samples mislabeled in field; results reflect switching the control sample and treated sample results.

⁶ Decline samples are from Trial 10.

⁷ Reanalysis of this sample in duplicate confirmed that a 7-day PHI sample had been switched with a 21-day PHI sample. The reported results reflect the average of the original result (2.67 ppm) and the average of duplicate reanalysis results (2.10 and 2.33 ppm; average of 2.22 ppm).

⁸ Reanalysis of this sample in duplicate confirmed that a 21-day PHI sample had been switched with a 7-day PHI sample. The reported results reflect the average of the original result (0.299 ppm) and the average of duplicate reanalysis results (0.170 and 0.223 ppm; average of 0.197 ppm).

⁹ Decline samples are from Trial 13.

¹⁰ TRT 4 (exaggerated rate) samples are from Trial 14.

Table B.7.6.1.1-7. Summary of Residues from Soybean Field Trials with Pethoxamid.										
Crop Matrix	Total Application Rate (lb ai/A) [kg ai/ha]	PHI (days)	n ¹	Pethoxamid Residues (ppm)						
				Min. ²	Max. ²	LAFT ³	HAFT ³	Median ³	Mean ³	SD ³
Soybean forage	3.96-4.12 [4.44-4.62]	6-7	18	0.154	23.3	0.245	21.1	1.45	3.70	5.50
Soybean hay		6-7	18	0.083	21.4	0.295	16.1	2.99	4.48	4.76
Soybean seed		86-112	18	<0.01	<0.01	<0.01	<0.01	0.01	0.01	N/A

¹ n = number of field trials.

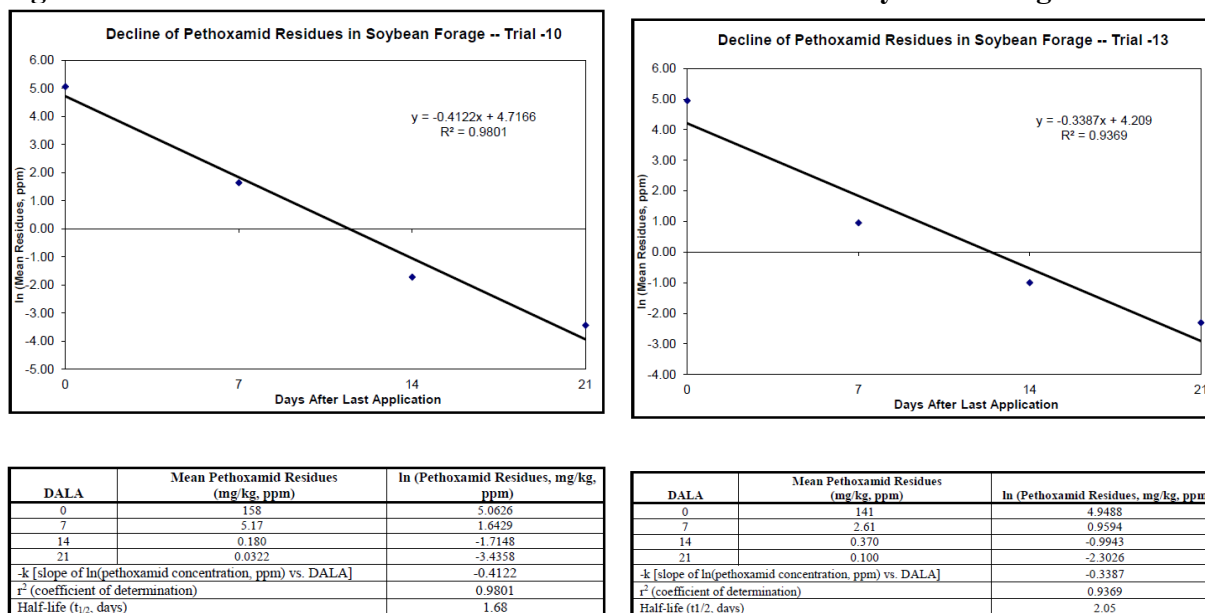
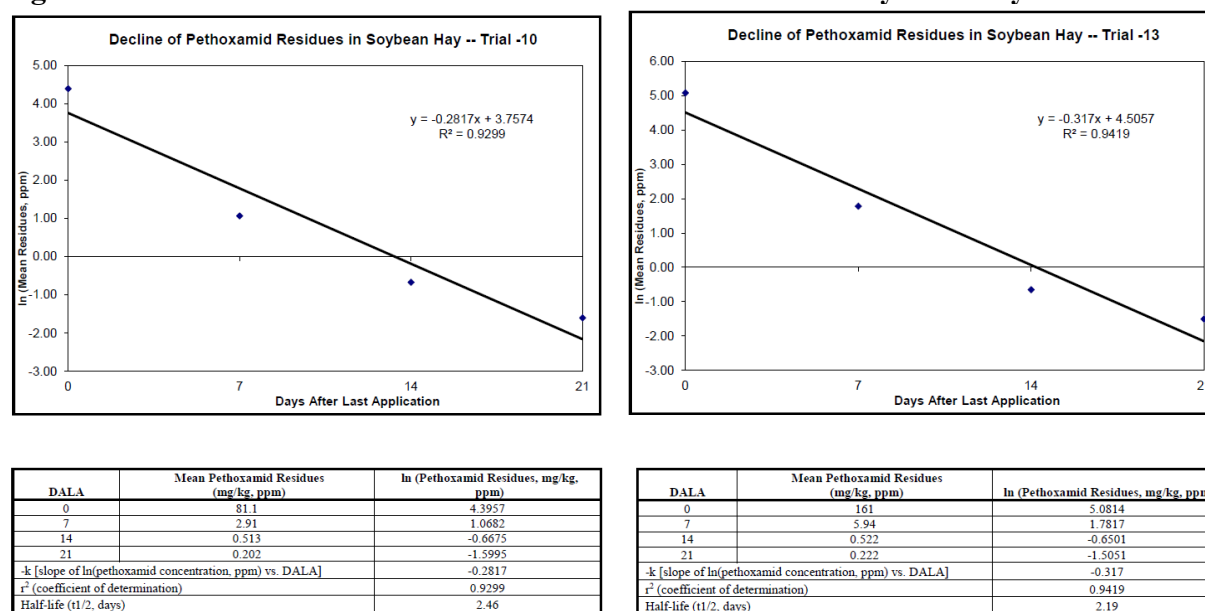
² Values based on residues in individual samples.

³ Values based on per-trial averages. LAFT = lowest average field trial, HAFT = highest average field trial, SD = standard deviation. For computation of the LAFT, HAFT, median, mean, and standard deviation, values <LOQ are assumed to be at the LOQ (0.01 ppm). N/A = Not applicable.

Table 5.3.4.1. Estimated Residue for Soybean Forage, Soybean Hay, Corn Forage and Corn Hay using Proportionality						
Matrix	PHI	Application rate from crop field trials (kg ai/ha)	Proposed GAP (kg ai/ha)	Scaling Factor ¹	HAFT (ppm)	Scaled HAFT ² (ppm)
Soybean forage	7	4.6	1.68	0.3652	21.1	7.7
Soybean hay	7	4.6	1.68	0.3652	16.1	5.9
Corn forage	7	3.35	1.68	0.5015	0.014	0.01
Corn hay	7	3.35	1.68	0.5015	0.019	0.01

¹ Scaling factor = Proposed GAP / Application rate from crop field trials

² Scaled HAFT= HAFT * Scaling Factor

Figure B.7.6.1.1-1. Decline of Residues of Pethoxamid in/on Soybean Forage.**Figure B.7.6.1.1-2. Decline of Residues of Pethoxamid in/on Soybean Hay.**

III. CONCLUSIONS

The soybean field trials are considered scientifically acceptable. Following a preplant or preemergence application and subsequent foliar application at a total rate of 3.96-4.12 lb ai/A (4.44-4.62 kg ai/ha), residues of pethoxamid were 0.154-23.3 ppm in/on forage and 0.083-21.4 ppm in/on hay harvested at a PHI of 6-7 days. Residues of pethoxamid were below the LOQ (<0.01 ppm) in/on all soybean seed samples harvested at a PHI of 86-112 days. Residues were also below the LOQ in/on soybean seed samples from the exaggerated-rate (5x) plot; therefore, a processing study was not conducted.

In the residue decline studies, residues of pethoxamid were below the LOQ (<0.01 ppm) in/on soybean seed samples from all sampling intervals; therefore, residue decline could not be evaluated. Residues in/on forage and hay declined with increasing PHIs. Average residues in/on forage declined from 141-158 ppm at the 0-day PHI to 0.032-0.100 ppm at the 21-day PHI; average residues in/on hay declined from 81.1-161 ppm to 0.210-0.222 ppm over the same interval.

An acceptable method was used for residue quantitation, and adequate storage stability data were submitted to support sample storage durations and conditions.

REFERENCES

- Document ID:** MRID Nos. 49813540 - 49813541; PMRA Study # xxxxxx, PMRA Review # xxxxxx
- Report:** Determination of Storage Stability of Pethoxamid in Crops under Freezer Storage Conditions. Study No. 1354 PXA. Unpublished study prepared by Eurofins Agrosience Services Chem SAS sponsored by Cheminova A/S in Denmark and submitted by FMC Corporation. 40 p.
Sorensen. L (2015). Tessier, V. (2014) Supplementary Data to Determination of Storage Stability of Pethoxamid in Crops Under Freezer Storage Conditions. Cheminova A/s in Denmark. Doc. No, 1354 PXA, Supplemental Data and submitted by FMC Corporation. 17 p.
- Document ID:** MRID Nos. 49813544 and 49813545; PMRA Study # xxxxxx, PMRA Review # xxxxxx
- Report:** Brewin. S (2000). The Development and Validation of a Method of Analysis for the Determination of Residues in Soybean (Green Plant, Seed, and Straw). Study No. 45 PXA. Unpublished study prepared by Huntingdon Life Sciences Ltd in United Kingdom sponsored by Cheminova A/S in Denmark and submitted by FMC Corporation. 71 p.

Sorensen. L (2000). Supplementary Data to TKC-94 The Development and Validation of a Method of Analysis for the Determination of Residues in Soybean (Green Plant, Seed, and Straw). Cheminova Doc. No 45 PXA. Unpublished study prepared by Cheminova A/S in Denmark and submitted by FMC Corporation. 33 p.

**B.7.6 Residues Resulting from Supervised Trials
(Annex IIA 6.3; Annex IIIA 8.3)**

B.7.6.1 Stability of Residues Prior to Analysis

B.7.6.1.2. Storage Stability

Document ID: MRID No. 49813540- 49813541

PMRA No. Not available

Report: Tessier. V (2014). Determination of Storage Stability of Pethoxamid in Crops under Freezer Storage Conditions. Study No. 1354 PXA. Unpublished study prepared by Eurofins Agroscience Services Chem SAS sponsored by Cheminova A/S in Denmark and submitted by FMC Corporation. 40 p.

Sorensen. L (2015). Tessier, V. (2014) Supplementary Data to Determination of Storage Stability of Pethoxamid in Crops Under Freezer Storage Conditions. Cheminova A/s in Denmark. Doc. No, 1354 PXA, Supplemental Data and submitted by FMC Corporation. 17 p.

Guidelines: EPA OCSPP Harmonized Test Guideline 860.1380 Storage Stability Data (August 1996)

PMRA Regulatory Directive DIR98-02 – Residue Chemistry Guidelines, Section 5 – Storage Stability

OECD Guideline 506 Stability of Pesticide Residues in Stored Commodities (October 2007)

GLP Compliance: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Acceptability: The study is considered scientifically acceptable.

Evaluator: Sheila Piper, Chemist, HED, RAB6 *Sheila Piper*

Secondary Evaluator: Robert Lanthier, PMRA

EXECUTIVE SUMMARY

Samples of maize (corn) grain, oilseed rape seed and soybean matrices were fortified with pethoxamid at 0.1 ppm and were placed in frozen storage at ≤ -20 °C. Stored samples and freshly fortified samples were analyzed for residues of pethoxamid at nominal intervals of 0, 3, 6, 9, and 12 months. The study was conducted by Eurofins Agroscience Service Chem SAS (Vergeze, France).

The supplemental report (MRID 49813541) indicates the dates of sample fortification, extraction, and analysis in the storage stability study of pethoxamid residues in maize grain, oilseed rape seed and soybean.

The analytical method (AGR/MOA/PTX-8) involves the extraction of pethoxamid by agitation with acetonitrile in presence of magnesium sulfate, sodium chloride, sodium citrate dibasic sesquihydrate and sodium citrate tribasic dihydrate. Samples were centrifuged, and purified with a mixture of PSA, C18EC and magnesium sulphate and diluted with water for analysis by

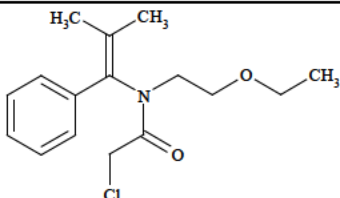
LC/MS/MS. The LOQ (determined as the LLMV in the associated method validation study) was 0.01 ppm for pethoxamid for all matrices.

After 12 months of storage at -20°C, corrected recoveries were soybean 89- 109%, 70- 117% for oilseed rape seed and 100- 108% for corn grain.

The results of the study showed that residues of pethoxamid are stable for at least 12 months in corn grain, oilseed rape seed and soybean stored at ≤ -20 °C.

I. MATERIALS AND METHODS

A. MATERIALS

Table B.7.6.2.1-1. Nomenclature for Pethoxamid.	
Common name	Pethoxamid
Identity	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide
CAS registry number	106700-29-2
Company experimental name	TKC-94
	

B. Study Design

1. Test Procedure

Frozen field samples (5 g) of corn grain, oilseed rape seed and soybean were homogenized with dry ice and stored in polypropylene boxes. Five replicate samples were analyzed at zero time and two replicated samples were removed from frozen storage after 3, 6, 9 and 12 months storage and analyzed along with freshly prepared samples fortified at 0.1 ppm for each matrix.

Not all samples were analyzed within 24 hours after extraction. The maximum extract storage time was 5 days from extraction to analysis. The recoveries for the final extracts were 81-91% after 5 days of storage. The results of the fortified samples at 0.01 ppm (LOQ) at the zero time are shown in Table B.7.6.2.1-2.

Table B.7.6.2.1-2. Fortified Samples at LOQ level (0.01 ppm) at Zero Time		
Matrix	Uncorrected Pethoxamid Conc.(ppm)	Freshly Prepared Recovery (%)
Maize (corn) grain	0.010	97.9
Oilseed rape seed	0.009	90.1
Soybean	0.009	91.7

Description of Analytical Procedures

The analytical method used to analyze the samples for pethoxamid was AGR/MOA/PTX-8. The method involves the extraction of pethoxamid by agitation with acetonitrile in the presence of magnesium sulfate, sodium chloride, sodium citrate dibasic sesquihydrate and sodium citrate tribasic dihydrate. Samples were centrifuged, and purified with a mixture of PSA, C18EC and magnesium sulphate and diluted with water for analysis by LC/MS/MS. The LOQ (determined as the LLMV in the associated method validation study) was 0.01 ppm for pethoxamid for all matrices.

II. RESULTS AND DISCUSSION

Method performance was evaluated by use of concurrent recovery samples of corn grain, oilseed rape seed, and soybean fortified with pethoxamid at 0.1 ppm. The 0-day recoveries represent method validation recoveries. All recoveries were within the acceptable range of 70-120%; therefore, the method was considered valid for the determination of pethoxamid residues in corn, rapeseed oil, and soybean matrices (Table B.7.6.2.1-3). After 12 months of storage at -20°C, uncorrected recoveries were 79- 85% and corrected recoveries were 96-101%.

Representative chromatograms of control and fortified samples were provided. The control chromatograms generally had no peaks of interest above the chromatographic background. The fortified sample chromatograms contained only the analyte of interest, and peaks were symmetrical and well defined.

Table B.7.6.2.1-3: Summary of Storage Stability of Pethoxamid						
Matrix	Storage Interval (months)	Mean Uncorrected Pethoxamid Conc. After Storage (ppm)	Mean Corrected Pethoxamid Conc. After Storage (ppm) ¹	Mean Freshly Prepared	Mean Recovery Uncorrected Pethoxamid Conc (ppm) ²	Mean Recovery Corrected Pethoxamid Conc (ppm) ³
Soybeans	0	0.09	0.10	--	100	100
	3	0.09	0.10	91	98	109
	6	0.07	0.08	88	78	89
	9	0.09	0.09	94	99	105
	12	0.07	0.09	82	79	96
Maize (corn) grain	0	0.09	0.10	--	100	100
	3	0.08	0.09	90	97	108
	6	0.08	0.09	93	93	100
	9	0.09	0.09	104	105	101
	12	0.07	0.09	84	85	101
Oilseed (rape seed)	0	0.09	0.10	--	100	100
	3	0.08	0.10	81	94	117
	6	0.06	0.06	99	70	70
	9	0.07	0.08	91	80	87
	12	0.07	0.09	84	83	99

¹ Corrected concentration= uncorrected concentration/ mean procedural recovery *100

² Recovery concentration= uncorrected concentration after storage/uncorrected concentration at T0 *100

³ Recovery corrected concentration= corrected concentration after storage/uncorrected concentration at T0 *100

III. CONCLUSIONS

The storage stability study with corn grain, oilseed rape seed and soybean is considered scientifically acceptable. The results of the study showed that residues of pethoxamid are stable for at least 12 months in corn grain, oilseed rape seed and soybean stored at ≤ -20 °C.

REFERENCES

None

**B.7.6 Residues Resulting from Supervised Trials
(Annex IIA 6.3; Annex IIIA 8.3)**

B.7.6.1 Stability of Residues Prior to Analysis

B.7.6.1.2. Storage Stability

Document ID: MRID No. 49813542 - 49813543

PMRA No. Not available

Report: Brewin. S (2000). The Development and Validation of a Method of Analysis for the Determination of Residues in Maize (Grain, Green Plant and Straw. Study No. 59 PXA. Unpublished study prepared by Huntingdon Life Sciences Ltd in United Kingdom sponsored by Cheminova A/S in Denmark and submitted by FMC Corporation. 69 p.

Sorensen. L (2000). Supplementary Data to TKC-94 The Development and Validation of a Method of Analysis for the Determination of Residues in Maize (Grain, Green Plant and Straw). Cheminova Doc. No 59 PXA. Unpublished study prepared by Cheminova A/S in Denmark and submitted by FMC Corporation. 46 p.

Guidelines: EPA OCSPP Harmonized Test Guideline 860.1380 Storage Stability Data (August 1996)

PMRA Regulatory Directive DIR98-02 – Residue Chemistry Guidelines, Section 5 – Storage Stability

OECD Guideline 506 Stability of Pesticide Residues in Stored Commodities (October 2007)

GLP Compliance: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Acceptability: The study is considered scientifically acceptable.

Evaluator: Sheila Piper, Chemist, HED, RAB6 

Secondary Evaluator: Robert Lanthier, PMRA

EXECUTIVE SUMMARY

A method of analysis for pethoxamid (TKC-94) in corn maize (grain, green plant and straw) was conducted by Huntingdon Life Sciences in the United Kingdom in 1998. In addition, samples of corn grain, cobs and straw fortified in duplicate by Stahler Agrochemie in 1997 were analyzed to confirm the stability of pethoxamid in crop matrix stored at approximately $\leq -20^{\circ}\text{C}$ for a period of approximately 2 years.

The supplemental report (MRID 49813543) indicates the dates of sample fortification, extraction, and analysis in the storage stability study of pethoxamid residues in corn matrices (whole plant/cob, grain and straw).

The analytical method used was validated by Huntingdon Life Sciences (report number TON 073/002233) from field trial F98001R for the determination of pethoxamid in corn (grain, green plant, and straw). The method consisted of extraction with a methanol:water mixture followed by a liquid:liquid partition and a silica solid phase extraction (SPE) cartridge. Quantitation was performed using gas chromatography with electron capture detection (GC-ECD). The limit of quantitation (LOQ; determined as the lowest level of method validation, LLMV, in the associated method validation study) was 0.01 ppm for pethoxamid in all matrices. The method was validated from the range of 0.01- 1.0 ppm and the recovery range was 74- 102% grain, 84- 108% in green plant and 80- 106% in straw.

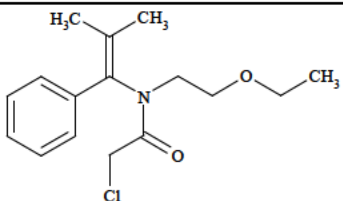
The chromatograms generally had no peaks of interest above the chromatographic background, except after 8 months storage (T8) some interference was shown in the retention time. In the control samples of corn (whole plant and grain), interference seen was equivalent to 14.6% and 14.4% of the peak area of the spiked batches. This interference may be due to matrix or solvent. Also, the control sample of *corn straw* after 8 months is not available. Therefore, a mean interference of 14.5% was used for correction of the recovery in straw samples.

Note: An independent laboratory validation has approved the LC/MS/MS method where the extraction and analysis procedures are based on the QuEChERS method. The GC-ECD method (Huntingdon Life Sciences report number TON 073/002233) used in this study has shown acceptable recovery, although this method has not been validated.

The results of the study showed that residues of pethoxamid are stable for at least 2 years in corn grain, green plant and straw stored at $\leq -20^{\circ}\text{C}$.

I. MATERIALS AND METHODS

A. MATERIALS

Table B.7.6.2.1-1. Nomenclature for Pethoxamid.	
Common name	Pethoxamid
Identity	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide
CAS registry number	106700-29-2
Company experimental name	TKC-94
	

B. Study Design

1. Test Procedure

Untreated samples (10 g) of corn (grain, green plant, and straw) was conducted by Huntingdon Life Sciences in the United Kingdom in 1998 report number TON 073/002233. In addition, samples of corn grain, cobs and straw fortified in duplicate by Stahler Agrochemie in 1997 were analyzed to confirm the stability of pethoxamid in crop matrix stored at approximately $\leq -20^{\circ}\text{C}$ for a period of approximately 2 years.

In the first set, corn matrices were fortified at a concentration of 0.5 ppm at study initiation (10/8/99). The samples designated "T0" were extracted and analyzed 35 days after fortification, but storage conditions for the T0 fortified samples are not specified in the study report or in the raw data.

In the second set, corn matrices were fortified by the sponsor (Stahler) at a concentration of 2 ppm in 1997. No analysis was made at time of fortification. Samples arrived in frozen conditions at Huntingdon 18th of August 1998 and were stored at $\leq -20^{\circ}\text{C}$ until extraction and analysis. After storage for approximately 2 years, the samples were analyzed twice. The raw data covering fortification of samples at Stahler for the 2-year set is not included in the raw data received from Huntingdon. It was concluded that storage period was between 22 and 34 months based on the original study report (1997). All samples were extracted and analyzed within 24 hours after extraction.

Description of Analytical Procedures

The analytical method used was validated by Huntingdon Life Sciences (report number TON 073/002233) from field trial F98001R for the determination of pethoxamid in corn (grain, green plant, and straw). Samples were analyzed for residues of pethoxamid using GC-ECD. Pethoxamid was extracted from corn grain, green plant, and straw using methanol:water (70:30 v:v). The supernatant was decanted and the crop residue remaining was extracted with methanol:water (70:30; v:v), centrifuged and the methanol:water phase was combined. The total volume was diluted with methanol and an aliquot was reduced by rotary evaporation at 40°C . The aqueous phase remaining was transferred with 5% sodium chloride solution and hexane. The sample was shaken vigorously and the upper hexane phase was transferred into a flask via a drying cartridge. The aqueous phase was re-partitioned with an aliquot of hexane as before and the hexane phases combined. The sample volume was reduced to $<5\text{ml}$ by rotary evaporation at $<40^{\circ}\text{C}$, prior to clean up by silica cartridge.

The cartridge was conditioned with aliquots of diethyl ether and hexane. The sample was loaded onto the conditioned cartridge and eluted through slowly discarding the eluate. The round bottom flask was rinsed with an aliquot of hexane, followed by an aliquot of diethyl ether: hexane (25:75; v:v) each time loading the rinse onto the cartridge slowly and discarding the eluate. The cartridge was eluted with an aliquot of diethyl ether:hexane (60:40; v:v), collecting the eluate in a polypropylene centrifuge tube. The solvent was removed under nitrogen at approximately 35°C and the residue reconstituted in an appropriate volume of hexane prior to

quantitation by GC-ECD. The limit of detection (LOD) of the method was 0.005 ppm and the limit of quantitation (LOQ) was 0.01 ppm.

Samples were fortified at 0.01, 0.1 and 1.0 ppm at the end of the storage period (8 months), one control and two of the spiked stability samples along with a procedural recovery were analyzed for each matrix type.

II. RESULTS AND DISCUSSION

Method performance was evaluated by use of concurrent recovery samples of corn (grain, green plant, and straw) fortified with pethoxamid at 0.01, 0.1 and 1.0 ppm. The 0-day recoveries represent method validation recoveries. The method was validated from the range of 0.01- 1.0 ppm and the recovery range was 74- 102% grain, 84-108% in green plant and 80- 106% in straw (Table B.7.6.2.1-2). Concurrent recoveries were used to correct the residues in the stored samples.

Table B.5.2.1.1-2. Validation of Pethoxamid Residues in Corn Commodities.					
Matrix	Fortification Level (ppm)	Validation Recovery (%)			RSD (%)
		Individual	Mean	Range	
Corn grain	0.01	74, 82, 90, 82, 96	85	74- 96	10
	0.1	102, 98, 98, 97, 99	99	97- 102	1.9
	1.0	93, 96, 96, 101, 99	97	93- 101	2.9
Corn green plant	0.01	108, 94, 84, 102, 84	94	84- 108	11.4
	0.1	93, 89, 99, 103, 101	97	89- 103	6.0
	1.0	95, 89, 91, 91, 93	92	89- 95	2.5
Corn straw	0.01	105, 101, 106, 80, 96	98	80- 106	10.9
	0.1	98, 92, 93, 98, 95	95	92- 98	2.9
	1.0	83, 87, 90, 88, 85	87	83- 90	3.1

Samples of corn grain, cobs and straw conducted by Stahler Agrochemie in 1997 were analyzed to confirm the stability of pethoxamid in crop matrix stored at approximately $\leq -20^{\circ}\text{C}$ for a period of approximately 2 years. The raw data covering fortification of samples at Stahler for the 2-year set is not included in the raw data received from Huntingdon. It was concluded that storage period was between 22 and 34 months based on the original study report. Also, the information regarding the storage conditions for the T0 samples is not available for the first set.

Representative chromatograms of control and fortified samples were provided. The control chromatograms generally had no peaks of interest above the chromatographic background, except in the control samples of corn whole plant and grain, the interference seen was equivalent to 14.6% and 14.4% of the peak area of the spiked batches. This interference may be due to matrix or solvent. Although a control sample of corn straw after 8 months is not available, the interference from corn whole plant and grain was almost identical, indicating that the interference seen in the T8 samples was similar for corn matrices.

Table B.5.2.1.1-3. Summary of 8-Month Storage Stability of Pethoxamid				
Sampling Time	Type of Results (ppm)	Corn Whole Plant	Corn Grain	Corn Straw
T0 (35 days)	Procedural recovery ¹	99%	109%	86%
	Sample conc. ¹	0.464	0.468	0.387
		0.468	0.455	0.402
	Mean conc.	0.466	0.461	0.394
	Recovery	93%	92%	79%
	Sample adjusted conc. ²	0.469	0.429	0.450
		0.473	0.417	0.467
T8 (8 months)	Mean adjusted conc.	0.471	0.423	0.459
	Recovery Adjusted	94%	85%	92%
	Procedural recovery ¹	93%	97%	925
	Sample conc. ¹	0.525	0.504	0.399
		0.462	0.488	0.461
	Mean conc.	0.493	0.496	0.430
	Recovery	99%	99%	86%
	Sample adjusted conc. ²	0.564	0.519	0.434
		0.497	0.503	0.501
	Mean adjusted conc.	0.531	0.511	0.468
	Recovery Adjusted	106%	102%	94%
	Relative contribution from control/blank samples	14.6	14.4	14.5 ³
	Mean double adjusted conc.	0.453	0.438	0.400
	Recovery double adjusted	91%	88%	80%

¹ Procedural recoveries and sample conc. (table 12 and 13 MRID 49813542 page 37-38)

² Adjusted conc. are cited from table 5 (MRID 4981543 in this study report on page 23)

³ A mean of the blank values from maize whole plant and grain was used for correction of recovery in corn straw.

Table B.5.2.1.1-4. Summary of 2-Year Storage Stability of Pethoxamid							
Sampling Time	Types of Results	Corn Cobs		Corn Grain		Corn Straw	
		T2a	T2b	T2a	T2b	T2a	T2b
T2a (~2 years)	Procedural recovery ¹	82%	88%	106%	100%	100%	95%
	Sample conc. ¹	0.0797 0.0811	0.0807 0.0892	0.0771 0.0925	0.0856 0.0932	0.0949 0.1002	0.1285 0.0910
	Mean conc.	0.0827		0.0871		0.1037	
	Recovery	83%		87%		104%	
	Sample adjusted conc.	0.0972 0.0989	0.0917 0.1014	0.0727 0.0873	0.0856 0.0932	0.0949 0.1002	0.1353 0.0958
	Mean adjusted conc.	0.0973		0.0847		0.1065	
	Recovery Adjusted	97%		85%		107%	

¹ Procedural recoveries and sample conc. (table 10 and 11 MRID 49813542 page 35-36)

III. CONCLUSIONS

The results of the study showed that residues of pethoxamid are stable for at least 2 years in corn grain, green plant and straw stored at ≤ -20 °C.

Note: The method from Huntingdon Life Sciences (report number TON 073/002233) has not been validated. An independent laboratory validation has approved the LC/MS/MS method where the extraction and analysis procedures are based on the QuEChERS method. There is some concern with the 8 month storage stability study where there was some interference found in the corn straw which may be from the matrix or solvent. A corrected recovery (14.6%) was used for all corn matrices and acceptable recovery was shown (70-120%).

REFERENCES

None

**B.7.6 Residues Resulting from Supervised Trials
(Annex IIA 6.3; Annex IIIA 8.3)**

B.7.6.1 Stability of Residues Prior to Analysis

B.7.6.1.2. Storage Stability

Document ID: MRID No. 49813544- 49813545

PMRA No. Not available

Report: Brewin. S (2000). The Development and Validation of a Method of Analysis for the Determination of Residues in Soybean (Green Plant, Seed, and Straw). Study No. 45 PXA. Unpublished study prepared by Huntingdon Life Sciences Ltd in United Kingdom sponsored by Cheminova A/S in Denmark and submitted by FMC Corporation. 71 p.

Sorensen. L (2000). Supplementary Data to TKC-94 The Development and Validation of a Method of Analysis for the Determination of Residues in Soybean (Green Plant, Seed, and Straw). Cheminova Doc. No 45 PXA. Unpublished study prepared by Cheminova A/S in Denmark and submitted by FMC Corporation. 33 p.

Guidelines: EPA OCSPP Harmonized Test Guideline 860.1380 Storage Stability Data (August 1996)
PMRA Regulatory Directive DIR98-02 – Residue Chemistry Guidelines, Section 5 – Storage Stability
OECD Guideline 506 Stability of Pesticide Residues in Stored Commodities (October 2007)

GLP Compliance: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Acceptability: The study is considered scientifically acceptable.

Evaluator: Sheila Piper, Chemist, HED, RAB6 *Sheila Piper*

Secondary Evaluator: Robert Lanthier, PMRA

EXECUTIVE SUMMARY

Samples of soybean (green plant, seed, and straw) were fortified with pethoxamid at 0.01, 0.1 and 1.0 ppm and put into -storage at $\leq -20^{\circ}\text{C}$. Stored samples and freshly fortified samples were analyzed for residues of pethoxamid at nominal intervals of 0 and 8 months. The study was conducted by Huntingdon Life Sciences in the United Kingdom in 1998.

The supplemental report (MRID 49813545) indicates the dates of sample fortification, extraction, and analysis in the storage stability study of pethoxamid residues in soybean. Pethoxamid was extracted from soybean green plant, seed and straw using methanol:water (70:30; v:v). Clean-up was performed using liquid:liquid partition and a silica solid phase extraction (SPE) cartridge. Quantitation was performed using gas chromatography with electron capture detection (GC-ECD) for green plant and seed extracts. Straw was quantified using liquid chromatography with mass spectrometric detection (LC-MS). The limit of detection (LOD) was

0.005 ppm for grain and green plant and 0.001 ppm for straw. LOQ (determined as the LLMV in the associated method validation study) was 0.01 ppm for pethoxamid for all matrices. Acceptable concurrent recoveries (70- 100%) were obtained from samples of soybean (green plant, seed, and straw) fortified with pethoxamid at 0.1-1.0 ppm.

The results of the study showed that residues of pethoxamid are stable for at least 8 months in soybean (green plant, seed, and straw) stored at ≤ -20 °C.

I. MATERIALS AND METHODS

A. MATERIALS

Table B.7.6.2.1-1. Nomenclature for Pethoxamid.	
Common name	Pethoxamid
Identity	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide
CAS registry number	106700-29-2
Company experimental name	TKC-94

B. Study Design

1. Test Procedure

Frozen field samples (10 g) of soybean (green plant, seed and straw) were homogenized and stored in polypropylene boxes. The developed method was applied to samples of control soybean from untreated plots from the 1998 field trial F98001R (Huntingdon Life Sciences report number TON 074/002234) were supplied by Stahler Agrochemie. At time 0 and at the end of the storage period (8 months), one control and two of each of the spiked stability samples along with a procedural recovery (a control spiked on the day of analysis) were analyzed for each matrix type.

Samples of all soybean matrices were fortified at a concentration of 0.5 ppm at study initiation (10/8/99). The samples designated "T0" were extracted and analyzed 38-39 days after fortification, but storage conditions for the T0 fortified samples are not specified in the study report or in the raw data. "T8" were stored under frozen conditions at -18°C for 8 months following fortification prior to extraction and analysis. All samples were extracted and analyzed on the same day, with the exception of the T8 soybean straw samples were analyzed 4 days after extraction.

Description of Analytical Procedures

Samples (10 g) of soybeans were weighed, fortified and an aliquot of methanol:water (70:30; v:v) was added and the sample was macerated using a Ultra Turrax blender until homogenous. The probe of the blender was rinsed with aliquots of methanol:water (70:30) and the sample was centrifuged to separate the phases. The supernatant was decanted through a glass funnel and crop residue remaining was extracted with methanol:water (70:30), centrifuged and the methanol:water phase was combined. The total volume was diluted with methanol and volume was reduced by rotary evaporation. The aqueous phase remaining was transferred using aliquots of 5% sodium chloride solution and hexane. The upper hexane phase was transferred to flask via a drying cartridge. The aqueous phase was re-partitioned with aliquot of hexane and the sample was reduced by rotary evaporation prior to clean up by silica cartridge.

The cartridge was conditioned with aliquots (3 ml) of diethyl ether and hexane. The sample was loaded onto the conditioned cartridge and eluted through slowly discarding the eluate. The round bottom flask was rinsed with an aliquot of hexane, followed by an aliquot of diethyl ether:hexane (25:75; v:v) each time loading the rinsate onto the cartridge slowly and discarding the eluate. The cartridge was eluted with an aliquot of diethyl ether:hexane (60:40; v:v), collecting the eluate in a polypropylene centrifuge tube. The solvent was removed under nitrogen at approximately 35°C and the residue reconstituted in an appropriate volume of hexane prior to quantitation by GC-ECD. Straw samples were reconstituted in methanol:water (50:50; v:v) prior to quantitation by GC-ECD.

The LOD was 0.005 ppm for grain and green plant and 0.001 ppm for straw. LOQ (determined as the LLMV in the associated method validation study) was 0.01 ppm for pethoxamid for all matrices.

II. RESULTS AND DISCUSSION

Method performance was evaluated during method validation and by use of concurrent recovery samples soybean (green plant, seed and straw) fortified with pethoxamid at 0.01, 0.1 and 1.0 ppm. The 0-day recoveries represent method validation recoveries. The method was validated from the range of 0.01- 1.0 ppm and the recovery range was 76- 110% green plant, 73-100% in seed, and 70- 85% in straw (Table B.5.2.1.1-2.). Concurrent recoveries were used to correct the residues in the stored samples.

Table B.5.2.1.1-2.Validation of Pethoxamid Residues in Soybean Commodities.					
Matrix	Fortification Level (ppm)	Validation Recovery (%)			RSD (%)
		Individual	Mean	Range	
Soybean green plant	0.01	104, 110, 105, 108, 109	107	104- 110	2.4
	0.1	77, 76, 87, 90, 93	85	76- 93	9.1
	1.0	84, 93, 87, 86, 85	87	84- 93	4.1
Soybean seed	0.01	75, 80, 73, 87, 78	79	73- 87	6.9
	0.1	98, 92, 97, 94, 86	93	86- 98	5.1
	1.0	84, 82, 100, 100, 95	92	82- 100	9.4

Table B.5.2.1.1-2. Validation of Pethoxamid Residues in Soybean Commodities.

Matrix	Fortification Level (ppm)	Validation Recovery (%)			RSD (%)
		Individual	Mean	Range	
Soybean straw	0.01	71, 72, 79, 70, 72	73	70- 79	4.9
	0.1	77, 82, 81, 80, 76	79	76- 82	3.3
	1.0	78, 85, 79, 79, 85	81	78- 85	4.3

Table B.5.2.1.1-3. Summary of Storage Stability of Pethoxamid

Sampling Time	Type of Results (ppm)	Soybean Whole Plant	Soybean Seed	Soybean Straw
T0 (38-39 days)	Procedural recovery ¹	91%	88%	75%
	Sample conc. ¹	0.448	0.440	0.354
		0.433	0.465	0.374
	Mean conc.	0.441	0.452	0.364
	Recovery	88%	90%	73%
	Sample adjusted conc. ²	0.0492	0.500	0.471
		0.476	0.528	0.499
	Mean adjusted conc.	0.484	0.514	0.485
T8 (8 months)	Recovery Adjusted	97%	103%	97%
	Procedural recovery ¹	91%	104%	85%
	Sample conc. ¹	0.0433	0.374	0.419
		0.521	0.529	0.406
	Mean conc.	0.477	0.451	0.413
	Recovery	92%	90%	83%
	Sample adjusted conc. ²	0.476	0.359	0.493
		0.573	0.508	0.478
	Mean adjusted conc.	0.525	0.434	0.486
	Recovery Adjusted	105%	87%	97%

¹ Procedural recoveries and sample conc. are cited from table 9-12 (pages 37-38; MRID 49813544)

² Adjusted conc. are cited from table 6 (page 2; MRID 49813544)

III. CONCLUSIONS

The storage stability study with soybean (green plant, seed and straw) is considered scientifically acceptable. The results of the study showed that residues of pethoxamid are stable for at least 8 months in soybean stored at ≤ -20 °C.

REFERENCES

None

**B.7.6 Residues Resulting from Supervised Trials
(Annex IIA 6.3; Annex IIIA 8.3)**

B.7.6.1 Residues in Target Crops

B.7.6.1.2 Field, Pop, and Sweet Corn

Document ID: MRID No. 49813546

PMRA No. Not Available

Report: Carringer, S.J. (2015) Magnitude and Decline of Pethoxamid Residues in/on Field Corn, Pop Corn, and Sweet Corn Raw Agricultural and Processed Commodities Following One Pre-plant or Pre-emergence Application and One Post-emergence Application of Pethoxamid 600 g/L EC (2014). Study Numbers: 1505 PXA; TCI-14-401; 81557. Unpublished study prepared by The Carringers, Inc., sponsored by Cheminova A/S, and submitted by FMC Corporation. 693 p.

Guidelines: EPA OCSPP Harmonized Test Guideline 860.1500 Crop Field Trials (August 1996)
PMRA Regulatory Directive DIR98-02 – Residue Chemistry Guidelines, Section 9 – Crop Field Trials
PMRA Regulatory Directive DIR2010-05 – Revisions to the Residue Chemistry Crop Field Trial Requirements
OECD Guideline 509 Crop Field Trial (September 2009)

GLP Compliance: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Acceptability: The study is considered scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP#452109.

Evaluator: Sheila Piper, Chemist, HED, RAB6 *Sheila Piper*

Secondary Evaluator: Robert Lanthier, PMRA

Note: This Data Evaluation Record (DER) was prepared under contract by CDM/CSS-Dynamac Joint Venture (submitted 2/16/18). The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies. The DER has been reviewed by HED and revised as necessary to reflect current Office of Pesticide Programs (OPP) policies.

EXECUTIVE SUMMARY

Seventeen trials for pethoxamid on field, pop, and sweet corn were conducted in Canada and/or United States encompassing North American Free Trade Agreement (NAFTA) Growing Zones 1 (PA; 1 trial), 2 (NC; 1 trial), 5 (IA, IL, MB, MI, MN, MO, NE, ON, and WI; 13 trials), 6 (OK; 1 trial), and 8 (TX; 1 trial); three trials were conducted with popcorn in Zone 5 (IA, IL, and NE); and 15 trials were conducted with sweet corn in Zones 1 (NY and PA; 2 trials), 2 (NC; 1 trial), 3 (FL; 1 trial), 5 (IA, IL, MB, MI, MO, NE, and ON; 7 trials), 7 (SK; 1 trial), 10 (CA; 1 trial), 11 (WA; 1 trial), and 12 (OR; 1 trial) during the 2014 growing season. At four pairs of field corn test sites (in IA, IL, MN, and NE), trials were determined to be replicates; therefore, residues

were averaged, and each pair of sites was taken to be one independent trial, which is reflected in the above trial count.

At each site consisted of one untreated plot and one treated plot reflecting a preplant or preemergence application to the soil followed by a foliar broadcast application targeting the 40-inch growth stage (prior to beginning of tasseling). Applications were made with a 5.0 lb ai/gal (600 g ai/L) emulsifiable concentrate (EC) formulation of pethoxamid at 1.42-1.56 lb ai/A/application (1.59-1.75 kg ai/ha/application), with 32- to 65-day retreatment intervals, for total seasonal rates of 2.88-3.12 lb ai/A (3.23-3.50 kg ai/ha). Applications were made using ground equipment in spray volumes of 5-20 gal/A (45-187 L/ha). At one field corn site, an additional plot was treated at an exaggerated rate (~5x the field trial rates) for a total application rate of 15.0 lb ai/A (16.8 kg ai/ha) for the collection of field corn grain for possible processing. An adjuvant (nonionic surfactant or crop oil concentrate) was added to the spray mixture for all foliar applications, and for preemergence/preplant applications at five trial sites. For field corn, forage was harvested at the late dough to early dent stage and grain and stover were harvested at maturity, at preharvest intervals (PHIs) of 42-78 days for forage and 71-125 days for grain and stover. For popcorn, grain and stover were harvested at maturity at a PHI of 97-107 days. For sweet corn, forage, kernel plus cob with husk removed (K+CWHR), and stover were harvested at the milk stage at a PHI of 14-42 days; stover was dried for 2-12 days prior to collection. At two field corn trials, one popcorn trial, and one sweet corn trial, additional RAC samples were harvested 6-8 days before, and 6-8 and 13-14 days after nominal harvest to assess residue decline.

Samples were maintained frozen at the field sites, during shipping, and at the laboratory prior to analysis. The maximum storage intervals for samples between harvest/collection and extraction for analysis were ≤ 7.8 months for field and sweet corn forage; 9.4 months for sweet corn K+CWHR; ≤ 6.1 months for field and popcorn grain; and ≤ 8.8 months for field, pop, and sweet corn stover. Samples were analyzed within 9 days of extraction. Acceptable storage stability data were submitted indicating that residues of pethoxamid are stable during frozen storage for up to 10 months in sweet corn K+CWHR, corn flour, corn meal, corn refined oil, and 12 months in corn grain (refer to MRIDs 49813534 and 49813540). These data are adequate to support the sample storage conditions and durations from the submitted study.

Samples were analyzed for residues of pethoxamid using a high performance liquid chromatography method with tandem mass spectrometry detection (LC/MS/MS), adapted from Eurofins Agrosience Services (EAS) Method No. AGR/MOA/PTX-8, the proposed enforcement method for crop commodities (refer to the DER for MRID 49813534, B.5.2.1.1). The limit of quantitation (LOQ; determined as the lowest level of method validation, LLMV) was 0.01 ppm for pethoxamid in corn matrices. Acceptable method validation and concurrent recoveries were obtained from samples of field corn forage fortified with pethoxamid at 0.01-200 ppm; field, pop, and sweet corn stover fortified at 0.01-1.0 ppm; field corn grain and sweet corn K+CWHR fortified at 0.01-0.50 ppm; and popcorn grain and sweet corn forage fortified at 0.01 and 0.10 ppm. The fortification levels were adequate to bracket measured residue levels.

Following a preplant or preemergence application and subsequent foliar application at a total rate of 2.88-3.12 lb ai/A (3.23-3.50 kg ai/ha), residues of pethoxamid were below the LOQ (<0.01

ppm) in/on all samples of field corn grain harvested at a PHI of 71-125 days, all samples of popcorn grain and stover harvested at a PHI of 97-107 days, and all samples of sweet corn K+CWHR harvested at a PHI of 14-42 days. Residues were also below the LOQ (<0.01 ppm) in/on field corn grain samples from the exaggerated-rate (5x) plot; therefore, the processing study was not conducted.

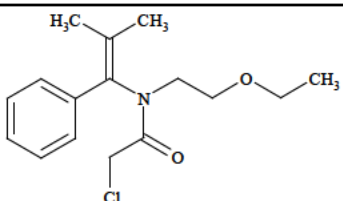
Residues of pethoxamid were below the LOQ (<0.01 ppm) in/on all field corn forage and stover samples harvested at PHIs of 42-78 and 71-125 days, respectively, except for samples from one trial which bore quantifiable residues of 0.013 and 0.015 ppm in/on forage and 0.016 and 0.022 ppm in/on stover. Per-trial average residues were <0.01-0.014 ppm in/on forage and <0.01-0.019 ppm in/on stover.

Residues of pethoxamid were below the LOQ (<0.01 ppm) in/on all sweet corn forage and stover samples harvested at a PHI of 14-42 days, except for samples from two trials which bore quantifiable residues of 0.072-0.391 ppm in/on forage and 0.087-0.759 ppm in/on stover; the second application at these trials was made at a later growth stage than specified in the protocol, resulting in shorter PHIs (14 and 21 days). Per-trial average residues were <0.01-0.350 ppm in/on forage and <0.01-0.467 ppm in/on stover.

In the residue decline studies, residues of pethoxamid were below the LOQ (<0.01 ppm) in/on field corn forage, grain, and stover; popcorn grain and stover; and sweet corn forage, K+CWHR, and stover samples from all sampling intervals; therefore, residue decline could not be evaluated.

I. MATERIALS AND METHODS

A. MATERIALS

Table B.7.6.1.2-1. Nomenclature for Pethoxamid.	
Common name	Pethoxamid
Identity	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide
CAS registry number	106700-29-2
Company experimental name	TKC-94
	

B. Study Design

1. Test Procedure

A total of thirty-five field trials on field, pop, and sweet corn were conducted with a 5.0 lb ai/gal (600 g ai/L) EC formulation of pethoxamid during the 2014 growing season. Field trial locations by NAFTA growing zone are summarized in Table B.7.6.1.2-2.

All trials, except for those listed in the table below, were separated by ≥ 20 miles and are therefore considered independent (568_Criteria for Independence of Trials 4/23/13 (EPA and PMRA)). The trials separated by < 20 miles have been assessed for independence as detailed in the table below. HED has determined that four pairs of field corn trials conducted in IA, IL, MN, and NE should each be considered a single trial with replicate samples for purposes of 860.1500 data requirements.

Table B.7.6.1.1-2 Independent Trial Determination¹			
Crop	Trial Nos. TCI-14-401-	Differences	Decision
Field corn	09 and 10 (IL)	<u>Location:</u> Separated by 8.4 miles <u>Variety:</u> NK N68B-3122 vs. FS 66JV1 RIB <u>Timing:</u> 1 st (and 2 nd) applications separated by 4 days <u>Spray volume:</u> 12 vs. 15-18 gal/A	Replicates
	11 and 13 (IA)	<u>Location:</u> Separated by 16.3 miles <u>Variety:</u> P0987AMX vs. P1360HR <u>Timing:</u> 1 st applications separated by 13 days; 2 nd applications same day <u>Spray volume:</u> 17-19 vs. 18 gal/A	Replicates
	15 and 16 (MN)	<u>Location:</u> Separated by 4 miles <u>Variety:</u> P9834AMX vs. DKC48-12RIB <u>Timing:</u> 1 st applications separated by 4 days; 2 nd applications separated by 13 days <u>Spray volume:</u> 14-18 vs. 14-17 gal/A	Replicates
	17 and 18 (NE)	<u>Location:</u> Same <u>Variety:</u> DKC65-79RIB vs. P0876CHR <u>Timing:</u> 1 st applications separated by 31 days; 2 nd applications separated by 13 days. Harvests for forage separated by 28 days; grain and stover separated by 9 days <u>Spray volume:</u> 14-19 vs. 19 gal/A	Replicates

¹ All assessments are based on the replicate trial guidance presented in draft memo 568_Criteria for Independence of Trials 4/23/13 (EPA and PMRA).

Table B.7.6.1.2-2. Trial Numbers and Geographical Locations.																
Crop	No. Trials	NAFTA Growing Zone														Total
		1	2	3	4	5	5B	6	7	7A	8	9	10	11	12	
Field corn	Sub.	1	1			13 ¹		1			1 ²					17
	U.S. Req. ³	1/1	1/1			17/12		1/1								20/15
	Can. Req. ⁴					9	7									16
Popcorn	Sub.					3										3
	U.S. Req. ⁵															3
	Can. Req. ⁴					3										3
Sweet corn	Sub.	2	1	1		7			1 ⁶				1	1	1	15
	U.S. Req. ³	2/1	1/1	1/1		5/3							1/1	1/1	1/1	12/9
	Can. Req. ⁴					4	3			1						8

¹ HED has determined that four pairs of trials conducted in Zone 5 are each replicates.

² The trial conducted in Zone 8 was located near the border with Zone 6.

³ As per Table 5 of 860.1500 for field and sweet corn; the second number reflects a 25% reduction in the number of field trials allowed for the crop as a representative commodity in support of a crop group/subgroup tolerance as applicable (refer to Tables 2 and 3) or when application results in no quantifiable residues.

⁴ As per Table 1.2 of PMRA Regulatory Directive DIR2010-05 for corn, grain; corn, pop; and corn, sweet.

⁵ As per Table 1 of 860.1500 for popcorn. Field trial locations are not specified for crops requiring ≤ 3 trials; however, 91% of the U.S. production of popcorn occurs in Zone 5 (Table 6 of 860.1500).

⁶ The trial conducted in Zone 7 was irrigated to represent Zone 7A.

Locations and detailed use patterns for the trials are provided in Table B.7.6.1.2-3. Preplant and preemergence applications were made to a variety of soil types: loam (Trials 01, 03, 05, 21, 25, 26, 29, 34, and 37), loamy sand (Trials 02, 19, 27, and 38), silt loam (Trials 04, 07, 08, 09, 10, 12, 14, 17, 22, 30, 31, and 39), sandy clay loam (Trials 06, 15, and 35), silty clay loam (Trials 11, 13, 18, 23, 24, 32, 33, and 36), clay loam (Trial 16), silty clay (Trial 20), and sand (Trial 28).

Table B.7.6.1.2-3. Study Use Pattern.								
Location: City, State/Province; Year (Trial ID TCI-14-401-)	End-use Product ¹	TRT No. ²	Method of Application; Timing of Application	Volume (gal/A) [L/ha]	Rate per Application (lb ai/A) [kg ai/ha]	Retreatment Interval (days)	Total Rate (lb ai/A) [kg ai/ha]	Surfactant/ Adjuvant ³
Field Corn								
Germansville, PA; 2014 (01)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	15 [140]	1.53 [1.71]	--	3.03 [3.40]	None
			2. Foliar broadcast; 36-45" crop, BBCH 35-37 (V10-V12)	15 [140]	1.50 [1.68]	47		NIS @ 0.25% v/v
Seven Springs, NC; 2014 (02)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, on day of planting	17 [159]	1.50 [1.68]	--	3.05 [3.42]	None
			2. Foliar broadcast; 39-40" crop, BBCH 38 (vegetative)	19 [178]	1.55 [1.74]	42		COC @ 1.0% v/v
Conklin, MI; 2014 (03)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	4.9 [46]	1.50 [1.68]	--	3.00 [3.36]	None
			2. Foliar broadcast; 36-42" crop, BBCH 18-20 (V8-V10, majority 40" tall)	5.1 [48]	1.50 [1.68]	40		NIS @ 0.25% v/v
Delavan, WI; 2014 (04)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	18 [168]	1.50 [1.68]	--	2.99 [3.35]	None
			2. Foliar broadcast; 39-40" crop, BBCH 19 (40" tall to top of leaf arch)	19 [178]	1.49 [1.67]	50		COC @ 1.0% v/v
Branchton, ON; 2014 (05)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, 3 days after planting	4.8 [45]	1.45 [1.63]	--	2.90 [3.25]	None
			2. Foliar broadcast; 39-40" crop, BBCH 19-20 (V8, 9-10 leaf)	4.8 [45]	1.45 [1.63]	40		NIS @ 0.25% v/v
Portage la Prairie, MB; 2014 (06)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	11 [103]	1.52 [1.70]	--	3.02 [3.39]	None
			2. Foliar broadcast; 35-45" crop, BBCH 34-39 (~40" tall)	11 [103]	1.50 [1.68]	53		COC @ 1.0% v/v
Wyoming, IL; 2014 (07)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, 5 days after planting	15 [140]	1.53 [1.71]	--	3.00 [3.36]	None
			2. Foliar broadcast; 40" crop, BBCH 32 (V10-V11)	14 [131]	1.47 [1.65]	36		NIS @ 0.25% v/v
Grantfork, IL; 2014 (08)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	18 [168]	1.51 [1.69]	--	3.02 [3.39]	COC @ 1.0% v/v
			2. Foliar broadcast; 38-42" crop, BBCH 18 (8 leaves unfolded)	16 [150]	1.51 [1.69]	35		COC @ 0.5% v/v
Shattuc, IL; 2014 (09)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	12 [112]	1.52 [1.70]	--	3.01 [3.37]	NIS @ 0.25% v/v
			2. Foliar broadcast; 38-40" crop, BBCH 17-18 (V7-V8)	12 [112]	1.49 [1.67]	34		NIS @ 0.25% v/v

Table B.7.6.1.2-3. Study Use Pattern.								
Location: City, State/Province; Year (Trial ID TCI-14-401-)	End-use Product ¹	TRT No. ²	Method of Application; Timing of Application	Volume (gal/A) [L/ha]	Rate per Application (lb ai/A) [kg ai/ha]	Retreatment Interval (days)	Total Rate (lb ai/A) [kg ai/ha]	Surfactant/ Adjuvant ³
Carlyle, IL; 2014 (10)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, on day of planting	18 [168]	1.50 [1.68]	--	3.00 [3.36]	COC @ 1.0% v/v
			2. Foliar broadcast; 36-44" crop, BBCH 18	15 [140]	1.50 [1.68]	39		COC @ 1.0% v/v
Richland, IA; 2014 (11)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, 3 days after planting	19 [178]	1.50 [1.68]	--	3.00 [3.36]	None
			2. Foliar broadcast; 38-40" crop, BBCH 17	17 [159]	1.50 [1.68]	32		NIS @ 0.25% v/v
		4	1. Broadcast to soil; preemergence, 3 days after planting	19 [178]	7.50 [8.41]	--	15.0 [16.8]	None
			2. Foliar broadcast; 38-40" crop, BBCH 17	17 [159]	7.50 [8.41]	32		NIS @ 0.25% v/v
Kirksville, MO; 2014 (12)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, 7 days after planting	5.1 [48]	1.50 [1.68]	--	2.99 [3.35]	None
			2. Foliar broadcast; 39-42" crop, BBCH 11	4.9 [46]	1.49 [1.67]	45		COC @ 1.0% v/v
Hedrick, IA; 2014 (13)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, 5 days after planting	18 [168]	1.52 [1.70]	--	3.03 [3.40]	None
			2. Foliar broadcast; 40-41" crop, BBCH 17	18 [168]	1.51 [1.69]	45		NIS @ 0.25% v/v
Grandview, IA; 2014 (14)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	17 [159]	1.49 [1.67]	--	2.99 [3.35]	None
			2. Foliar broadcast; 39-40" crop, BBCH 18 (V7-V8)	15 [140]	1.50 [1.68]	50		COC @ 1.0% v/v
Geneva, MN; 2014 (15)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	14 [131]	1.52 [1.70]	--	3.03 [3.40]	None
			2. Foliar broadcast; 38-42" crop, BBCH 19-20 (V9-V10)	18 [168]	1.51 [1.69]	45		NIS @ 0.25% v/v
Ellendale, MN; 2014 (16)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, on day of planting	14 [131]	1.51 [1.69]	--	3.01 [3.37]	None
			2. Foliar broadcast; 38-42" crop, BBCH 20-21 (V10-V11)	17 [159]	1.50 [1.68]	54		COC @ 1.0% v/v
York, NE; 2014 (17)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	14 [131]	1.50 [1.68]	--	3.01 [3.37]	None
			2. Foliar broadcast; 37-41" crop, BBCH 19 (V9-V10)	19 [178]	1.51 [1.69]	34		NIS @ 0.25% v/v
York, NE; 2014 (18)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	19 [178]	1.51 [1.69]	--	3.01 [3.37]	None
			2. Foliar broadcast; 38-42" crop, BBCH 19 (V9-V10)	19 [178]	1.50 [1.68]	52		COC @ 1.0% v/v
Osceola, NE; 2014 (19)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, on day of planting	19 [178]	1.48 [1.66]	--	2.98 [3.34]	None
			2. Foliar broadcast; 38-42" crop, BBCH 16 (V6)	18 [168]	1.50 [1.68]	54		NIS @ 0.25% v/v

Table B.7.6.1.2-3. Study Use Pattern.								
Location: City, State/Province; Year (Trial ID TCI-14-401-)	End-use Product ¹	TRT No. ²	Method of Application; Timing of Application	Volume (gal/A) [L/ha]	Rate per Application (lb ai/A) [kg ai/ha]	Retreatment Interval (days)	Total Rate (lb ai/A) [kg ai/ha]	Surfactant/ Adjuvant ³
Wall, TX; 2014 (20)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, on day of planting	14 [131]	1.49 [1.67]	--	2.99 [3.35]	None
			2. Foliar broadcast; 38-42" crop, BBCH 39 (9 or more nodes)	14 [131]	1.50 [1.68]	59		COC @ 1.0% v/v
Hinton, OK; 2014 (21)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	16 [150]	1.51 [1.69]	--	3.04 [3.41]	None
			2. Foliar broadcast; 38-42" crop, BBCH 36-38	19 [178]	1.53 [1.71]	49		NIS @ 0.25% v/v
Popcorn								
Carlyle, IL; 2014 (22)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, on day of planting	18 [168]	1.50 [168]	--	2.99 [3.35]	COC @ 1.0% v/v
			2. Foliar broadcast; 38-42" crop, BBCH 17 (V7)	15 [140]	1.49 [1.67]	39		COC @ 1.0% v/v
Richland, IA; 2014 (23)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	15 [140]	1.47 [1.65]	--	2.98 [3.34]	None
			2. Foliar broadcast; 38-40" crop, BBCH 33-34	18 [168]	1.51 [1.69]	49		NIS @ 0.25% v/v
York, NE; 2014 (24)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	5.1 [48]	1.50 [1.68]	--	2.97 [3.33]	None
			2. Foliar broadcast; 38-42" crop, BBCH 19 (V10-V11)	4.8 [45]	1.47 [1.65]	47		COC @ 1.0% v/v
Sweet Corn								
Germansville, PA; 2014 (25)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, 3 days after planting	15 [140]	1.49 [1.67]	--	3.01 [3.37]	None
			2. Foliar broadcast; 38-41" crop, BBCH 44-51 (V14/VT)	15 [140]	1.52 [1.70]	44		NIS @ 0.25% v/v
Alton, NY; 2014 (26)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, 1 day after planting	20 [187]	1.51 [1.69]	--	3.02 [3.39]	None
			2. Foliar broadcast; 30-40" crop, BBCH 39-51 (10% of plants beginning tassel emergence)	20 [187]	1.51 [1.69]	50		COC @ 1.0% v/v
Seven Springs, NC; 2014 (27)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, on day of planting	17 [159]	1.51 [1.69]	--	3.02 [3.39]	None
			2. Foliar broadcast; 21-40" crop, BBCH 15-51	18 [168]	1.51 [1.69]	54		NIS @ 0.25% v/v
Oviedo, FL; 2014 (28)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	20 [187]	1.46 [1.64]	--	2.88 [3.23]	None
			2. Foliar broadcast; 19-30" crop, BBCH 18 (7-10 leaf stage)	19 [178]	1.42 [1.59]	46		COC @ 1.0% v/v
Conklin, MI; 2014 (29)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	4.9 [46]	1.49 [1.67]	--	2.98 [3.34]	None
			2. Foliar broadcast; majority 40" crop, BBCH 18-20	5.0 [47]	1.49 [1.67]	46		NIS @ 0.25% v/v

Table B.7.6.1.2-3. Study Use Pattern.								
Location: City, State/Province; Year (Trial ID TCI-14-401-)	End-use Product ¹	TRT No. ²	Method of Application; Timing of Application	Volume (gal/A) [L/ha]	Rate per Application (lb ai/A) [kg ai/ha]	Retreatment Interval (days)	Total Rate (lb ai/A) [kg ai/ha]	Surfactant/ Adjuvant ³
Carlyle, IL; 2014 (30)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, on day of planting	18 [168]	1.48 [1.66]	--	2.97 [3.33]	COC @ 1.0% v/v
			2. Foliar broadcast; 35-45" crop, BBCH 17 (V7)	15 [140]	1.49 [1.67]	39		COC @ 1.0% v/v
Kirkville, MO; 2014 (31)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, on day of planting	4.8 [45]	1.47 [1.65]	--	2.99 [3.35]	None
			2. Foliar broadcast; 36-42" crop (majority 40"), BBCH 51 (beginning of tasseling)	4.9 [46]	1.52 [1.70]	46		NIS @ 0.25% v/v
Richland, IA; 2014 (32)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	15 [140]	1.51 [1.69]	--	3.02 [3.39]	None
			2. Foliar broadcast; 39-40" crop, BBCH 33-34	18 [168]	1.51 [1.69]	49		COC @ 1.0% v/v
York, NE; 2014 (33)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	19 [178]	1.49 [1.67]	--	2.99 [3.35]	None
			2. Foliar broadcast; 38-42" crop, BBCH 19 (V11)	15 [140]	1.50 [1.68]	48		NIS @ 0.25% v/v
Branchton, ON; 2014 (34)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	20 [187]	1.50 [1.68]	--	3.00 [3.36]	None
			2. Foliar broadcast; 38-42" crop, BBCH 19 (10 leaves)	20 [187]	1.50 [1.68]	43		COC @ 1.0% v/v
Portage la Prairie, MB; 2014 (35)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, 1 day after planting	11 [103]	1.56 [1.75]	--	3.12 [3.50]	None
			2. Foliar broadcast; 43-50" crop, BBCH 55-63	11 [103]	1.56 [1.75]	58		NIS @ 0.25% v/v
Broderick, SK; 2014 (36)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, 2 days after planting	11 [103]	1.54 [1.73]	--	3.04 [3.41]	None
			2. Foliar broadcast; 38-42" crop, BBCH 33-51 (3 nodes to beginning tassel emergence)	11 [103]	1.50 [1.68]	65		COC @ 1.0% v/v
Porterville, CA; 2014 (37)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	15 [140]	1.50 [1.68]	--	3.00 [3.36]	None
			2. Foliar broadcast; 34-48" crop (average 40"), BBCH 51 (beginning tassel emergence)	16 [150]	1.50 [1.68]	55		NIS @ 0.25% v/v
Ephrata, WA; 2014 (38)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preemergence, on day of planting	15 [140]	1.51 [1.69]	--	3.02 [3.39]	None
			2. Foliar broadcast; 37-41" crop, BBCH 37-38 (V7-V8, 7-8 collars)	15 [140]	1.51 [1.69]	49		COC @ 1.0% v/v

Table B.7.6.1.2-3. Study Use Pattern.								
Location: City, State/Province; Year (Trial ID TCI-14-401-)	End-use Product ¹	TRT No. ²	Method of Application; Timing of Application	Volume (gal/A) [L/ha]	Rate per Application (lb ai/A) [kg ai/ha]	Retreatment Interval (days)	Total Rate (lb ai/A) [kg ai/ha]	Surfactant/ Adjuvant ³
Hillsboro, OR; 2014 (39)	5.0 lb ai/gal EC	2	1. Broadcast to soil; preplant, on day of planting	20 [187]	1.47 [1.65]	--	2.96 [3.32]	None
			2. Foliar broadcast; 35-40" crop, BBCH 35 (35-40")	19 [178]	1.49 [1.67]	60		NIS @ 0.25% v/v

¹ A 5.0 lb ai/gal (600 g ai/L) EC formulation of pethoxamid.

² Treated plots received a preplant/preemergence soil application followed by a foliar application; TRT 2 plots were treated at total rates of 2.88-3.12 lb ai/A (3.23-3.50 kg ai/ha) and the TRT 4 plot was treated at exaggerated rates totaling 15.0 lb ai/A (16.8 kg ai/ha).

³ NIS = Nonionic surfactant; COC = Crop oil concentrate.

Field, pop, and sweet corn were grown and maintained using typical agricultural practices. Irrigation was used at field corn trial sites 01, 02, 17, 18, 19, 20, and 21; popcorn trial site 24; and sweet corn trial sites 25, 27, 33, 36, 37, 38, and 39. Despite variations in weather conditions reported at some trial sites, no unusual weather conditions were reported to have adversely affected crop production or yield during the study, except at Trial 36, where yields were reduced (thin crop stand) by rainfall early in June (near time of first application) that hindered crop emergence and may have drowned out a percentage of the population. Phytotoxicity (delayed/reduced crop emergence, reduced crop stand, stunting, or buggy whipping/epinasty) was observed 2-4 weeks after the first application at Trials 01, 02, 04, 10, 11, 14, 18, 22, 24, 27, 28, and 39; crops typically recovered, however reduction in the crop stand was observed at 6 of the affected trials. Phytotoxicity (slight growth stunting) after the second application was observed at Trial 38 resulting in a ~4-inch height difference in the treated plot crop compared to the untreated plot crop. At Trial 28, it was reported that crop growth stopped after the second application; however, yield was reported to be normal.

Sample Handling and Preparation

Single control and duplicate treated samples were collected from each trial site. For field corn, forage (with ears) was harvested at the late dough to early dent stage at a PHI of 42-78 days, and grain and stover were harvested at maturity at a PHI of 71-125 days. For popcorn, grain and stover were harvested at maturity at a PHI of 97-107 days. For sweet corn, forage, K+CWHR, and stover were harvested at the milk stage at a PHI of 14-42 days; stover was dried for 2-12 days prior to collection to a moisture content of 18-73% (drying conditions were not reported). Bulk samples of untreated (246 kg) and treated (244 kg) field corn grain were collected from the control and exaggerated-rate plots at Trial 11 for possible processing. At two field corn trials, one popcorn trial, and one sweet corn trial, additional RAC samples were harvested 6-8 days before, and 6-8 and 13-14 days after nominal harvest to assess residue decline. Samples were placed into frozen storage within 4 hours of collection and were stored frozen (temperatures not reported) at the field sites prior to shipment by ACDS freezer truck to the analytical laboratory, ABC Laboratories, Inc. (Columbia, MO). At the laboratory, samples were homogenized in the presence of dry ice and stored frozen (*ca.* -20 °C) until extraction for analysis.

2. Description of Analytical Procedures

Samples of corn were analyzed for residues of pethoxamid using an LC/MS/MS method adapted from EAS Method No. AGR/MOA/PTX-8, as described in “Validation of an Analytical Method for the Determination of Pethoxamid Residues in Various Crops.” The method is the proposed enforcement method for crop commodities (refer to the DER for MRID 49813534, B.5.2.1.1).

Briefly, samples were extracted with acetonitrile (ACN). QuEChERS extraction salts (magnesium sulfate:sodium chloride:sodium citrate tribasic dihydrate:sodium citrate dibasic sesquihydrate; 4:1:1:0.5, w:w:w:w) were added, and samples were shaken and centrifuged. A portion of the supernatant was purified by the addition of cleanup salts (primary secondary amine:C18EC:magnesium sulfate; 1:1:6, w:w:w). Samples were shaken and centrifuged, and the supernatant was diluted with ACN:water (1:9, v:v) for analysis by LC/MS/MS. The method monitors the following ion transitions for pethoxamid: m/z 296 $\rightarrow$ 131 (quantitation) and m/z 296 $\rightarrow$ 250 (confirmation). The LOQ (determined as the LLMV) was 0.01 ppm for pethoxamid, and the estimated limit of detection (LOD) was 0.003 ppm (1/3 LOQ).

II. RESULTS AND DISCUSSION

Method performance was evaluated by use of method validation and concurrent recovery samples of field corn forage fortified with pethoxamid at 0.01-200 ppm; field, pop, and sweet corn stover fortified at 0.01-1.0 ppm; field corn grain and sweet corn K+CWHR fortified at 0.01-0.50 ppm; and popcorn grain and sweet corn forage fortified at 0.01 and 0.10 ppm. Recoveries were within the acceptable range of 70-120%; therefore, the method was considered valid for the determination of pethoxamid residues in corn matrices (Table B.7.6.1.2-4). The fortification levels bracketed the measured residues. Concurrent recoveries were corrected for any apparent residues in controls.

The detector response was linear (coefficient of determination, $r^2 \geq 0.9971$) within the range of 0.01-2 ng/mL. Representative chromatograms of control samples, fortified samples, and treated samples were provided. The control chromatograms generally had no peaks of interest above the chromatographic background. The fortified sample chromatograms contained only the analyte of interest, and peaks were symmetrical and well defined. Apparent residues in/on controls were nondetectable (<0.003 ppm).

Table B.7.6.1.2-4. Summary of Method Validation and Concurrent Recoveries of Pethoxamid from Corn Matrices.				
Matrix	Fortification Level (ppm)	Sample Size (n)	Recoveries ¹ (%)	Mean $\pm$ Std. Dev. (%)
Method Validation				
Field corn forage	0.01, 0.50	6	94-103	99 $\pm$ 3
Field corn grain	0.01, 0.50	6	80-97	90 $\pm$ 7
Field corn stover	0.01, 0.50	6	85-96	91 $\pm$ 4
Sweet corn K+CWHR	0.01, 0.50	6	94-106	101 $\pm$ 4
Concurrent Recoveries				
Field corn forage	0.01-200	16	82-109	97 $\pm$ 8
Field corn grain	0.01, 0.10	16	79-103	90 $\pm$ 8
Field corn stover	0.01-1.0	14	89-104	95 $\pm$ 5
Popcorn grain	0.01, 0.10	4	73-96	84 $\pm$ 10

Table B.7.6.1.2-4. Summary of Method Validation and Concurrent Recoveries of Pethoxamid from Corn Matrices.

Matrix	Fortification Level (ppm)	Sample Size (n)	Recoveries ¹ (%)	Mean $\pm$ Std. Dev. (%)
Popcorn stover	0.01, 1.0	4	82-100	90 $\pm$ 8
Sweet corn forage	0.01, 0.10	12	91-107	101 $\pm$ 5
Sweet corn K+CWHR	0.01, 0.10	12	82-109	99 $\pm$ 9
Sweet corn stover	0.01, 1.0	12	89-120	98 $\pm$ 8

¹ Concurrent recoveries were corrected for any apparent residues in controls.

The maximum storage intervals for samples between harvest/collection and extraction for analysis were ≤ 7.8 months for field and sweet corn forage; 9.4 months for sweet corn K+CWHR; ≤ 6.1 months for field and popcorn grain; and ≤ 8.8 months for field, pop, and sweet corn stover (Table B.7.6.1.2-5). Samples were analyzed within 9 days of extraction. Acceptable storage stability data were submitted indicating that residues of pethoxamid are stable during frozen storage for up to 24 months in corn grain, cobs and straw, and up to 10 months in corn flour and corn meal (refer to the DERs for MRIDs 49813534 and 49813540-49813543; B.7.6.2).

These data are adequate to support the sample storage conditions and durations from the submitted study.

Table B.7.6.1.2-5. Summary of Storage Conditions.

Matrix	Storage Temperature (°C)	Actual Storage Duration ¹	Interval of Demonstrated Storage Stability
Field corn forage	≤ -20 °C	104-237 days (3.4-7.8 months)	Residues of pethoxamid are stable during frozen storage for up to 24 months in corn grain, cobs and straw, and up to 10 months in corn flour and corn meal ²
Field corn grain		57-186 days (1.9-6.1 months)	
Field corn stover		72-192 days (2.4-6.3 months)	
Popcorn grain		134-176 days (4.4-5.8 months)	
Popcorn stover		142-165 days (4.7-5.4 months)	
Sweet corn forage		92-229 days (3.0-7.5 months)	
Sweet corn K+CWHR		162-286 days (5.3-9.4 months)	
Sweet corn stover		160-269 days (5.3-8.8 months)	

¹ Interval from harvest/collection to extraction. Samples were analyzed within 0-9 days of extraction.

² Refer to the DERs for MRIDs 49813534 and 49813540-49813543; B.7.6.2

The results from the submitted field trials are presented in Table B.7.6.1.2-6 and summarized in Table B.7.6.1.2-7. Following a preplant or preemergence application and subsequent foliar application at a total rate of 2.88-3.12 lb ai/A (3.23-3.50 kg ai/ha), residues of pethoxamid were highest in/on sweet corn forage (<0.01 -0.391 ppm; overall average 0.047 ppm) and stover (<0.01 -0.759 ppm; overall average 0.046 ppm). Residues in/on field corn forage and stover were near or below the LOQ (<0.01 -0.015 and <0.01 -0.022 ppm, respectively; overall averages of 0.010 and 0.011 ppm), and residues were below the LOQ (<0.01 ppm) in/on all field corn grain, popcorn grain and stover, and sweet corn K+CWHR samples, including samples of field corn grain from the exaggerated-rate (5x) plot.

In the residue decline studies, residues of pethoxamid were below the LOQ in/on field corn forage, grain, and stover; popcorn grain and stover; and sweet corn forage, K+CWHR, and stover samples from all sampling intervals; therefore, residue decline could not be evaluated.

Table B.7.6.1.2-6. Residue Data from Field, Pop, and Sweet Corn Field Trials with Pethoxamid.¹							
Location: City, State/Province; Year (Trial ID TCI-14-401-)	Zone	Crop Variety	TRT No. ²	Rate (lb ai/A) [kg ai/ha]	Matrix	PHI ³ (days)	Residues ⁴ (ppm) [Average]
Field Corn							
Germansville, PA; 2014 (01)	1	TA264-13VPRIB	2	3.03 [3.40]	Forage	46	ND, ND [<0.01]
					Grain	75	ND, ND [<0.01]
					Stover	75	ND, ND [<0.01]
Seven Springs, NC; 2014 (02)	2	DKC64-69	2	3.05 [3.42]	Forage	46	ND, ND [<0.01]
					Grain	87	ND, ND [<0.01]
					Stover	87	ND, ND [<0.01]
Conklin, MI; 2014 (03)	5	DKC46-20RIB	2	3.00 [3.36]	Forage	68	ND, ND [<0.01]
					Grain	109	ND, ND [<0.01]
					Stover	109	ND, ND [<0.01]
Delavan, WI; 2014 (04)	5	DKC49-94RIB	2	2.99 [3.35]	Forage	78	ND, ND [<0.01]
					Grain	125	ND, ND [<0.01]
					Stover	125	ND, ND [<0.01]
Branchton, ON; 2014 (05)	5	Maizex 35-15	2	2.90 [3.25]	Forage	69	ND, ND [<0.01]
					Grain	105	ND, ND [<0.01]
					Stover	105	ND, ND [<0.01]
Portage la Prairie, MB; 2014 (06)	5	DKC26-28 RIB	2	3.02 [3.39]	Forage	62	ND, ND [<0.01]
					Grain	87	ND, ND [<0.01]
					Stover	87	ND, ND [<0.01]
Wyoming, IL; 2014 (07)	5	Golden Harvest G09H57-3111	2	3.00 [3.36]	Forage	64	ND, ND [<0.01]
					Grain	113	ND, ND [<0.01]
					Stover	113	ND, ND [<0.01]
Grantfork, IL; 2014 (08)	5	33T55	2	3.02 [3.39]	Forage	64	ND, ND [<0.01]
					Grain	104	ND, ND [<0.01]
					Stover	104	ND, ND [<0.01]
Shattuc, IL; 2014 (09) and Carlyle, IL; 2014 ⁵ (10)	5	NK N68B-3122; FS 66JV1 RIB	2	3.01 [3.37]; 3.00 [3.36]	Forage	49	ND, ND [<0.01]
						63; 56	ND, ND, ND, ND [<0.01]
						63	ND, ND [<0.01]
						70	ND, ND [<0.01]
					Grain	97	ND, ND [<0.01]
						103; 105	ND, ND, ND, ND [<0.01]
						111	ND, ND [<0.01]
						118	ND, ND [<0.01]
					Stover	97	ND, ND [<0.01]
						103; 105	ND, ND, ND, ND [<0.01]
						111	ND, ND [<0.01]
						118	ND, ND [<0.01]
Richland, IA; 2014 (11) and Hedrick, IA; 2014 (13)	5	P0987AMX; P1360HR	2	3.00 [3.36]; 3.03 [3.40]	Forage	62; 56	ND, ND, ND, ND [<0.01]
					Grain	122; 102	ND, ND, ND, ND [<0.01]
					Stover	122; 102	ND, ND, ND, ND [<0.01]
			4 ⁶	15.0 [16.8]	Grain	122	ND, ND [<0.01]

Table B.7.6.1.2-6. Residue Data from Field, Pop, and Sweet Corn Field Trials with Pethoxamid. ¹							
Location: City, State/Province; Year (Trial ID TCI-14-401-)	Zone	Crop Variety	TRT No. ²	Rate (lb ai/A) [kg ai/ha]	Matrix	PHI ³ (days)	Residues ⁴ (ppm) [Average]
Kirksville, MO; 2014 (12)	5	P1498AM	2	2.99 [3.35]	Forage	55	ND, ND [<0.01]
					Grain	102	ND, ND [<0.01]
					Stover	102	ND, ND [<0.01]
Grandview, IA; 2014 (14)	5	P1360CHR	2	2.99 [3.35]	Forage	58	ND, ND [<0.01]
					Grain	109	ND, ND [<0.01]
					Stover	109	ND, ND [<0.01]
Geneva, MN; 2014 (15) and Ellendale, MN; 2014 (16)	5	P9834AMX; DKC48-12RIB	2	3.03 [3.40]; 3.01 [3.37]	Forage	66, 53	ND, ND, ND, ND [<0.01]
					Grain	115; 104	ND, ND, ND, ND [<0.01]
					Stover	115; 104	ND, ND, ND, ND [<0.01]
York, NE; 2014 ⁷ (17) and York, NE; 2014 (18)	5	DKC65-79RIB; P0876CHR	2	3.01 [3.37]; 3.01 [3.37]	Forage	60	ND, ND [<0.01]
						67 ; 52	ND, (0.005), ND, ND [<0.01]
						74	ND, ND [<0.01]
						81	ND, ND [<0.01]
					Grain	113	ND, ND [<0.01]
						119 ; 123	ND, ND, ND, ND [<0.01]
						126	ND, ND [<0.01]
						133	ND, ND [<0.01]
					Stover	113	ND, ND [<0.01]
						119 ; 123	ND, ND, ND, ND [<0.01]
						126	ND, ND [<0.01]
						133	ND, ND [<0.01]
Osceola, NE; 2014 (19)	5	P0876CHR	2	2.98 [3.34]	Forage	51	ND, ND [<0.01]
					Grain	98	ND, ND [<0.01]
					Stover	98	ND, ND [<0.01]
Wall, TX; 2014 (20)	8	Dyna-Gro D57VP75	2	2.99 [3.35]	Forage	42	0.015, 0.013 [0.014]
					Grain	71	ND, ND [<0.01]
					Stover	71	0.022, 0.016 [0.019]
Hinton, OK; 2014 (21)	6	Channel 209-85VT3PRIB	2	3.04 [3.41]	Forage	44	ND, ND [<0.01]
					Grain	79	ND, ND [<0.01]
					Stover	79	ND, ND [<0.01]
Popcorn							
Carlyle, IL; 2014 (22)	5	AP2501 F113SUCR	2	2.99 [3.35]	Grain	90	ND, ND [<0.01]
						97	ND, ND [<0.01]
						105	ND, ND [<0.01]
						111	ND, ND [<0.01]
					Stover	90	ND, ND [<0.01]
						97	ND, ND [<0.01]
						105	ND, ND [<0.01]
						111	ND, ND [<0.01]
Richland, IA; 2014 (23)	5	Robust 997 F1	2	2.98 [3.34]	Grain	100	ND, ND [<0.01]
					Stover	100	ND, ND [<0.01]
York, NE; 2014 (24)	5	Robust Yellow	2	2.97 [3.33]	Grain	107	ND, ND [<0.01]
					Stover	107	ND, ND [<0.01]

Table B.7.6.1.2-6. Residue Data from Field, Pop, and Sweet Corn Field Trials with Pethoxamid.¹							
Location: City, State/Province; Year (Trial ID TCI-14-401-)	Zone	Crop Variety	TRT No. ²	Rate (lb ai/A) [kg ai/ha]	Matrix	PHI ³ (days)	Residues ⁴ (ppm) [Average]
Sweet Corn							
Germansville, PA; 2014 (25)	1	Mirai 421	2	3.01 [3.37]	Forage	28	ND, ND [<0.01]
					K+CWHR	28	ND, ND [<0.01]
					Stover	28 (5)	ND, ND [<0.01]
Alton, NY; 2014 (26)	1	Precious Gem	2	3.02 [3.39]	Forage	35	ND, ND [<0.01]
					K+CWHR	35	ND, ND [<0.01]
					Stover	35 (9)	ND, ND [<0.01]
Seven Springs, NC; 2014 (27)	2	Sweet G90	2	3.02 [3.39]	Forage	29	ND, ND [<0.01]
					K+CWHR	29	ND, ND [<0.01]
					Stover	29 (10)	ND, ND [<0.01]
Oviedo, FL; 2014 (28)	3	Munition	2	2.88 [3.23]	Forage	29	ND, ND [<0.01]
					K+CWHR	29	ND, ND [<0.01]
					Stover	29 (6)	ND, ND [<0.01]
Conklin, MI; 2014 (29)	5	Luscious	2	2.98 [3.34]	Forage	30	ND, ND [<0.01]
					K+CWHR	30	ND, ND [<0.01]
					Stover	30 (7)	ND, ND [<0.01]
Carlyle, IL; 2014 (30)	5	Obsession II	2	2.97 [3.33]	Forage	35	ND, ND [<0.01]
					K+CWHR	35	ND, ND [<0.01]
					Stover	35 (3)	ND, ND [<0.01]
Kirksville, MO; 2014 (31)	5	Early Sunglow Hybrid 5093	2	2.99 [3.35]	Forage	21	ND, ND [<0.01]
					K+CWHR	21	ND, ND [<0.01]
					Stover	21 (4)	ND, ND [<0.01]
Richland, IA; 2014 (32)	5	Xtra-Tender 2573 F1 (sh2)	2	3.02 [3.39]	Forage	27	ND, ND [<0.01]
						34	ND, ND [<0.01]
						41	ND, ND [<0.01]
						48	ND, ND [<0.01]
					K+CWHR	27	ND, ND [<0.01]
						34	ND, ND [<0.01]
						41	ND, ND [<0.01]
						48	ND, ND [<0.01]
					Stover	27 (3)	ND, ND [<0.01]
						34 (4)	ND, (0.003) [<0.01]
						41 (3)	ND, ND [<0.01]
						48 (2)	ND, ND [<0.01]
York, NE; 2014 (33)	5	Obsession II	2	2.99 [3.35]	Forage	28	ND, ND [<0.01]
					K+CWHR	28	ND, ND [<0.01]
					Stover	28 (5)	ND, ND [<0.01]
Branchton, ON; 2014 (34)	5	Ambrosia	2	3.00 [3.36]	Forage	37	ND, ND [<0.01]
					K+CWHR	37	ND, ND [<0.01]
					Stover	37 (12)	ND, ND [<0.01]
Portage la Prairie, MB; 2014 (35)	5	Earlivee	2	3.12 [3.50]	Forage	14 ⁸	0.308, 0.391 [0.350]
					K+CWHR	14 ⁸	ND, ND [<0.01]
					Stover	14 ⁸ (11)	0.087, 0.105 [0.096]
Broderick, SK; 2014 (36)	7	Northern Extra Hybrid	2	3.04 [3.41]	Forage	41	ND, ND [<0.01]
					K+CWHR	41	ND, ND [<0.01]
					Stover	41 (5)	ND, ND [<0.01]

Table B.7.6.1.2-6. Residue Data from Field, Pop, and Sweet Corn Field Trials with Pethoxamid.¹							
Location: City, State/Province; Year (Trial ID TCI-14-401-)	Zone	Crop Variety	TRT No. ²	Rate (lb ai/A) [kg ai/ha]	Matrix	PHI ³ (days)	Residues ⁴ (ppm) [Average]
Porterville, CA; 2014 (37)	10	Bodacious	2	3.00 [3.36]	Forage	21 ⁹	0.381, 0.072 [0.226]
					K+CWHR	21 ⁹	ND, ND [<0.01]
					Stover	21 ⁹ (7)	0.175, 0.759 [0.467]
Ephrata, WA; 2014 (38)	11	Serendipity	2	3.02 [3.39]	Forage	42	ND, ND [<0.01]
					K+CWHR	42	ND, ND [<0.01]
					Stover	42 (10)	ND, ND [<0.01]
Hillsboro, OR; 2014 (39)	12	Honey 'N Pearl	2	2.96 [3.32]	Forage	35	ND, ND [<0.01]
					K+CWHR	35	ND, ND [<0.01]
					Stover	35 (7)	ND, ND [<0.01]

¹ A 5.0 lb ai/gal (600 g ai/L) EC formulation of pethoxamid was used.

² Treated plots received a preplant/preemergence soil application followed by a foliar application; TRT 2 plots were treated at total rates of 2.88-3.12 lb ai/A (3.23-3.50 kg ai/ha) and the TRT 4 plot was treated at exaggerated rates totaling 15.0 lb ai/A (16.8 kg ai/ha).

³ The target PHI is **bolded** for the decline studies. The number of days sweet corn stover was dried prior to collection is reported in parentheses.

⁴ ND = Not detected (<LOD). The LOD was 0.003 ppm and the LOQ was 0.01 ppm. Values between the LOQ and LOD are reported in parentheses. Per-trial averages were calculated by the study reviewer using the LOQ for all residues reported as <LOQ.

⁵ Decline samples are from Trial 10.

⁶ TRT 4 (exaggerated rate) samples are from Trial 11.

⁷ Decline samples are from Trial 17.

⁸ Samples were harvested at a shorter PHI because the second application was made when crops were 43-50" tall, which was later than the protocol timing (~40" tall).

⁹ Samples were harvested at a shorter PHI because the second application was made when crops were 34-48" tall (average 40").

Table B.7.6.1.2-7. Summary of Residues from Field, Pop, and Sweet Corn Field Trials with Pethoxamid.										
Crop Matrix	Total Application Rate (lb ai/A) [kg ai/ha]	PHI (days)	n ¹	Pethoxamid Residues (ppm)						
				Min. ²	Max. ²	LAFT ³	HAFT ³	Median ³	Mean ³	SD ³
Field corn forage	2.90-3.05 [3.25-3.42]	42-78	17	<0.01	0.015	<0.01	0.014	0.010	0.010	0.001
Field corn grain		71-125	17	<0.01	<0.01	<0.01	<0.01	0.01	0.01	N/A
Field corn stover		71-125	17	<0.01	0.022	<0.01	0.019	0.010	0.011	0.002
Popcorn grain	2.97-2.99 [3.33-3.35]	97-107	3	<0.01	<0.01	<0.01	<0.01	0.01	0.01	N/A
Popcorn stover		97-107	3	<0.01	<0.01	<0.01	<0.01	0.01	0.01	N/A
Sweet corn forage	2.88-3.12 [3.23-3.50]	14-42	15	<0.01	0.391	<0.01	0.350	0.010	0.047	0.101
Sweet corn K+CWHR		14-42	15	<0.01	<0.01	<0.01	<0.01	0.01	0.01	N/A
Sweet corn stover		14-42	15	<0.01	0.759	<0.01	0.467	0.010	0.046	0.118

¹ n = number of field trials.

² Values based on residues in individual samples.

³ Values based on per-trial averages. LAFT = lowest average field trial, HAFT = highest average field trial, SD = standard deviation. For computation of the LAFT, HAFT, median, mean, and standard deviation, values <LOQ are assumed to be at the LOQ (0.01 ppm). N/A = Not applicable.

III. CONCLUSIONS

The corn field trials are considered scientifically acceptable. Following a preplant or preemergence application and subsequent foliar application at a total rate of 2.88-3.12 lb ai/A (3.23-3.50 kg ai/ha), residues of pethoxamid were below the LOQ (<0.01 ppm) in/on all samples of field corn grain harvested at a PHI of 71-125 days, all samples of popcorn grain and stover

harvested at a PHI of 97-107 days, and all samples of sweet corn K+CWHR harvested at a PHI of 14-42 days. Residues were also below the LOQ (<0.01 ppm) in/on field corn grain samples from the exaggerated-rate (5x) plot; therefore, the processing study was not conducted.

Residues of pethoxamid were below the LOQ (<0.01 ppm) in/on all field corn forage and stover samples harvested at PHIs of 42-78 and 71-125 days, respectively, except for samples from one trial which bore quantifiable residues of 0.013 and 0.015 ppm in/on forage and 0.016 and 0.022 ppm in/on stover.

Residues of pethoxamid were below the LOQ (<0.01 ppm) in/on all sweet corn forage and stover samples harvested at a PHI of 14-42 days, except for samples from two trials which bore quantifiable residues of 0.072-0.391 ppm in/on forage and 0.087-0.759 ppm in/on stover; the second application at these trials was made at a later growth stage than specified in the protocol resulting in shorter PHIs (14 and 21 days).

In the residue decline studies, residues of pethoxamid were below the LOQ (<0.01 ppm) in/on field corn forage, grain, and stover; popcorn grain and stover; and sweet corn forage, K+CWHR, and stover samples from all sampling intervals; therefore, residue decline could not be evaluated.

An acceptable method was used for residue quantitation, and adequate storage stability data were submitted to support sample storage durations and conditions.

REFERENCES

Document ID: MRID 49813534; PMRA Study # xxxxxx, PMRA Review # xxxxxx
Report: Bitter, J. (2017) S-2399: Freezer Storage Stability of S-2399 and Metabolites in Processed Fractions. Report No. 201700305; Laboratory Project ID VP-40066. Unpublished study sponsored by Valent USA LLC. 836 p.

Document ID: MRIDs 49813540-49813541; PMRA Study # xxxxxx, PMRA Review # xxxxxx
Report: Tessier, V (2014). Determination of Storage Stability of Pethoxamid in Crops under Freezer Storage Conditions. Study No. 1354 PXA. Unpublished study prepared by Eurofins Agrosience Services Chem SAS sponsored by Cheminova A/S in Denmark and submitted by FMC Corporation. 40 p.
Sorensen, L (2015). Tessier, V. (2014) Supplementary Data to Determination of Storage Stability of Pethoxamid in Crops Under Freezer Storage Conditions. Cheminova A/s in Denmark. Doc. No, 1354 PXA, Supplemental Data and submitted by FMC Corporation. 17 p.

**B.7.6 Residues Resulting from Supervised Trials
(Annex IIA 6.3; Annex IIIA 8.3)**

B.7.6.1 Residues in Target Crops

B.7.6.1.3 Cotton

Document ID: MRID No. 49813548
PMRA No. Not Available

Report: Carringer, S.J. (2015) Magnitude and Decline of Pethoxamid Residues in/on Cotton Raw Agricultural and Processed Commodities Following One Pre-plant or Pre-emergence Application and One Post-emergence Application of Pethoxamid 600 g/L EC (2014). Study Numbers: 1506 PXA; TCI-14-402; 81558. Unpublished study prepared by The Carringers, Inc., sponsored by Cheminova A/S, and submitted by FMC Corporation. 418 p.

Guidelines: EPA OCSPP Harmonized Test Guideline 860.1500 Crop Field Trials (August 1996)
PMRA Regulatory Directive DIR98-02 – Residue Chemistry Guidelines, Section 9 – Crop Field Trials
PMRA Regulatory Directive DIR2010-05 – Revisions to the Residue Chemistry Crop Field Trial Requirements
OECD Guideline 509 Crop Field Trial (September 2009)

GLP Compliance: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Acceptability: The study is considered scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP#452109.

Evaluator: Sheila Piper, Chemist, HED, RAB6 *Sheila Piper*

Secondary Evaluator: Robert Lanthier, PMRA

Note: This Data Evaluation Record (DER) was prepared under contract by CDM/CSS-Dynamac Joint Venture (submitted 2/16/18). The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies. The DER has been reviewed by HED and revised as necessary to reflect current Office of Pesticide Programs (OPP) policies.

EXECUTIVE SUMMARY

Eleven field trials for pethoxamid on cotton were conducted in the Canada and/or the United States encompassing North American Free Trade Agreement (NAFTA) Growing Zones 2 (GA; 1 trial), 4 (AR, LA, and MO; 3 trials), 6 (OK and TX; 2 trials), 8 (TX; 3 trials), and 10 (CA; 2 trials) during the 2014 growing season. At one pair of test sites (in CA), trials were determined to be replicates; therefore, residues were averaged, and the pair of sites was taken to be one independent trial, which is reflected in the above trial count. This submission also includes data for cotton processed commodities (refer to the B.7.7.3.1 DER part 2 for MRID 49813548).

At each site consisted of one untreated plot and one treated plot reflecting a preplant or preemergence application to the soil followed by a foliar broadcast application targeting the first white flower growth stage (~BBCH 60). Applications were made with a 5.0 lb ai/gal (600 g ai/L) emulsifiable concentrate (EC) formulation of pethoxamid at 1.43-1.56 lb ai/A/application (1.60-1.75 kg ai/ha/application), with 50- to 84-day retreatment intervals, for total seasonal rates of 2.96-3.05 lb ai/A (3.32-3.42 kg ai/ha). Applications were made using ground equipment in spray volumes of 5-18 gal/A (45-168 L/ha). An adjuvant (nonionic surfactant or crop oil concentrate) was added to the spray mixture for all foliar applications, and for the preplant/preemergence application at two sites. Duplicate treated samples of seed cotton were harvested at maturity by hand (7 trials) or using stripper equipment (4 trials) at a preharvest interval (PHI) of 67-185 days and ginned to produce undelinted cotton seed (all trials) and gin byproducts (at 3 stripper harvest equipment trials). At one trial, additional undelinted cotton seed and gin byproducts samples were collected 12 days before and 7, 14, and 22 days after nominal harvest to assess residue decline.

Samples were maintained frozen at the field sites, during shipping, and at the laboratory prior to analysis. The maximum storage intervals for samples between collection and extraction for analysis were 6.2 months for undelinted seed and 5.0 months for gin byproducts. Samples were analyzed within 4 days of extraction. Acceptable storage stability data were submitted indicating that residues of pethoxamid are stable during frozen storage (-20 °C) for 5.1 months in cotton undelinted seed and up to 10 months in gin byproducts, but residues in undelinted seed declined an average of 36% after 10 months of storage (MRID 49813534). Although decline was observed in undelinted seed between the 5- and 10-month sampling intervals, acceptable recoveries would be expected at the maximum storage interval of samples from the subject study. These data are adequate to support the sample storage conditions and durations from the submitted study.

Samples were analyzed for residues of pethoxamid using a high performance liquid chromatography method with tandem mass spectrometry detection (LC/MS/MS), adapted from Eurofins Agrosience Services (EAS) Method No. AGR/MOA/PTX-8, the proposed enforcement method for crop commodities (refer to the DER for MRID 49813534, B.5.2.1.1). The limit of quantitation (LOQ; determined as the lowest level of method validation, LLMV) was 0.01 ppm for pethoxamid in cotton matrices. Acceptable method validation and concurrent recoveries were obtained from samples of cotton undelinted seed and gin byproducts fortified with pethoxamid at 0.01-0.5 ppm. The fortification levels were adequate to bracket measured residue levels.

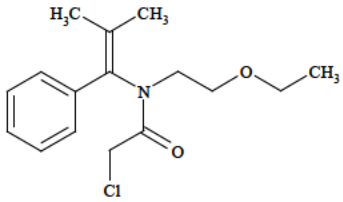
Following a preplant or preemergence application and subsequent foliar application at a total rate of 2.96-3.05 lb ai/A (3.32-3.42 kg ai/ha), residues of pethoxamid were below the LOQ (<0.01 ppm) in/on all undelinted seed samples collected at a PHI of 67-185 days. In the corresponding gin byproduct samples, collected at three trials reflecting a PHI of 67-112 days, individual sample (and per-trial average) residues of pethoxamid were <0.01-0.047 (<0.01-0.039) ppm.

In the residue decline study, residues of pethoxamid were below the LOQ (<0.01 ppm) in/on cotton undelinted seed samples from all sampling intervals; therefore, residue decline could not

be evaluated. Residues in/on cotton gin byproducts were too variable and low (all $\leq 2 \times \text{LOQ}$) to assess decline.

I. MATERIALS AND METHODS

A. MATERIALS

Table B.7.6.1.3-1. Nomenclature for Pethoxamid.	
Common name	Pethoxamid
Identity	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide
CAS registry number	106700-29-2
Company experimental name	TKC-94
	

B. Study Design

1. Test Procedure

A total of eleven field trials on cotton were conducted with a 5.0 lb ai/gal (600 g ai/L) EC formulation of pethoxamid during the 2014 growing season. Field trial locations by NAFTA growing zone are summarized in Table B.7.6.1.3-2.

All trials, except for those listed in the table below, were separated by ≥ 20 miles and are therefore considered independent (568_Criteria for Independence of Trials 4/23/13 (EPA and PMRA)). The trials separated by < 20 miles have been assessed for independence as detailed in the table below. HED has determined that two trials conducted in CA should be considered a single trial with replicate samples for purposes of 860.1500 data requirements.

Table B.7.6.1.1-1. Independent Trial Determination ¹		
Trial Nos. TCI-14-402-	Differences	Decision
11 and 12 (CA)	<u>Location:</u> Separated by 2 miles <u>Variety:</u> Same <u>Timing:</u> 1 st applications separated by 6 days; 2 nd applications separated by 14 days <u>Spray volume:</u> 16-18 gal/A vs. 5.1-5.3 gal/A	Replicates

¹ All assessments are based on the replicate trial guidance presented in draft memo 568_Criteria for Independence of Trials 4/23/13 (EPA and PMRA).

Table B.7.6.1.1-2. Trial Numbers and Geographical Locations.

Crop	No. Trials	NAFTA Growing Zone												Total
		1	2	3	4	5	6	7	8	9	10	11	12	
Cotton	Sub.		1		3		2 ¹		3		2 ²			11
	Req. ³		1/1		3/2		1/1		4/3		3/2			12/9

¹ The study author noted that one trial conducted in Zone 6 was ~25 miles from the border between Zones 6 and 8 and intended to represent both zones.

² HED has determined that two trials conducted in Zone 10 are replicates.

³ As per Table 5 of 860.1500 for cotton; the second number reflects a 25% reduction in the number of field trials allowed for the crop as a representative commodity in support of a crop group/subgroup tolerance as applicable (refer to Tables 2 and 3) or when application results in no quantifiable residues. Per OCSPP 860.1000 Supplement (6/30/08), for cotton gin byproducts, cotton must be harvested by commercial equipment to provide an adequate representation of plant residue for the ginning process; three field trials for harvesting of stripper cotton are needed (no data are needed for picker cotton).

Locations and detailed use patterns for the trials are provided in Table B.7.6.1.3-3. Preplant and preemergence applications were made to a variety of soil types: loamy sand (Trial 01), silt loam (Trials 02 and 04), fine sand (Trial 03), clay (Trials 05 and 06), clay loam (Trial 07), sandy loam (Trials 08, 09, and 11), silty clay loam (Trial 10), and loam (Trial 12).

Table B.7.6.1.1-3. Study Use Pattern.

Location: City, State; Year (Trial ID TCI-14-402-)	End-use Product ¹	Method of Application; Timing of Application	Volume (gal/A) [L/ha]	Rate per Application (lb ai/A) [kg ai/ha]	Retreatment Interval (days)	Total Rate (lb ai/A) [kg ai/ha]	Surfactant/ Adjuvant ²	Harvest Procedure ³
Chula, GA; 2014 (01)	5.0 lb ai/gal EC	1. Broadcast to soil; preplant, on day of planting	17 [159]	1.50 [1.68]	--	3.00 [3.36]	None	Hand picked
		2. Foliar broadcast; first white flower, BBCH 60	17 [159]	1.50 [1.68]	50		NIS @ 0.25% v/v	
Proctor, AR; 2014 (02)	5.0 lb ai/gal EC	1. Broadcast to soil; preemergence, on day of planting	12 [112]	1.50 [1.68]	--	3.00 [3.36]	None	Hand picked with shop vacuum
		2. Foliar broadcast; first white flower, BBCH 60	15 [140]	1.50 [1.68]	74		COC @ 1.0% v/v	
Fisk, MO; 2014 (03)	5.0 lb ai/gal EC	1. Broadcast to soil; preemergence, 6 days after planting	5.0 [47]	1.51 [1.69]	--	3.01 [3.37]	NIS @ 0.25% v/v	Hand picked
		2. Foliar broadcast; first white flower, BBCH 60	5.0 [47]	1.50 [1.68]	67		NIS @ 0.25% v/v	
Cheneyville, LA; 2014 (04)	5.0 lb ai/gal EC	1. Broadcast to soil; preplant, on day of planting	16 [150]	1.52 [1.70]	--	2.99 [3.35]	None	Hand Picked
		2. Foliar broadcast; first white flower, BBCH 60	15 [140]	1.47 [1.65]	63		COC @ 1.0% v/v	
Uvalde, TX; 2014 (05)	5.0 lb ai/gal EC	1. Broadcast to soil; preplant, on day of planting	16 [150]	1.48 [1.66]	--	2.98 [3.34]	None	Hand Picked
		2. Foliar broadcast; first white flower, BBCH 60	17 [159]	1.50 [1.68]	66		NIS @ 0.25% v/v	
Wall, TX; 2014 (06)	5.0 lb ai/gal EC	1. Broadcast to soil; preemergence, on day of planting	14 [131]	1.51 [1.69]	--	3.00 [3.36]	None	Stripper
		2. Foliar broadcast; first white flower, BBCH 60	14 [131]	1.49 [1.67]	65		COC @ 1.0% v/v	

Table B.7.6.1.1-3. Study Use Pattern.								
Location: City, State; Year (Trial ID TCI-14-402-)	End-use Product ¹	Method of Application; Timing of Application	Volume (gal/A) [L/ha]	Rate per Application (lb ai/A) [kg ai/ha]	Retreatment Interval (days)	Total Rate (lb ai/A) [kg ai/ha]	Surfactant/ Adjuvant ²	Harvest Procedure ³
Edmonson, TX; 2014 (07)	5.0 lb ai/gal EC	1. Broadcast to soil; preemergence, 1 day after planting	14 [131]	1.51 [1.69]	--	3.00 [3.36]	None	Stripper
		2. Foliar broadcast; first white flower, BBCH 60	14 [131]	1.49 [1.67]	66		NIS @ 0.25% v/v	
Hinton, OK; 2014 (08)	5.0 lb ai/gal EC	1. Broadcast to soil; preplant, on day of planting	4.8 [45]	1.43 [1.60]	--	2.96 [3.32]	None	Stripper
		2. Foliar broadcast; first white flower, BBCH 60	5.1 [48]	1.53 [1.71]	75		COC @ 1.0% v/v	
Levelland, TX; 2014 (09)	5.0 lb ai/gal EC	1. Broadcast to soil; preplant, on day of planting	15 [140]	1.50 [1.68]	--	3.00 [3.36]	NIS @ 0.25% v/v	Stripper
		2. Foliar broadcast; first white flower, BBCH 60	15 [140]	1.50 [1.68]	72		NIS @ 0.25% v/v	
Blythe, CA; 2014 (10)	5.0 lb ai/gal EC	1. Broadcast to soil; preemergence, 34 days after planting	15 [140]	1.48 [1.66]	--	2.96 [3.32]	None	Hand Picked
		2. Foliar broadcast; first white flower, BBCH 60	15 [140]	1.48 [1.66]	61		COC @ 1.0% v/v	
Porterville, CA; 2014 (11)	5.0 lb ai/gal EC	1. Broadcast to soil; preemergence; 1 day after planting	18 [168]	1.52 [1.70]	--	3.02 [3.39]	None	Hand Picked
		2. Foliar broadcast; first white flower, BBCH 60	16 [150]	1.50 [1.68]	76		NIS @ 0.25% v/v	
Porterville, CA; 2014 (12)	5.0 lb ai/gal EC	1. Broadcast to soil; preplant, on day of planting	5.1 [48]	1.49 [1.67]	--	3.05 [3.42]	None	Hand Picked
		2. Foliar broadcast; first white flower, BBCH 60	5.3 [50]	1.56 [1.75]	84		COC @ 1.0% v/v	

¹ A 5.0 lb ai/gal (600 g ai/L) EC formulation of pethoxamid.

² NIS = Nonionic surfactant; COC = Crop oil concentrate.

Cotton was grown and maintained using typical agricultural practices. Irrigation was used at all sites, except at Trials 02 and 04. Despite variations in weather conditions reported at some trial sites, no unusual weather conditions were reported to have adversely affected crop production or yield during the study, except at Trials 07 and 08, which were restarted due to heavy rains after the first application. At Trial 07, yields were slightly lower than normal due to the later planting date, and at Trial 08, yields and crop growth and development were normal. Phytotoxicity was observed after the first and/or second application (slow crop emergence or speckling of leaves) at Trials 01, 04, and 05, but the crops recovered and yields were normal.

Sample Handling and Preparation

Single control and duplicate treated samples of seed cotton were harvested at maturity by hand (Trials 01-05 and 10-12) or using stripper equipment (Trials 06-09) at a PHI of 67-185 days. On the day of harvest, seed cotton was ginned to produce undelinted cotton seed (all trials) and gin byproducts (at three stripper harvest equipment trials; Trials 06, 08, and 09). At one trial (06),

additional undelinted cotton seed and gin byproducts samples were collected 12 days before, and 7, 14, and 22 days after nominal harvest to assess residue decline. Samples were placed into frozen storage within 2.8 hours of collection and were stored frozen (temperatures not reported) at the field sites prior to shipment by ACDS freezer truck to the analytical laboratory, ABC Laboratories, Inc. (Columbia, MO). At the laboratory, samples were homogenized in the presence of dry ice and stored frozen (*ca.* -20 °C) until extraction for analysis.

2. Description of Analytical Procedures

Samples of cotton were analyzed for residues of pethoxamid using an LC/MS/MS method adapted from EAS Method No. AGR/MOA/PTX-8, as described in “Validation of an Analytical Method for the Determination of Pethoxamid Residues in Various Crops.” The method is the proposed enforcement method for crop commodities (refer to the DER for MRID 49813534, B.5.2.1.1).

Briefly, samples were extracted with acetonitrile (ACN). QuEChERS extraction salts (magnesium sulfate:sodium chloride:sodium citrate tribasic dihydrate:sodium citrate dibasic sesquihydrate; 4:1:1:0.5, w:w:w:w) were added, and samples were shaken and centrifuged. A portion of the supernatant was purified by the addition of cleanup salts (primary secondary amine:C18EC:magnesium sulfate; 1:1:6, w:w:w). Samples were shaken and centrifuged, and the supernatant was diluted with ACN:water (1:9, v:v) for analysis by LC/MS/MS. The method monitors the following ion transitions for pethoxamid: m/z 296 $\rightarrow$ 131 (quantitation) and m/z 296 $\rightarrow$ 250 (confirmation). The LOQ (determined as the LLMV) was 0.01 ppm for pethoxamid, and the estimated limit of detection (LOD) was 0.003 ppm (1/3 LOQ).

II. RESULTS AND DISCUSSION

Method performance was evaluated by use of method validation and concurrent recovery samples of cotton undelinted seed and gin byproducts fortified with pethoxamid at 0.01-0.5 ppm. Recoveries were generally within the acceptable range of 70-120%; therefore, the method was considered valid for the determination of pethoxamid residues in cotton matrices (Table B.7.6.1.3-4). It is noted that acceptable method validation recoveries were also obtained for cotton matrices using the confirmatory ion transition; these data are not presented herein. The fortification levels bracketed the measured residues. Concurrent recoveries were corrected for apparent residues in controls.

The detector response was linear (coefficient of determination, $r^2 \geq 0.9985$) within the range of 0.01-2 ng/mL. Representative chromatograms of control samples, fortified samples, and treated samples were provided. The control chromatograms generally had no peaks of interest above the chromatographic background. The fortified sample chromatograms contained only the analyte of interest, and peaks were symmetrical and well defined. Apparent residues in/on controls were nondetectable (<0.003 ppm), except in one undelinted seed untreated sample (Trial 10) which bore detectable residues of pethoxamid below the LOQ (0.008 ppm). Residues in the treated samples from the same trial were nondetectable.

Table B.7.6.1.3-4. Summary of Method Validation and Concurrent Recoveries of Pethoxamid from Cotton Matrices.				
Matrix	Fortification Level (ppm)	Sample Size (n)	Recoveries ¹ (%)	Mean ± Std. Dev. (%)
Method Validation				
Undelinted seed	0.01, 0.5	6	99-113	104 ± 5
Gin byproducts	0.01, 0.5	6	93-103	98 ± 4
Concurrent Recoveries				
Undelinted seed	0.01, 0.10	10	81-112; 121	103 ± 13
Gin byproducts	0.01, 0.10	4	95-103	100 ± 4

¹ Concurrent recoveries were corrected for apparent residues in controls.

The maximum storage intervals for samples between collection and extraction for analysis were 6.2 months for undelinted seed and 5.0 months for gin byproducts (Table B.7.6.1.3-5). Samples were analyzed within 4 days of extraction. Acceptable storage stability data were submitted indicating that residues of pethoxamid are stable during frozen storage (-20 °C) for 5.1 months in cotton undelinted seed and up to 10 months in gin byproducts, but residues in undelinted seed declined an average of 36% after 10 months of storage (refer to the DER for MRID 49813534, B.7.6.2). Although decline was observed in undelinted seed between the 5- and 10-month sampling intervals, acceptable recoveries would be expected at the maximum storage interval of samples from the subject study. In addition, residues were below the LOQ in/on all undelinted seed samples. These data are adequate to support the sample storage conditions and durations from the submitted study.

Table B.7.6.1.3-5. Summary of Storage Conditions.			
Matrix	Storage Temperature (°C)	Actual Storage Duration ¹	Interval of Demonstrated Storage Stability
Undelinted seed	≤-20 °C	76-188 days (2.5-6.2 months)	Residues of pethoxamid are stable during frozen storage (-20 °C) for 5.1 months in cotton undelinted seed, but declined 36% after 10 months of storage. ²
Gin byproducts		118-153 days (3.9-5.0 months)	Residues of pethoxamid are stable during frozen storage (-20 °C) for at least 10 months in gin byproducts. ²

¹ Interval from collection to extraction. Samples were analyzed within 0-4 days of extraction.

² Refer to the DER for MRID 49813534, B.7.6.2.

The results from the submitted field trials are presented in Table B.7.6.1.3-6 and summarized in Table B.7.6.1.3-7. Following a preplant or preemergence application and subsequent foliar application at a total rate of 2.96-3.05 lb ai/A (3.32-3.42 kg ai/ha), residues of pethoxamid were below the LOQ (<0.01 ppm) in/on all undelinted seed samples collected at a PHI of 67-185 days. In the corresponding gin byproduct samples, collected at three trials reflecting a PHI of 67-112 days, residues of pethoxamid were <0.01-0.047 ppm.

In the residue decline study, residues of pethoxamid were below the LOQ (<0.01 ppm) in/on cotton undelinted seed samples from all sampling intervals; therefore, residue decline could not be evaluated. Residues in/on gin byproducts were too low (all ≤2xLOQ) and variable to assess decline (Figure B.7.6.1.3-1).

Table B.7.6.1.3-6. Residue Data from Cotton Field Trials with Pethoxamid.¹						
Location: City, State; Year (Trial ID TCI-14-402-)	Zone	Crop Variety	Rate (lb ai/A) [kg ai/ha]	Matrix	PHI ² (days)	Residues ³ (ppm) [Average]
Chula, GA; 2014 (01)	2	FM 1944	3.00 [3.36]	Undelinted seed	84	ND, ND [<0.01]
Proctor, AR; 2014 (02)	4	ST4946	3.00 [3.36]	Undelinted seed	79	ND, ND [<0.01]
Fisk, MO; 2014 (03)	4	PHY 375 WRF	3.01 [3.37]	Undelinted seed	97	ND, ND [<0.01]
Cheneyville, LA; 2014 (04)	4	Phytogen 499	2.99 [3.35]	Undelinted seed	85	ND, ND [<0.01]
Uvalde, TX; 2014 (05)	6	Phytogen 499 WRF	2.98 [3.34]	Undelinted seed	88	ND, ND [<0.01]
Wall, TX; 2014 (06)	8	FM 2334GLT	3.00 [3.36]	Undelinted seed	100	ND, ND [<0.01]
					112	ND, ND [<0.01]
					119	ND, ND [<0.01]
					126	ND, ND [<0.01]
					134	ND, ND [<0.01]
				Gin byproducts	100	(0.009), 0.011 [<0.010]
					112	0.011, 0.018 [0.014]
					119	0.016, 0.024 [0.020]
					126	(0.006), (0.009) [<0.01]
					134	0.014, 0.023 [0.018]
Edmonson, TX; 2014 (07)	8	FM1944GLB2	3.00 [3.36]	Undelinted seed	100	ND, ND [<0.01]
Hinton, OK; 2014 (08)	6	FM 9160B2F	2.96 [3.32]	Undelinted seed	67	(0.004), ND [<0.01]
				Gin byproducts	67	0.047, 0.031 [0.039]
Levelland, TX; 2014 (09)	8	FM 9063B2F	3.00 [3.36]	Undelinted seed	106	ND, ND [<0.01]
				Gin byproducts	106	(0.006), (0.006) [<0.01]
Blythe, CA; 2014 (10)	10	D&PL 1359	2.96 [3.32]	Undelinted seed	185	ND, ND [<0.01]
Porterville, CA; 2014 (11) and Porterville, CA; 2014 (12)	10	PHY 339	3.02 [3.39]; 3.05 [3.42]	Undelinted seed	96; 95	ND, ND, ND, ND [<0.01]

¹ A 5.0 lb ai/gal (600 g ai/L) EC formulation of pethoxamid was used.

² The target PHI is **bolded** for the decline study.

³ ND = Not detected (<LOD). The LOD was 0.003 ppm and the LOQ was 0.01 ppm. Values between the LOD and LOQ are reported in parentheses. Per-trial averages were calculated by the study reviewer using the LOQ for all residues reported as <LOQ.

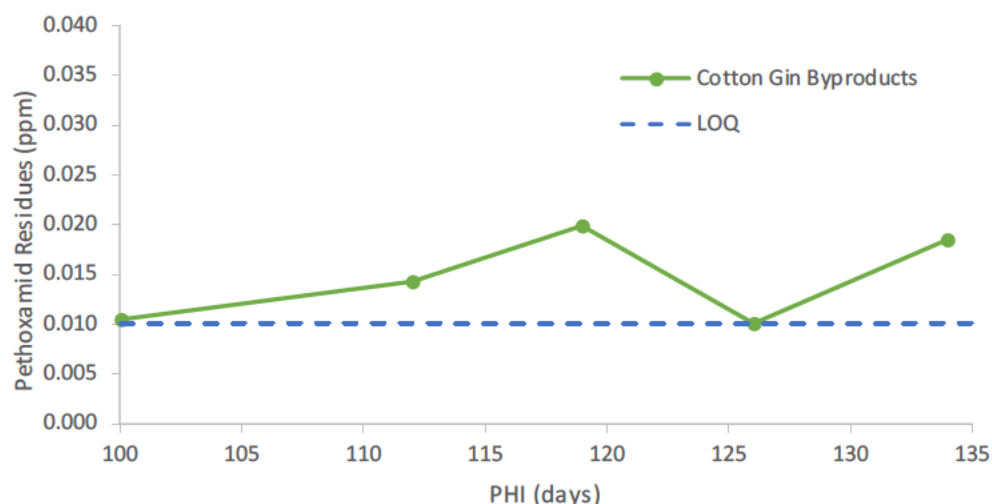
Table B.7.6.1.3-7. Summary of Residues from Cotton Field Trials with Pethoxamid.										
Crop Matrix	Total Application Rate (lb ai/A) [kg ai/ha]	PHI (days)	n ¹	Pethoxamid Residues (ppm)						
				Min. ²	Max. ²	LAFT ³	HAFT ³	Median ³	Mean ³	SD ³
Cotton undelinted seed	2.96-3.05 [3.32-3.42]	67-185	11	<0.01	<0.01	<0.01	<0.01	0.01	0.01	N/A
Gin byproducts	2.96-3.00 [3.32-3.36]	67-112	3	<0.01	0.047	<0.01	0.039	0.014	0.021	0.016

¹ n = number of field trials.

² Values based on residues in individual samples.

³ Values based on per-trial averages. LAFT = lowest average field trial, HAFT = highest average field trial, SD = standard deviation. For computation of the LAFT, HAFT, median, mean, and standard deviation, values <LOQ are assumed to be at the LOQ (0.01 ppm). N/A = Not applicable.

Figure B.7.6.1.3-1. Residues of Pethoxamid in/on Cotton Gin Byproducts at Various PHIs.



III. CONCLUSIONS

The cotton field trials are considered scientifically acceptable. Following a preplant or preemergence application and subsequent foliar application at a total rate of 2.96-3.05 lb ai/A (3.32-3.42 kg ai/ha), residues of pethoxamid were below the LOQ (<0.01 ppm) in/on all undelinted seed samples collected at a PHI of 67-185 days. In the corresponding gin byproduct samples, collected at three trials reflecting a PHI of 67-112 days, residues of pethoxamid were <0.01-0.047 ppm.

In the residue decline study, residues of pethoxamid were below the LOQ (<0.01 ppm) in/on cotton undelinted seed samples from all sampling intervals; therefore, residue decline could not be evaluated. Residues in/on cotton gin byproducts were too variable and low (all $\leq 2 \times \text{LOQ}$) to assess decline.

An acceptable method was used for residue quantitation, and adequate storage stability data were submitted to support sample storage durations and conditions.

REFERENCES

Document ID: MRID 49813534; PMRA Study # xxxxxx, PMRA Review # xxxxxx
Report: Sears, K. (2015) Method Validation and 10-Month Storage Stability of Pethoxamid in Various Crops. Study Numbers: 1495 PXA; 2793. Unpublished study prepared by Pyxant Labs Inc., sponsored by Cheminova A/S, and submitted by FMC Corporation. 600 p.

B.7.6 Residues Resulting from Supervised Trials (Annex IIA 6.3; Annex IIIA 8.3)

B.7.6.2 Stability of Residues Prior to Analysis

B.7.6.2.1 Corn, Cotton, and Soybean Matrices

Document ID: MRID No. 49813534
PMRA No. Not Available

Report: Sears, K. (2015) Method Validation and 10-Month Storage Stability of Pethoxamid in Various Crops. Study Numbers: 1495 PXA; 2793. Unpublished study prepared by Pyxant Labs Inc., sponsored by Cheminova A/S, and submitted by FMC Corporation. 600 p.

Guidelines: EPA OCSPP Harmonized Test Guideline 860.1380 Storage Stability Data (August 1996)
PMRA Regulatory Directive DIR98-02 – Residue Chemistry Guidelines, Section 5 – Storage Stability Data
OECD Guideline 506 Stability of Pesticide Residues in Stored Commodities (October 2007)

GLP Compliance: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Acceptability: The study is considered scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP#452109.

Evaluator: Sheila Piper, Chemist, HED, RAB6

Secondary Evaluator: Robert Lanthier, PMRA

Note: This Data Evaluation Record (DER) was originally prepared under contract by CDM/CSS-Dynamac Joint Venture (submitted 2/16/18). The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies. The DER has been reviewed by HED and revised as necessary to reflect current Office of Pesticide Programs (OPP) policies.

EXECUTIVE SUMMARY

Samples of untreated sweet corn kernel plus cob with husk removed (K+CWHR), corn flour, corn meal, corn refined oil, cotton gin byproducts, cotton undelinted seed, cotton hulls, cotton meal, soybean hulls, and soybean meal were fortified with pethoxamid at 0.1 ppm and were placed in frozen storage at ≤ -20 °C. Stored samples and freshly fortified samples were analyzed for residues of pethoxamid at nominal intervals of 0, 1, 3, 5, and 10 months. The study was conducted by Pyxant Labs Inc. (Colorado Springs, CO). The submission also includes method validation data for the proposed enforcement method for plant commodities, Eurofins Agrosience Services (EAS) Method No. AGR/MOA/PTX-8 (refer to the B.5.2.1.1 DER part 1 for MRID 49813534).

At each storage interval, pethoxamid was determined using a high performance liquid chromatography method with tandem mass spectrometry detection (LC/MS/MS), EAS Method No. AGR/MOA/PTX-8, the proposed enforcement method for crop commodities. The limit of

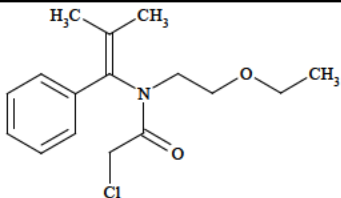
quantitation (LOQ; determined as the lowest level of method validation, LLMV, in the associated method validation study) was 0.01 ppm for pethoxamid in all matrices. Acceptable concurrent recoveries were obtained from samples of sweet corn K+CWHR, corn flour, corn meal, corn refined oil, cotton gin byproducts, cotton undelinted seed, cotton hulls, cotton meal, soybean hulls, and soybean meal fortified with pethoxamid at 0.1 ppm.

The results of the study showed that residues of pethoxamid are stable for at least 10 months in sweet corn K+CWHR, corn flour, corn meal, corn refined oil, cotton gin byproducts, cotton hulls, cotton meal, soybean hulls, and soybean meal stored at ≤ -20 °C. Residues of pethoxamid in cotton undelinted seed were stable for 5.1 months, but declined at the 10-month storage interval (61% corrected average recovery). To confirm the 10-month results, additional samples were analyzed following 10.3 months storage. The corrected average recovery of 67% in these samples was similar to the 10-month recovery, indicating a decline of an average of 36% in undelinted cotton seed after 10 months frozen storage.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

Table B.7.6.2.1-1. Nomenclature for Pethoxamid.	
Common name	Pethoxamid
Identity	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide
CAS registry number	106700-29-2
Company experimental name	TKC-94
	

2. Test Commodity

Matrix: Sweet corn K+CWHR, corn flour, corn meal, corn refined oil, cotton gin byproducts, cotton undelinted seed, cotton hulls, cotton meal, soybean hulls, and soybean meal

B. STUDY DESIGN

1. Test Procedure

Untreated samples of sweet corn K+CWHR, corn flour, corn meal, corn refined oil, cotton gin byproducts, cotton undelinted seed, cotton hulls, cotton meal, soybean hulls, and soybean meal were acquired from the study sponsor or purchased. The samples were transferred from freezer storage and homogenized with dry ice using a Robot Coupe blender, Hobart food cutter, or

coffee grinder. The samples were returned to freezer storage after processing and left to allow dissipation of the dry ice. Homogenized samples were transferred to centrifuge tubes and fortified with pethoxamid in acetonitrile (ACN):water (90:10, v:v) at 0.1 ppm; two fortified samples for each storage interval plus contingency fortified samples were prepared. The fortified samples were placed in frozen storage (*ca.* ≤ -20 °C). Unfortified samples for control and fresh fortification samples were stored frozen along with the fortified samples. Samples were analyzed following approximately 0, 1, 3, 5, and 10 months of frozen storage.

Each analysis set, except 0-day, included one reagent blank, one control, two fresh fortification samples, and two stored fortified samples. The 0-day analysis set included one reagent blank, one control, and three freshly fortified samples.

2. Description of Analytical Procedures

Samples were analyzed for residues of pethoxamid using an LC/MS/MS method, EAS Method No. AGR/MOA/PTX-8, as described in “Validation of an Analytical Method for the Determination of Pethoxamid Residues in Various Crops.” The method is the proposed enforcement method for crop commodities (refer to the B.5.2.1.1 DER for MRID 49813534).

Briefly, samples were extracted with ACN. QuEChERS extraction salts (magnesium sulfate:sodium chloride:sodium citrate tribasic dihydrate:sodium citrate dibasic sesquihydrate; 4:1:1:0.5, w:w:w:w) were added, and samples were shaken and centrifuged. A portion of the supernatant was purified by the addition of cleanup salts (primary secondary amine:C18EC:magnesium sulfate; 1:1:6, w:w:w). Samples were shaken and centrifuged, and the supernatant was diluted with water for analysis by LC/MS/MS. The method monitors the following ion transitions for pethoxamid: m/z 296.2 $\rightarrow$ 131.2 (quantitation) and m/z 296.2 $\rightarrow$ 250.2 (confirmation). The LOQ (determined as the LLMV in the associated method validation study) was 0.01 ppm for pethoxamid for all matrices, and the estimated limit of detection was 0.003 ppm (1/3 LOQ).

II. RESULTS AND DISCUSSION

Method performance was evaluated by use of concurrent recovery samples of sweet corn K+CWHR, corn flour, corn meal, corn refined oil, cotton gin byproducts, cotton undelinted seed, cotton hulls, cotton meal, soybean hulls, and soybean meal fortified with pethoxamid at 0.1 ppm. The 0-day recoveries represent method validation recoveries. All recoveries were generally within the acceptable range of 70-120%; therefore, the method was considered valid for the determination of pethoxamid residues in corn, cotton, and soybean matrices (Table B.7.6.2.1-2). Concurrent recoveries were used to correct the residues in the stored samples.

The detector response was linear (coefficient of determination, $r^2 \geq 0.9807$) within the range of 0.1-10.0 ng/mL. Representative chromatograms of control and fortified samples were provided. The control chromatograms generally had no peaks of interest above the chromatographic background. The fortified sample chromatograms contained only the analyte of interest, and peaks were symmetrical and well defined. Residues in controls were nondetectable (<0.003 ppm).

Table B.7.6.2.1-2. Summary of Concurrent Recoveries of Pethoxamid from Corn, Cotton, and Soybean Matrices.				
Matrix	Fortification Level (ppm)	Storage Interval (months)	Recoveries ¹ (%)	Mean $\pm$ Std. Dev. ² (%)
Sweet corn K+CWHR	0.10	0	98, 102, 107	102 $\pm$ 5
		1.0	119, 121	120
		3.2	88, 90	89
		5.1	89, 103	96
		10.0	100, 101	101
Corn flour	0.10	0	102, 102, 120	108 $\pm$ 10
		1.0	109, 115	112
		3.2	94, 95	94
		5.1	94, 97	96
		10.0	94, 96	95
Corn meal	0.10	0	101, 102, 103	102 $\pm$ 1
		1.0	99, 99	99
		3.2	92, 94	93
		5.1	105, 105	105
		10.0	94, 97	95
Corn refined oil	0.10	0	90, 91, 91	91 $\pm$ 1
		1.0	93, 98	96
		3.2	86, 88	87
		5.1	113, 115	114
		10.0	80, 85	82
Cotton gin byproducts	0.10	0	103, 106, 112	107 $\pm$ 5
		1.0	118, 119	119
		3.2	101, 109	105
		5.1	87, 100	94
		10.0	103, 108	106
Cotton undelinted seed	0.10	0	97, 101, 106	101 $\pm$ 5
		1.0	85, 88	87
		3.2	87, 87	87
		5.1	74, 93	83
		10.0	87, 94	91
		10.3	72, 99	85
Cotton hulls	0.10	0	90, 96, 100	95 $\pm$ 5
		1.0	98, 104	101
		3.2	99, 114	106
		5.1	106, 111	109
		10.0	103, 105	104
Cotton meal	0.10	0	88, 95, 99	94 $\pm$ 6
		1.0	111, 122	117
		3.2	90, 98	94
		5.1	90, 106	98
		10.0	91, 94	92
Soybean hulls	0.10	0	91, 97, 97	95 $\pm$ 3
		1.0	99, 104	101
		3.2	97, 98	98
		5.1	103, 113	108
		10.0	97, 102	100

Table B.7.6.2.1-2. Summary of Concurrent Recoveries of Pethoxamid from Corn, Cotton, and Soybean Matrices.				
Matrix	Fortification Level (ppm)	Storage Interval (months)	Recoveries ¹ (%)	Mean $\pm$ Std. Dev. ² (%)
Soybean meal	0.10	0	95, 99, 113	102 $\pm$ 9
		1.0	90, 106	98
		3.2	85, 90	88
		5.1	108, 113	111
		10.0	89, 96	92

¹ Concurrent recoveries were corrected for any apparent residues in controls.

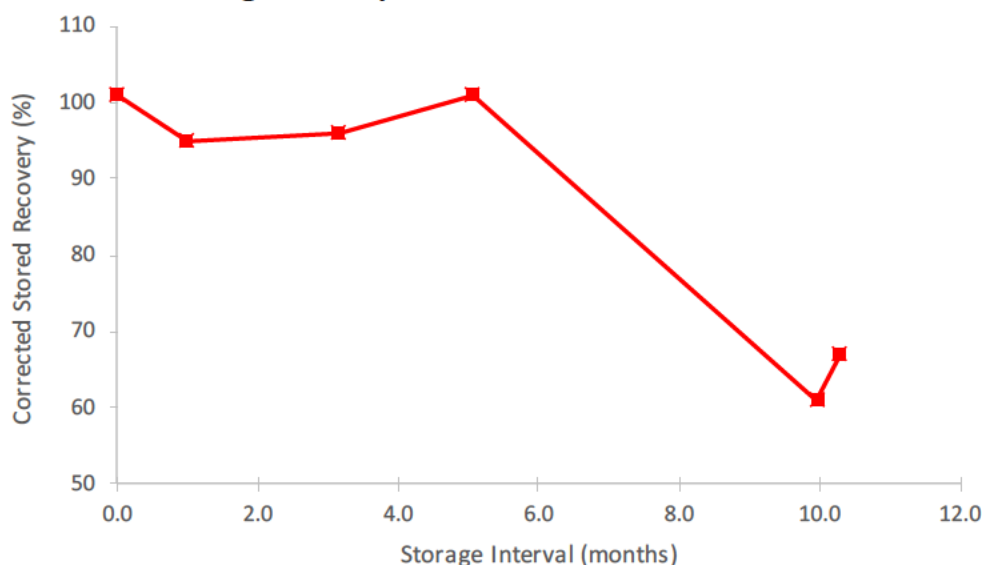
² Standard deviation is not calculated for sample sizes <3.

The results of the storage stability study are presented in Table B.7.6.2.1-3. Pethoxamid residues were stable in all tested matrices under frozen storage for up to 10 months, except for cotton undelinted seed. Residues in undelinted seed were stable for 5.1 months, but declined at the 10-month storage interval (61% corrected average recovery). To confirm the 10-month results, the contingency samples were analyzed following 10.3 months of storage; the corrected average recovery of 67% in these samples was similar to the 10-month recoveries, indicating a decline of an average of 36% in undelinted cotton seed after 10 months frozen storage. The storage stability of pethoxamid residues in cotton undelinted seed is presented graphically in Figure B.7.6.2.1-1.

Table B.7.6.2.1-3. Stability of Pethoxamid Residues in Corn, Cotton, and Soybean Matrices During Storage at $\leq 20^{\circ}\text{C}$.						
Commodity	Storage Duration, days (months)	Fortification Level (ppm)	Recovered Residues (ppm)	Mean Recovery (%)	Mean Concurrent Recovery (%)	Corrected Recovery ¹ (%)
Sweet corn K+CWHR	0	0.10	0.0978, 0.102, 0.107	102	--	--
	31 (1.0)		0.102, 0.113	108	120	90
	97 (3.2)		0.0792, 0.0858	83	89	93
	155 (5.1)		0.0889, 0.0903	90	96	93
	303 (10.0)		0.0927, 0.0953	94	101	93
Corn flour	0	0.10	0.102, 0.102, 0.120	108	--	--
	31 (1.0)		0.0974, 0.113	105	112	94
	97 (3.2)		0.0826, 0.0862	84	94	89
	155 (5.1)		0.0876, 0.0923	90	96	94
	303 (10.0)		0.0915, 0.0941	93	95	98
Corn meal	0	0.10	0.101, 0.102, 0.103	102	--	--
	31 (1.0)		0.0918, 0.102	97	99	98
	97 (3.2)		0.0823, 0.0836	83	93	89
	155 (5.1)		0.0901, 0.0936	92	105	87
	303 (10.0)		0.0916, 0.0991	95	95	100
Corn refined oil	0	0.10	0.0904, 0.0905, 0.0909	91	--	--
	31 (1.0)		0.0904, 0.0910	91	96	95
	97 (3.2)		0.0799, 0.0811	81	87	92
	155 (5.1)		0.0876, 0.0905	89	114	78
	303 (10.0)		0.0878, 0.0882	88	82	107

Table B.7.6.2.1-3. Stability of Pethoxamid Residues in Corn, Cotton, and Soybean Matrices During Storage at ≤20 °C.						
Commodity	Storage Duration, days (months)	Fortification Level (ppm)	Recovered Residues (ppm)	Mean Recovery (%)	Mean Concurrent Recovery (%)	Corrected Recovery ¹ (%)
Cotton gin byproducts	0	0.10	0.103, 0.106, 0.112	107	--	--
	30 (1.0)		0.0906, 0.103	97	119	82
	96 (3.2)		0.0930, 0.0991	96	105	91
	154 (5.1)		0.0849, 0.0988	92	94	98
	303 (10.0)		0.109, 0.115	112	106	106
Cotton undelinted seed	0	0.10	0.0971, 0.101, 0.106	101	--	--
	30 (1.0)		0.0811, 0.0826	82	87	95
	96 (3.2)		0.0809, 0.0851	83	87	96
	154 (5.1)		0.0826, 0.0859	84	83	101
	303 (10.0)		0.0460, 0.0643	55	91	61
	313 (10.3)		0.0489, 0.0646	57	85	67
Cotton hulls	0	0.10	0.0898, 0.0957, 0.100	95	--	--
	30 (1.0)		0.0939, 0.102	98	101	97
	96 (3.2)		0.0821, 0.0838	83	106	78
	154 (5.1)		0.0791, 0.0832	81	109	75
	303 (10.0)		0.0766, 0.0845	81	104	77
Cotton meal	0	0.10	0.0876, 0.0946, 0.0994	94	--	--
	30 (1.0)		0.103, 0.105	104	117	89
	96 (3.2)		0.0827, 0.0830	83	94	88
	154 (5.1)		0.0900, 0.0947	92	98	94
	303 (10.0)		0.0865, 0.0877	87	92	94
Soybean hulls	0	0.10	0.0912, 0.0969, 0.0973	95	--	--
	30 (1.0)		0.0894, 0.0897	90	101	88
	96 (3.2)		0.0859, 0.0900	88	98	90
	154 (5.1)		0.0851, 0.0876	86	108	80
	303 (10.0)		0.0874, 0.0918	90	100	90
Soybean meal	0	0.10	0.0947, 0.0991, 0.113	102	--	--
	31 (1.0)		0.0764, 0.0780	77	98	79
	97 (3.2)		0.0714, 0.0762	74	88	84
	155 (5.1)		0.0912, 0.0926	92	111	83
	303 (10.0)		0.0797, 0.0781	79	92	86

¹ Corrected for concurrent recovery by study author.

Figure B.7.6.2.1-1. Storage Stability of Pethoxamid Residues in Cotton Undelinted Seed.

III. CONCLUSIONS

The storage stability study with corn, cotton, and soybean matrices is considered scientifically acceptable. The results of the study showed that residues of pethoxamid are stable for at least 10 months in sweet corn K+CWHR, corn flour, corn meal, corn refined oil, cotton gin byproducts, cotton hulls, cotton meal, soybean hulls, and soybean meal stored at $\leq -20^{\circ}\text{C}$. Residues of pethoxamid in cotton undelinted seed were stable for 5.1 months, but declined at the 10-month storage interval (61% corrected average recovery). To confirm the 10-month results, the contingency samples were analyzed following 10.3 months of storage; the corrected average recovery of 67% in these samples was similar to the 10-month recovery, indicating a decline of an average of 36% in undelinted cotton seed after 10 months frozen storage.

REFERENCES

Document ID: MRID 49813534
Report: Bitter, J. (2017) S-2399: Freezer Storage Stability of S-2399 and Metabolites in Processed Fractions. Report No. 201700305; Laboratory Project ID VP-40066. Unpublished study sponsored by Valent USA LLC. 836 p.

**B.7.7 Effects of Industrial Processing and/or Household Preparation
(Annex IIA 6.5; Annex IIIA 8.5)**

**B.7.7.1 Nature of the Residue
(Annex IIA 6.5.1; Annex IIIA 8.5.1)**

**B.7.7.2 Distribution of the Residue in Peel/Pulp
(Annex IIA 6.5.2; Annex IIIA 8.5.2)**

**B.7.7.3 Magnitude of Residues on Set of Representative Processes
(Annex IIA 6.5.3; Annex IIIA 8.5.3)**

B.7.7.3.1 Cotton

Document ID: MRID No. 49813548

PMRA No. Not Available

Report: Carringer, S.J. (2015) Magnitude and Decline of Pethoxamid Residues in/on Cotton Raw Agricultural and Processed Commodities Following One Pre-plant or Pre-emergence Application and One Post-emergence Application of Pethoxamid 600 g/L EC (2014). Study Numbers: 1506 PXA; TCI-14-402; 81558. Unpublished study prepared by The Carringers, Inc., sponsored by Cheminova A/S, and submitted by FMC Corporation. 418 p.

Guidelines: EPA OCSPP Harmonized Test Guideline 860.1520 Processed Food/Feed (August 1996)
PMRA Regulatory Directive DIR98-02 – Residue Chemistry Guidelines, Section 10 – Processed Food/Feed
OECD Guideline 508 Magnitude of the Pesticide Residues in Processed Commodities (October 2008)

GLP Compliance: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Acceptability: The study is considered scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP#452109.

Evaluator: Sheila Piper, Chemist, HED, RAB6 *Sheila Piper*

Secondary Evaluator: Robert Lanthier, PMRA

Note: This Data Evaluation Record (DER) was prepared under contract by CDM/CSS-Dynamac Joint Venture (submitted 2/16/18). The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies. The DER has been reviewed by HED and revised as necessary to reflect current Office of Pesticide Programs (OPP) policies.

EXECUTIVE SUMMARY

A cotton field trial for pethoxamid was conducted in TX during the 2014 growing season. This submission also includes data for cotton field trials (refer to the B.7.6.1.3 DER for MRID 49813548).

At the trial, the treated plot received a preplant application to the soil followed by a foliar broadcast application at the first white flower growth stage (BBCH 60). Applications were made with a 5.0 lb ai/gal (600 g ai/L) emulsifiable concentrate (EC) formulation of pethoxamid at 7.47-7.50 lb ai/A/application (8.37-8.41 kg ai/ha/application), with a 66-day retreatment interval, for a total seasonal rate of 15.0 lb ai/A (16.8 kg ai/ha; ~5x the corresponding field trial rates). Applications were made using ground equipment in spray volumes of 16-17 gal/A (150-159 L/ha). A nonionic surfactant was added to the spray mixture for the foliar application. Single bulk samples of seed cotton were harvested by hand at a preharvest interval (PHI) of 88 days and ginned to produce undelinted seed, which was processed into meal, hulls, and refined, bleached, and deodorized (RBD) oil using simulated commercial practices at the processing facility, GLP Technologies (Navasota, TX). Adequate descriptions of the processing procedures were provided, including material balance summaries.

Ginned samples were frozen at the field facility and shipped frozen to the processing facility, then stored frozen prior to processing which was completed within 6.0 months of harvest. Following processing, samples were maintained frozen at the processor, during shipping to the laboratory, and at the analytical laboratory prior to analysis. The maximum storage intervals between collection and extraction for analysis were 6.4 months for undelinted seed, <1 month for hulls and oil, and 2.5 months for meal. Samples were analyzed within 1 day of extraction. Acceptable storage stability data were submitted indicating that residues of pethoxamid are stable during frozen storage (-20 °C) for up to 10 months in corn refined oil, cotton hulls, and cotton meal, and for 5.1 months in cotton undelinted seed (refer to the MRID 49813534). These data are adequate to support the sample storage conditions and durations from the submitted study.

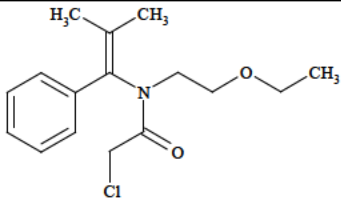
Samples were analyzed for residues of pethoxamid using a high performance liquid chromatography method with tandem mass spectrometry detection (LC/MS/MS), adapted from Eurofins Agrosience Services (EAS) Method No. AGR/MOA/PTX-8, the proposed enforcement method for crop commodities (refer to the DER for MRID 49813534, B.5.2.1.1). The limit of quantitation (LOQ; determined as the lowest level of method validation, LLMV) was 0.01 ppm for pethoxamid in all cotton matrices. Acceptable method validation and concurrent recoveries were obtained from samples of undelinted seed, meal, hulls, and RBD oil fortified with pethoxamid at 0.01-0.5 ppm. The fortification levels were adequate to bracket measured residue levels.

Following a preplant application to the soil and subsequent foliar application at exaggerated rates (~5x), average residues of pethoxamid were 0.016 ppm (just above the LOQ) in/on undelinted seed (RAC) collected at the field and below the LOQ (<0.01 ppm) in/on undelinted seed collected just prior to processing. Residues in/on the processed fractions (hulls, meal, and RBD oil) were below the LOQ (<0.01 ppm); therefore, processing factors could not be calculated.

The theoretical concentration factors for cotton are 3.8x for hulls, 2.2x for meal, and 6.3x for oil (based on separation into components; OCSPP 860.1520, Table 3).

I. MATERIALS AND METHODS

A. MATERIALS

Table B.7.7.3.1-1. Nomenclature for Pethoxamid.	
Common name	Pethoxamid
Identity	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide
CAS registry number	106700-29-2
Molecular weight	295.80
Company experimental name	TKC-94
	

B. Study Design

1. Test Procedure

The location and detailed use pattern information for the trial is provided in Table B.7.7.3.1-2.

Table B.7.7.3.1-2. Study Use Pattern.							
Location: City, State; Year (Trial ID)	End-use Product ¹	Method of Application; Timing of Application	Volume (gal/A) [L/ha]	Rate per Application (lb ai/A) [kg ai/ha]	Retreatment Interval (days)	Total Rate (lb ai/A) [kg ai/ha]	Surfactant/ Adjuvant ²
Uvalde, TX; 2014 (TCI-14-402-05)	5.0 lb ai/gal EC	1. Broadcast to soil; preplant, on day of planting	16 [150]	7.47 [8.37]	--	15.0 [16.8]	None
		2. Foliar broadcast; first white flower, BBCH 60	17 [159]	7.50 [8.41]	66		NIS @ 0.25% v/v

¹ A 5.0 lb ai/gal (600 g ai/L) EC formulation of pethoxamid.

² NIS = Nonionic surfactant.

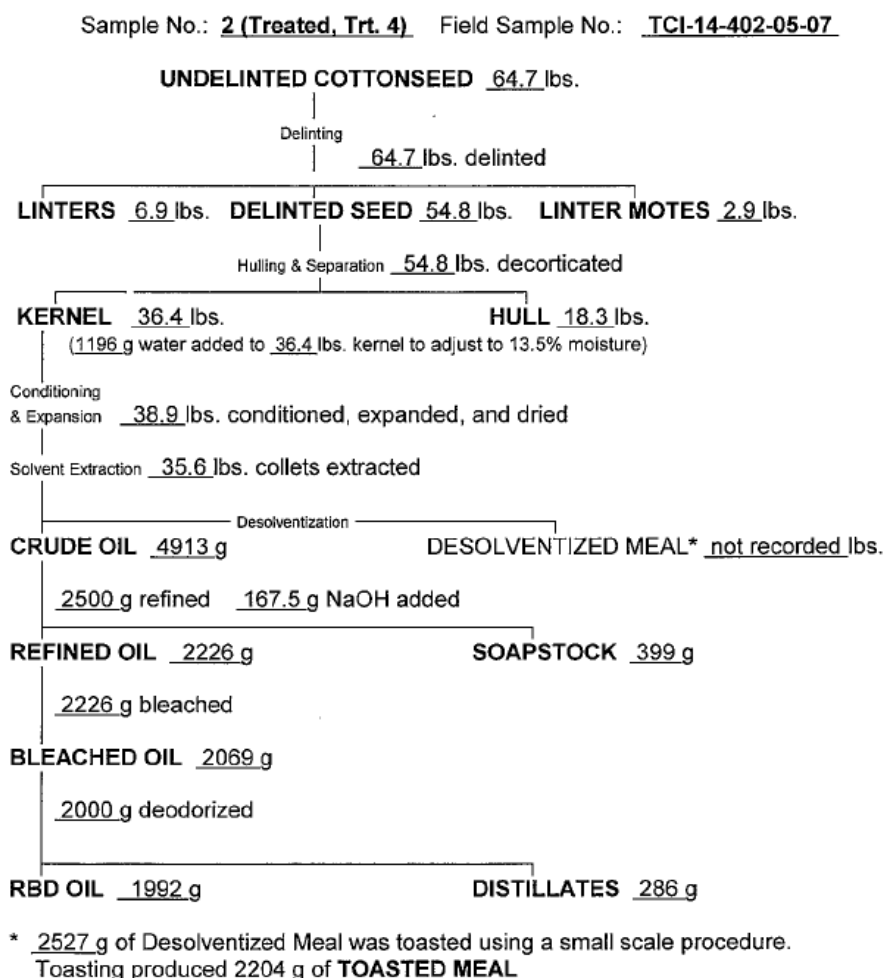
Sample Handling and Preparation

Single bulk samples of untreated (47.6 kg) and treated (32.2 kg) seed cotton were harvested by hand at maturity, at a PHI of 88 days. In addition, single untreated and duplicate treated samples (1.0 kg each) of seed cotton were collected in conjunction with the field trial study. Samples were ginned to produce undelinted seed on the day of harvest then stored frozen (temperatures not reported) prior to shipment by freezer truck to the analytical laboratory. ABC Laboratories, Inc. (Columbia, MO; field trial samples) or the processing facility, GLP Technologies (Navasota, TX; bulk samples). At the processing facility, samples were stored frozen (≤ -12 °C) prior to and following processing into hulls, meal, and RBD oil using simulated commercial practices. Processing was completed within 183 days of harvest. RAC and processed fractions were shipped on dry ice by overnight courier to the analytical laboratory. At the laboratory, samples were homogenized in the presence of dry ice and stored frozen (*ca.* -20 °C) until extraction for analysis; oil samples did not require homogenization.

Sample Processing

Undelinted cotton seed was processed using batch processing procedures to produce the required processed commodities of meal (toasted meal), hulls, and refined (RBD) oil. Adequate descriptions of the processing procedures were provided, including material balance summaries. A flowchart for undelinted cotton seed processing procedures is presented in Figure B.7.7.3.1-1. Single untreated and duplicate treated samples of the RAC and the processed fractions were collected at the processing facility.

Figure B.7.7.3.1-1. Cotton Processing Flowchart.



2. Description of Analytical Procedures

Samples of cotton were analyzed for residues of pethoxamid using a LC/MS/MS method adapted from EAS Method No. AGR/MOA/PTX-8, as described in "Validation of an Analytical Method for the Determination of Pethoxamid Residues in Various Crops." The method is the proposed enforcement method for crop commodities (refer to the DER for MRID 49813534, B.5.2.1.1).

Briefly, samples were extracted with acetonitrile (ACN). QuEChERS extraction salts (magnesium sulfate:sodium chloride:sodium citrate tribasic dihydrate:sodium citrate dibasic sesquihydrate; 4:1:1:0.5, w:w:w:w) were added, and samples were shaken and centrifuged. A portion of the supernatant was purified by the addition of cleanup salts (primary secondary amine:C18EC:magnesium sulfate; 1:1:6, w:w:w). Samples were shaken and centrifuged, and the supernatant was diluted with ACN:water (1:9, v:v) for analysis by LC/MS/MS. The method monitors the following ion transitions for pethoxamid: m/z 296 $\rightarrow$ 131 (quantitation) and m/z 296 $\rightarrow$ 250 (confirmation). The LOQ (determined as the LLMV) for each cotton matrix was 0.01 ppm for pethoxamid, and the estimated limit of detection (LOD) was 0.003 ppm (1/3 LOQ).

II. RESULTS AND DISCUSSION

Method performance was evaluated by use of method validation and concurrent recovery samples of undelinted seed fortified with pethoxamid at 0.01-0.5 ppm, and meal, hulls, and RBD oil fortified at 0.01 and 0.5 ppm. Recoveries were generally within the acceptable range of 70-120%; therefore, the method was considered valid for the determination of pethoxamid residues in cotton matrices (Table B.7.7.3.1-3). It is noted that acceptable method validation recoveries were also obtained for cotton matrices using the confirmatory ion transition; these data are not presented herein. The fortification levels bracketed the measured residues. Concurrent recoveries were corrected for any apparent residues in controls.

The detector response was linear (coefficient of determination, $r^2 \geq 0.9974$) within the range of 0.01-2.0 ng/mL. Representative chromatograms of control samples, fortified samples, and treated samples were provided. The control chromatograms generally had no peaks of interest above the chromatographic background. The fortified sample chromatograms contained only the analyte of interest, and peaks were symmetrical and well defined. Apparent residues in controls were nondetectable (<0.003 ppm).

Table B.7.7.3.1-3. Summary of Method Validation and Concurrent Recoveries of Pethoxamid from Cotton Matrices.				
Matrix	Fortification Level (ppm)	Sample Size (n)	Recoveries ¹ (%)	Mean $\pm$ Std. Dev. ² (%)
Method Validation				
Undelinted seed	0.01, 0.5	6	99-113	104 $\pm$ 5
Hulls	0.01, 0.5	6	83-104	93 $\pm$ 10
Meal	0.01, 0.5	6	104-110	107 $\pm$ 2
RBD oil	0.01, 0.5	6	94-100	97 $\pm$ 2
Concurrent Recoveries				
Undelinted seed	0.01, 0.1	4	85-112; 121	103 $\pm$ 17
Hulls	0.01, 0.5	2	99, 99	99
Meal	0.01, 0.5	2	97, 103	100
RBD oil	0.01, 0.5	2	95, 100	98

¹ Concurrent recoveries were corrected for any apparent residues in controls.

² Standard deviation is not calculated for sample sizes <3.

Processing was completed within 6.0 months of harvest. The maximum storage intervals between collection and extraction for analysis were 6.4 months for undelinted seed, <1 month for hulls and oil, and 2.5 months for meal (Table B.7.7.3.1-4). Samples were analyzed within 1 day of extraction. Acceptable storage stability data were submitted indicating that residues of

pethoxamid are stable during frozen storage (-20 °C) for up to 10 months in corn refined oil, cotton hulls, and cotton meal, and for 5.1 months in cotton undelinted seed; residues of pethoxamid in cotton undelinted seed declined an average of 36% after 10 months of storage (refer to the DER for MRID 49813534, B.7.6.2). Although decline was observed in undelinted seed between the 5- and 10-month sampling intervals, acceptable recoveries would be expected at the maximum storage interval of samples from the subject study. These data are adequate to support the sample storage conditions and durations from the submitted study.

Table B.7.7.3.1-4. Summary of Storage Conditions.

Matrix	Storage Temperature (°C)	Actual Storage Duration ¹	Interval of Demonstrated Storage Stability
Undelinted seed	≤-20 °C	126-194 days (4.1-6.4 months)	Residues of pethoxamid are stable during frozen storage (-20 °C) for 5.1 months in cotton undelinted seed, but declined 36% after 10 months of storage. ²
Hulls		25 days (0.8 months)	Residues of pethoxamid are stable during frozen storage (-20 °C) for up to 10 months in corn refined oil, cotton hulls, and cotton meal. ²
Meal		75 days (2.5 months)	
RBD oil		24 days (0.8 months)	

¹ Interval from collection to extraction. Samples were analyzed within 0-1 days of extraction.

² Refer to the DER for MRID 49813534, B.7.6.2.

The results from the processing study are presented in Table B.7.7.3.1-5. Average pethoxamid residues in/on undelinted cotton seed collected at the field were 0.016 ppm, and residues in/on undelinted seed collected prior to processing were nondetectable (<0.003 ppm). The study author stated that the cause of the apparent decline in residue levels in/on the RAC sample from the field to the processing facility was not understood. However, the study author hypothesized that the difference may have been due to thawing of the seed samples to ambient temperatures prior to the processing, which is the standard operating procedure.

Table B.7.7.3.1-5. Residue Data from Cotton Processing Study with Pethoxamid.

Commodity	Pethoxamid Residues ¹ (ppm) [Average]	Processing Factor ²	Median Processing Factor
Undelinted seed (RAC) (field sample)	0.018, 0.014 [0.016]	--	--
Undelinted seed (RAC) (processor samples)	ND, ND [<0.01]	--	--
Hulls	ND, ND [<0.01]	NC	Not applicable
Meal	ND, ND [<0.01]	NC	
RBD oil	ND, ND [<0.01]	NC	

¹ ND = Not detected (<LOD). The LOD and LOQ were 0.003 and 0.01 ppm, respectively. Average residues were calculated by the study reviewer using the LOQ for residues reported as <LOQ.

² Processing Factor = [Average residue for analyte in the processed fraction]/ [Average residue for analyte in the RAC from processor]. NC = Not calculated; residues were below the LOQ in the RAC and processed fraction.

III. CONCLUSIONS

The cotton processing study is considered scientifically acceptable. Following a preplant application to the soil and subsequent foliar application at exaggerated rates (~5x the rates used in the corresponding field trials), average residues of pethoxamid were 0.016 ppm (just above the LOQ) in/on undelinted seed (RAC) collected at the field and below the LOQ (<0.01 ppm) in/on undelinted seed collected just prior to processing. Residues in/on the processed fractions (hulls,

meal, and oil) were also below the LOQ (<0.01 ppm); therefore, processing factors could not be calculated.

The theoretical concentration factors for cotton are 3.8x for hulls, 2.2x for meal, and 6.3x for oil (based on separation into components; OCSPP 860.1520, Table 3).

An acceptable method was used for residue quantitation, and adequate storage stability data were submitted to support sample storage durations and conditions.

REFERENCES

Document ID: MRID 49813534; PMRA Study # xxxxxx, PMRA Review # xxxxxx
Report: Sears, K. (2015) Method Validation and 10-Month Storage Stability of Pethoxamid in Various Crops. Study Numbers: 1495 PXA; 2793. Unpublished study prepared by Pyxant Labs Inc., sponsored by Cheminova A/S, and submitted by FMC Corporation. 600 p.

**B.7.9 Residues in Succeeding Crops
(Annex IIA 6.6; Annex IIIA 8.6)**

B.7.9.1 Metabolism and Distribution Studies on Representative Crops (Confined Accumulation in Rotational Crops)

B.7.9.1.1 Carrot, Spinach, and Wheat; 2013-2015 Study

Document ID: MRID No. 49813552
PMRA No. Not available

Report: Mohajeri, S. and Zane, J. (2015) [¹⁴C]Pethoxamid Confined Rotational Crop Study (One Radiolabel). Study Numbers: 1477 PXA; 2419W; 2419W-1. Unpublished study prepared by PTRL West, sponsored by Cheminova A/S, and submitted by FMC Corporation. 518 p.

Guidelines: U.S. EPA Residue Chemistry Test Guideline OCSP 860.1850 Confined Accumulation in Rotational Crops (August 1996)
PMRA Regulatory Directive Dir98-02 – Residue Chemistry Guidelines, Section 13 - Confined Accumulation in Rotational Crops
OECD Guideline 502 Metabolism in Rotational Crops (January 2007)

GLP Compliance: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Acceptability: The study is considered scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP#452109

Evaluator: Sheila Piper, Chemist, HED, RAB6 *Sheila Piper*

Secondary Evaluator: Robert Lanthier, PMRA

Note: This Data Evaluation Record (DER) was prepared under contract by CDM/CSS-Dynamac Joint Venture (submitted 2/16/18). The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies. The DER has been reviewed by HED and revised as necessary to reflect current Office of Pesticide Programs (OPP) policies.

EXECUTIVE SUMMARY

The metabolism of pethoxamid was investigated in confined rotational crop study with [phenyl-¹⁴C]pethoxamid (specific activity 1.67 MBq/mg). The radiolabeled test substance was applied to bare sandy loam soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha). Rotational crops of carrot, spinach, and wheat were planted at plantback intervals (PBIs) of 30, 120, and 365 days. Crops were grown outdoors, and the following commodities were harvested: carrot roots and tops, immature and mature spinach, and wheat forage, hay, grain, and straw. The in-life phase of the study was conducted by Excel Research Services, Inc. (Fresno, CA), and the analytical phase of the study was conducted by PTRL West, Inc. (Hercules, CA).

Total radioactive residues (TRR) in all rotational crops were determined by combustion/LSC. For samples that were subjected to extraction and analysis, TRR were also calculated by summing extractable and nonextractable radioactivity following extraction with acetonitrile (ACN)/water and ACN, and these values were used for all further determinations. For all

rotational crop matrices, TRR were highest at the 30-day PBI and lowest at the 365-day PBI. Overall, TRR were lowest in carrot root and wheat grain and highest in spinach, wheat hay, and wheat straw.

TRR were >0.01 ppm in all rotational crop matrices except 365-day PBI carrot root. TRR in 30-, 120-, and 365-day PBI matrices, respectively, were: 0.071, 0.026, and 0.007 ppm in carrot root; 0.292, 0.104, and 0.031 ppm in carrot tops; 0.539, 0.335, and 0.069 ppm in immature spinach; 0.465, 0.156, and 0.069 ppm in mature spinach; 0.232, 0.212, and 0.130 ppm in wheat forage; 0.544, 0.269, and 0.145 ppm in wheat hay; 0.058, 0.046, and 0.031 ppm in wheat grain; and 1.099, 0.758, and 0.321 ppm in wheat straw. All rotational crop matrices except 365-day PBI carrot root were subjected to extraction procedures.

Extraction with ACN/water and ACN released the majority of the radioactivity (72-89% TRR) from rotational crop matrices except wheat grain; solvent extraction released 29-44% TRR from wheat grain. For 30-day PBI carrot roots and tops, immature spinach, and wheat hay and straw, and 365-day PBI carrot tops, immature spinach, and wheat straw, an aliquot of the combined extracts was subjected to acid hydrolysis, and both neutral and acid-hydrolyzed extracts were analyzed. For the post-extraction solids (PES), sequential mild and/or strong hydrolyses with HCl and KOH released a total of 56.5-61.3% TRR from wheat grain, and 3.8-23.3% TRR from the other rotational crop matrices; no attempts to further characterize this radioactivity were conducted. Remaining PES accounted for ≤0.047 ppm in all matrices except 30-day PBI spinach, for which PES were 11.6% TRR (0.054 ppm), and 30-day PBI wheat straw, for which PES were 10.7% TRR (0.118 ppm). The extraction and hydrolysis procedures adequately extracted the majority of residues from rotational crop matrices. Extraction results were normalized; therefore, accountabilities were ~100%.

Residues were quantified in the neutral and acid-hydrolyzed solvent extracts of rotational crop matrices by high performance liquid chromatography (HPLC) with UV detection and radiodetection. Selected extracts were also analyzed one-dimensional thin-layer chromatography (1D-TLC). Three compounds, designated RT28H, RT36H, and RT38H, were identified in the acid-hydrolyzed extract of 30-day PBI immature spinach using HPLC with mass spectrometry (MS) or tandem mass spectrometry (MS/MS) detection, and a fourth compound, RT24H, was putatively identified in the same manner. These compounds were tentatively identified in the hydrolyzed extracts of other rotational crop matrices by retention time comparisons. No attempts were made by the study author to correlate components in the hydrolyzed extracts with the corresponding components in the neutral extracts; thus, these components are considered to be unknowns in the neutral extracts. Samples of rotational crop commodities were stored frozen from harvest to initial HPLC analysis for metabolite profiling for ≤196 days; certain samples and extracts were stored frozen for up to 431 days prior to completion of analyses. Supporting storage stability data were provided which demonstrated that the metabolite profiles in mature spinach and wheat hay were generally stable during at least 401 days of frozen storage. Also, acceptable storage stability data supporting potato foliage for up to 631 days (21 months) and 596 days (20 months) in tubers (MRIDs 0 49813529). Because the results of initial HPLC analyses, generally conducted within 6 months of sample collection, showed that the metabolite profile in all matrices was multicomponent with no compounds that appeared to co-

chromatograph with parent or reference standards, no additional storage stability data will be required.

The neutral solvent extracts were co-chromatographed with up to nine reference standards, but the overall level of identification was low ($\leq 11\%$ TRR). Pethoxamid was initially detected in 30- and 120-day PBI carrot root, at 11.3% TRR (0.008 ppm) and 3.8% TRR (0.001 ppm), respectively, but was not conclusively identified. MET-30 was found in 30-day PBI immature spinach at very low levels ($<0.2\%$ TRR, <0.001 ppm). No other residues were identified in the initial solvent extracts. The remainder of the solvent-extractable radioactivity consisted of single or multi-component unknowns, each present at $\leq 15.9\%$ TRR (≤ 0.075 ppm) in hydrolyzed extracts, and at $\leq 21.9\%$ TRR (≤ 0.102 ppm).

No residues of pethoxamid or known metabolites were identified on co-chromatography of the hydrolyzed extracts with reference standards; however, the overall levels of identification of compounds RT24H, RT28H, RT36H, and RT38H (considered to be tentative identification for all matrices except 30-day PBI immature spinach) were 23.4-51.4% TRR in the hydrolyzed extracts of 30-day PHI matrices and 27.5-45.8% TRR in 365-day wheat straw. Compound RT24H was found in 30-day PBI hydrolyzed extracts as follows: 6.5% TRR (0.005 ppm) in carrot root; 19.5% TRR (0.057 ppm) in carrot tops; 15.2% TRR (0.082 ppm) in immature spinach; 13.6% TRR (0.074 ppm) in wheat hay; and 13.6% TRR (0.149 ppm) in wheat straw. RT24H was also found in the hydrolyzed extracts of 365-day PBI immature spinach (11.6% TRR, 0.008 ppm) and wheat straw (16.2% TRR, 0.052 ppm). RT28H was found in 30-day PBI hydrolyzed extracts as follows: 9.8% TRR (0.053 ppm) in immature spinach; 2.9% TRR (0.016 ppm) in wheat hay; and 5.4% TRR (0.059 ppm) in wheat straw. RT28H was also found in the hydrolyzed extracts of 365-day PBI immature spinach (5.8% TRR, 0.004 ppm) and wheat straw (11.2% TRR, 0.036 ppm). RT36H was found in 30-day PBI hydrolyzed extracts as follows: 5.2% TRR (0.004 ppm) in carrot root; 14.4% TRR (0.042 ppm) in carrot tops; 5.2% TRR (0.028 ppm) in immature spinach; 10.1% TRR (0.055 ppm) in wheat hay, and 8.0% TRR (0.088 ppm) in wheat straw. RT36H was also found in the hydrolyzed extracts of 365-day PBI immature spinach (8.7% TRR, 0.006 ppm) and wheat straw (11.8% TRR, 0.008 ppm). RT38H was found in 30-day PBI hydrolyzed extracts as follows: 11.7% TRR (0.009 ppm) in carrot root; 17.5% TRR (0.051 ppm) in carrot tops; 3.0% TRR (0.016 ppm) in immature spinach; 16.2% TRR (0.088 ppm) in wheat hay; and 12.9% TRR (0.142 ppm) in wheat straw. RT38H was also found in the hydrolyzed extracts of 365-day PBI immature spinach (1.4% TRR, 0.001 ppm) and wheat straw (6.5% TRR, 0.021 ppm). The unidentified compound RT54H comprised a significant amount of radioactivity in 30-day PBI carrot root (20.8% TRR, 0.016 ppm) and 365-day PBI carrot tops (87.1% TRR, 0.027 ppm) and was a minor component in 365-day immature spinach (8.7% TRR, 0.006 ppm). The remainder of the solvent-extractable radioactivity consisted of single or multicomponent unknowns, each present at $\leq 15.9\%$ TRR (≤ 0.075 ppm).

Based on the results of the study, the study authors proposed that the metabolism of pethoxamid in rotational carrots, spinach, and wheat is similar to that observed in the primary crops of potato, soybean, and corn. The first step is proposed to be the displacement of the chlorine atom by the thiolate anion of glutathione to yield a glutathione conjugate (not found in this study). The glutamic acid and glycine portions of glutathione are then cleaved to form the cysteine conjugate MET-30, which may be metabolized by a number of pathways common to cysteine conjugates,

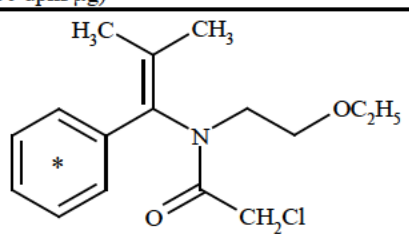
forming many products. The three components identified after hydrolysis of extracts (RT28H, RT36H, and RT38H) were found to be cleaved components of pethoxamid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

The radiolabeled test substance, dissolved in toluene, was isotopically diluted with non-labeled pethoxamid. The solvent was evaporated under nitrogen and mixed with blank emulsifiable concentrate formulation and water to a concentration of 57.3% pethoxamid. Radiochemical purity was measured before and after application to the test plots.

Table B.7.9.1.1-1. Pethoxamid Nomenclature.	
Common name	Pethoxamid
Identity	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide
CAS registry number	106700-29-2
Company experimental name	TKC-94
Radiolabeled compound	[phenyl-U- ¹⁴ C]pethoxamid
Lot/Batch #	CFQ41685, CFQ 41880
Radiochemical purity	97.1-99.72% (pre-application), 96.9-98.95% (post-application)
Specific activity as received	29 mCi/mmol; 31.0 mCi/mmol
Specific activity of dose	1.67 MBq/mg (100,000 dpm/μg)
Position of radiolabel	

2. Test Crops

Table B.7.9.1.1-2. Crop Information.				
Crop; Crop Group	Variety	PBIs (days)	Growth Stage at Harvest	Harvested Commodities
Carrot; Root and tuber vegetables	Danvers 126	30, 120, and 365	Mature	Roots and tops
Spinach; Leafy vegetables	Shasta	30, 120, and 365	Immature (~50% mature size) and mature	Leaves
Wheat; Cereal grains and Forage, fodder, and straw of cereal grains	Blanca Royale	30, 120, and 365	Immature: Forage (BBCH 30-39) and hay (BBCH 71-72) Mature: Grain and straw	Forage, hay, grain, and straw

3. Soil Type

Table B.7.9.1.1-3. Soil Physicochemical Properties.							
Soil Type	pH	Organic Matter (%)	Sand (%)	Silt (%)	Clay (%)	Moisture Holding Capacity (% at 1/3 bar)	Cation Exchange Capacity (meq/100 g)
Sandy loam	7.0	0.68	68	18	14	Not reported	10.5

B. STUDY DESIGN

Experimental Conditions

Rotational crops were grown outdoors in wooden boxes (1 m²) filled with sandy loam soil to a depth of ~32 cm. Two boxes were established for each PBI: one box for carrot and spinach and one box for wheat. The formulated test substance was applied to bare soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha) using a manually-operated, trigger-pulled pump sprayer. The test site was located near Madera, CA.

Rotational crops of carrot, spinach, and wheat were planted 30, 120, and 365 days after soil treatment. Weather conditions during the field phase of the study were reported to be typical of the area. Plants were watered by hand as needed and were grown using typical agronomic practices.

The study use pattern is summarized in Table B.7.9.1.1-4.

Table B.7.9.1.1-4. Use Pattern Information.	
Chemical name	[phenyl-U- ¹⁴ C]pethoxamid
Application method	The formulated test substance was applied to bare soil using a manually-operated trigger-pulled pump sprayer.
Application rate	Carrot and spinach: 1.16 lb ai/A (1.30 k g ai/ha) for 30-day PBI; 1.17 lb ai/A (1.31 kg ai/ha) for 120- and 365-day PBIs Wheat: 1.17 lb ai/A (1.31 kg ai/ha) for 30- and 120-day PBIs; 1.16 lb ai/A (1.30 k g ai/ha) for 365-day PBI
Number of applications	1
PBI (DAT ¹)	30, 120, and 365 days

¹ DAT = Days after treatment.

Sampling

Samples of each rotational crop commodity were harvested at the appropriate growth stage at each of the three PBIs. Immature spinach was collected when plants were approximately 50% of mature size. Wheat forage was collected at BBCH 30-39 and wheat hay was collected at early flower, BBCH 71-72. All other crop commodities were collected at maturity. Plant-to-harvest intervals were: 110-167 days for carrot roots and tops; 32-69 and 43-89 days for immature and mature spinach, respectively; 32-89 days for wheat forage; 47-130 days for wheat hay; and 71-200 days for wheat grain and straw. Carrot tops were cut from the plants and then roots were pulled from the soil; roots were washed with water to remove soil. Samples of spinach and

wheat forage and hay were harvested by cutting just above the soil surface; hay was collected fresh and was not dried in the field. Mature wheat heads were removed from the stalks and threshed, and grain was separated from the chaff using a moving blower. Straw samples were cut above the soil surface. Samples of rotational crop commodities were placed in frozen storage (temperature unspecified) immediately after sampling and were delivered frozen to the analytical laboratory, PTRL West (Hercules, CA) by courier or PTRL West vehicle (on dry ice), or by ACDS freezer truck. At the analytical laboratory, samples were maintained frozen (ca. -20 °C) prior to and following processing and were homogenized in the presence of dry ice for extraction and analysis. Wheat hay moisture content analyses were conducted at the analytical laboratory; moisture content in treated samples was 73-76%.

TRR Determination

TRR in the extracts and hydrolysates of rotational crop commodities were determined by direct LSC, and TRR in homogenized crop matrices and PES were determined by combustion/LSC. TRR in samples was also determined by summing radioactivity in extract and PES fractions. The limits of detection and quantitation for TRR determinations were generally 35 and 70 dpm, respectively.

Extraction and Analysis

Samples of all rotational crop matrices, with the exception of 365-day PBI carrot roots, were subjected to extraction and analysis. Homogenized rotational crop matrices were extracted sequentially with ACN:water (1:1, v:v; twice) and ACN by shaking for 30 minutes for each extraction. The extracts were isolated by centrifugation and combined for HPLC analysis.

For 30-day PBI carrot roots and tops, immature spinach, and wheat hay and straw, and 365-day PBI carrot tops, immature spinach, and wheat straw, an aliquot of the combined extracts was also subjected to acid hydrolysis. The aliquot was mixed with 4 N HCl to achieve an overall concentration of 2 N HCl, and the mixture was heated at 100 °C for 2 hours. The hydrolyzed extract was neutralized with 30% (w:v) ammonium hydroxide, concentrated (to remove ammonium hydroxide), and filtered through a 0.45-µm syringe filter for HPLC analysis. For comparison, [¹⁴C]pethoxamid was reportedly subjected to acid hydrolysis using the same procedures. Although no results of this hydrolysis were reported in the submission, the same experiment was conducted in the potato metabolism study submitted under the current action (refer to the DER for MRID 49813529; B.7.1.2) and showed little change to [¹⁴C]pethoxamid following hydrolysis under these conditions. The study authors noted that mature spinach extracts were not subjected to hydrolysis as immature spinach was the representative matrix for spinach. Similarly, wheat forage extracts were not hydrolyzed as wheat hay and straw were the representative matrices for wheat. Wheat grain extracts were not hydrolyzed due to low radioactivity levels.

Wheat straw extracts (30-day PBI) were subjected to the following additional hydrolyses: mild acid hydrolysis (in 1 N HCl at 100 °C for 15 minutes; mixture neutralized with ammonium hydroxide); protease hydrolysis (in phosphate buffer containing 10 mM CaCl₂ at 37 °C for 2 days); peroxide hydrolysis (extract evaporated to dryness and redissolved in methanol before the

addition of 30% hydrogen peroxide, followed by heating at 100 °C for 2 hours); meta-chloroperoxybenzoic acid hydrolysis (extract evaporated to dryness and redissolved in methanol, mixed with 3-chloroperbenzoic acid, and left at room temperature overnight); and base hydrolysis (in 2 N KOH at 100 °C for 2 hours; mixture neutralized with 12 M HCl). The mild acid, protease, and base hydrolysates were reportedly isolated by filtration or centrifugation for HPLC analysis.

The remaining PES were subjected to sequential hydrolyses to release additional ¹⁴C-residues. Minimal descriptions of the procedures were provided in the submission. The PES were reportedly hydrolyzed under acidic and basic conditions, such as 0.1 M HCl, 0.1 M KOH, 6 M HCl, 6 M KOH at ambient temperature, with the hydrolysates isolated by centrifugation. The PES were then subjected to hydrolysis using 6 M HCl and 6 M KOH at reflux for 2 hours, and the hydrolysates were isolated by centrifugation. Radioactivity was determined in the hydrolysates and the remaining PES; it did not appear that any hydrolysates were analyzed by HPLC.

Representative flowcharts of the general extraction procedures, the procedures for acid hydrolysis of extracts, and the procedures for hydrolysis of PES are presented in Figures B.7.9.1.1-1, B.7.9.1.1-2, and B.7.9.1.1-3, respectively.

Figure B.7.9.1.1-1. General Extraction Scheme for Rotational Crop Matrices.

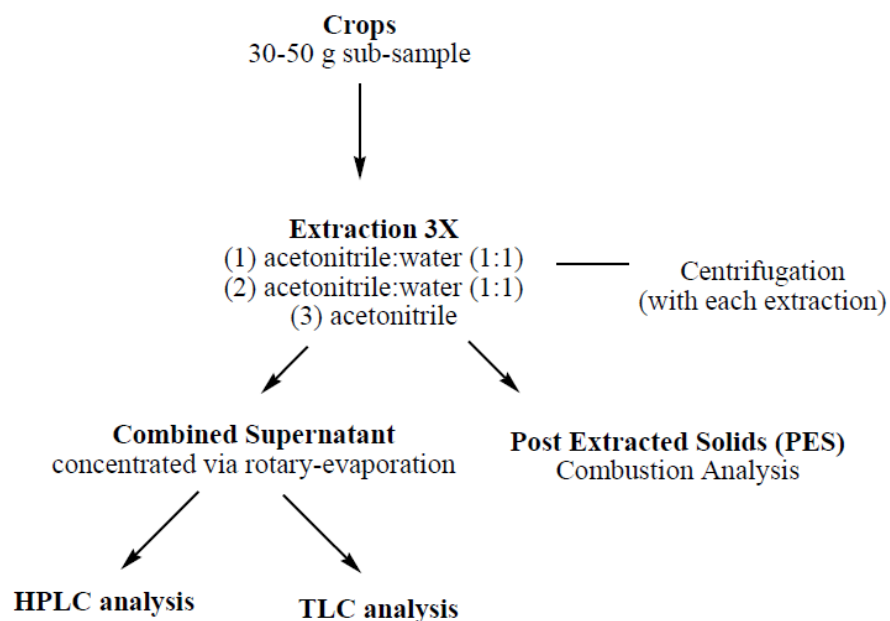


Figure B.7.9.1.1-2. Acid Hydrolysis Scheme for Rotational Crop Extracts.

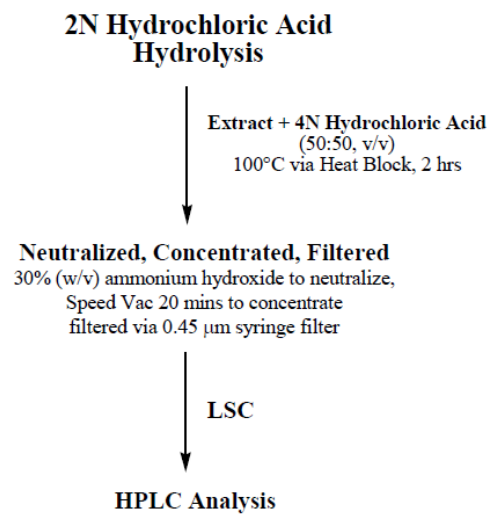
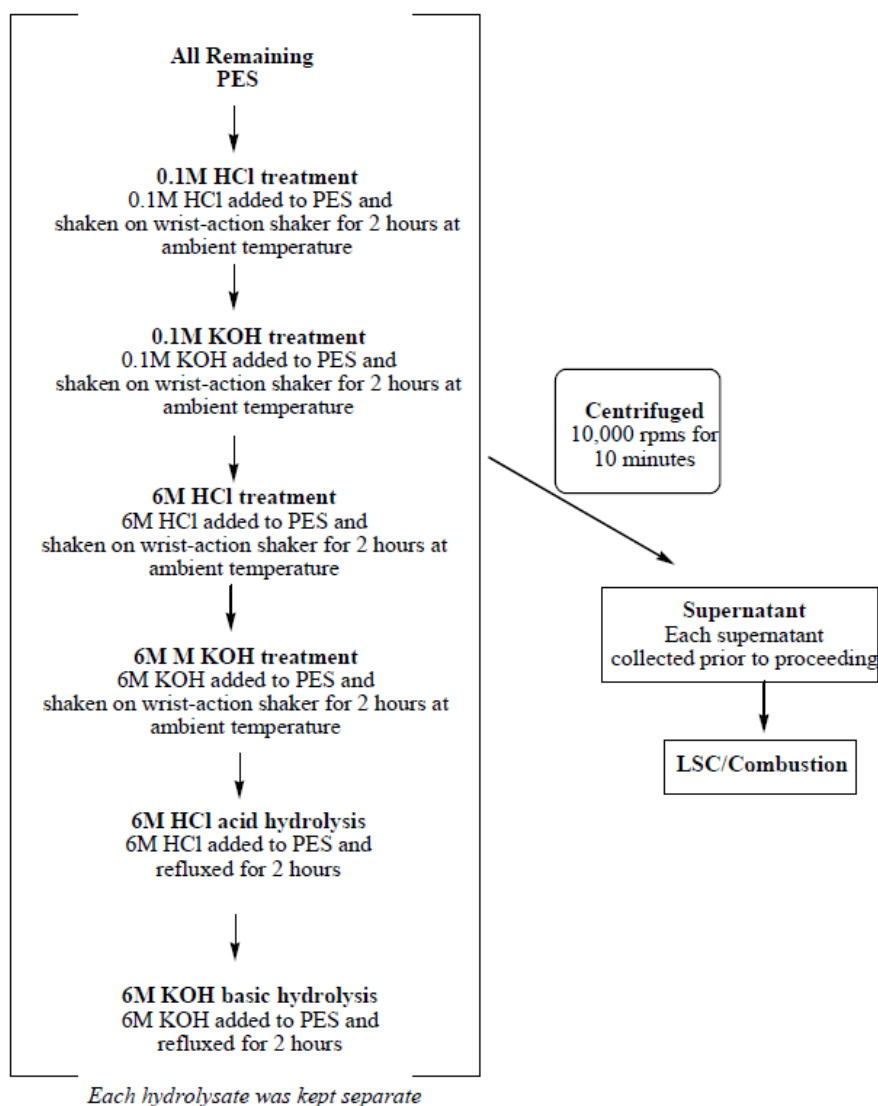


Figure B.7.9.1.1-3. Hydrolysis Scheme for Rotational Crop Matrix Post-Extraction Solids.



Identification and Characterization

Extracts were analyzed using reverse-phase HPLC with UV detection (254 nm) and radiodetection using either a radioisotope flow monitor or fraction collection with LSC. Peak assignments were based on co-elution with nine reference standards (see Table B.7.9.1.1-23) injected with each sample. The following HPLC methods were used:

- Method 2 (selected neutral extracts): C18 column (Capcell Pak) and gradient mobile phase of water and ACN, each containing 0.1% trifluoroacetic acid;
- Method 6 (neutral extracts): C18 column (Spherisorb ODS2) and gradient mobile phase of ACN and 0.01 M formate buffer (pH 3.0) in water;
- Method 6A (neutral extracts): C18 column (Synergy fusion-RP) and gradient mobile phase of ACN and 0.01 M formate buffer (pH 3.0) in water;

- Method CR (hydrolyzed extracts and select neutral extracts): reverse-phase column (Capcell CR, a 1:20 mixture of SCX and C18) and a gradient mobile phase of 0.1% formic acid in ACN and 10 mM ammonium acetate in water.

Method 2 was initially used for extract analysis but was determined to be ineffective at separating residues. Method 6 was used for the initial analysis of the neutral extracts of all 30- and 120-day PBI matrices, except 30-day PBI grain. Method 6A was used for the analysis of the extract of 30-day PBI grain and the neutral extracts of 365-day PBI matrices. Method CR was used to analyze the hydrolyzed extracts of 30-day PBI carrot roots and tops, immature spinach, and wheat hay and straw, as well as the neutral extracts of 30-day PBI matrices. The study authors reported that neutral extracts did not resolve any better with Method CR analysis (as compared to Method 6 analysis) but resolution was improved after hydrolysis. Peak names were assigned for selected peaks in Method CR analyses using “RT” followed by the approximate retention time (e.g., RT24); an H was added for peaks observed in hydrolyzed extracts (e.g., RT24H). We note that the results of Method CR analyses were the focus of study report; therefore, there was little discussion of the results of analyses of 120-day and 365-day PBI matrices.

Note to EPA Reviewer: The study authors listed RTxx and RTxxH components together in tables, indicating that they were the same components (i.e., RT24 found in a neutral extract is the same compound as RT24H found in a hydrolyzed extract) but they did not say this explicitly. In addition, because the extracts from each PBI for a crop matrix were generally analyzed using a different HPLC method (Method CR for 30-day PBI, Method 6 for 120-day PBI, and Method 6A for 365-day PBI) it is hard to compare metabolite peaks across PBIs for a matrix. We note that the reference standards that were used in this study include all the metabolites identified in the 30-day PBI CRC study (MRID 49813553) except MET-49.

The neutral extracts of 30-day PBI carrot roots, carrot tops, immature spinach, and wheat straw were also analyzed using 1D-TLC. Analyses were conducted on silica gel 60 Å F₂₅₄ plates using a solvent system of chloroform:methanol:acetic acid (7:3:0.3, v:v:v or 7:3:1, v:v:v). Radioactivity was detected using a phosphorimager and non-labeled standards were determined by quenching of UV light-induced fluorescence.

The hydrolyzed extract of 30-day PBI immature spinach was used to isolate five unknowns: RT24H, RT28H, RT31H, RT36H, and RT38H. The extract was cleaned up on a solid-phase extraction cartridge (Strata-X-Giga), which was eluted with 0.1% formic acid in water (3x), ACN (3x), and ethyl acetate (2x). The eluents were concentrated, and the fractions were isolated using HPLC Method CR. The isolated components were further purified using HPLC, on a reverse-phase column (Supelco F5) with a gradient mobile phase of water to ACN, each containing 0.1% trifluoroacetic acid, for LC/MS or LC/MS/MS analysis. Analyses were conducted using a reverse-phase column, a gradient mobile phase of ACN and 10 mM ammonium formate buffer in water, and electrospray ionization with constantly switching positive/negative polarity. A fraction collector was used for subsequent LSC measurements.

II. RESULTS AND DISCUSSION

A. Total Radioactive Residues

The TRR in rotational carrot, spinach, and wheat samples are summarized in Table B.7.9.1.1-5. TRR levels determined by combustion/LSC were similar to the levels determined by the summing of extractable and nonextractable radioactivity. For all matrices, TRR were highest at the 30-day PBI and lowest at the 365-day PBI. Overall, TRR were lowest in carrot root and wheat grain and highest in spinach, wheat hay, and wheat straw.

Table B.7.9.1.1-5. TRR in Rotational Carrot, Spinach, and Wheat.			
Matrix	Residues ¹ (ppm [¹⁴ C]pethoxamid equivalents)		
	30-day PBI ²	120-day PBI	365-day PBI
Carrot root	0.069 (0.071)	0.024 (0.026)	0.007
Carrot tops	0.304 (0.292)	0.104 (0.104)	0.036 (0.031)
Immature spinach	0.585 (0.539)	0.365 (0.335)	0.073 (0.069)
Mature spinach	0.523 (0.465)	0.189 (0.156)	0.069 (0.069)
Wheat forage	0.272 (0.232)	0.241 (0.212)	0.178 (0.130)
Wheat hay	0.605 (0.544)	0.290 (0.269)	0.158 (0.145)
Wheat grain	0.067 (0.058)	0.055 (0.046)	0.030 (0.031)
Wheat straw	1.077 (1.099)	0.814 (0.758)	0.334 (0.321)

¹ Residues in all matrices were determined by combustion/LSC. For matrices bearing residues ≥ 0.01 ppm, residues were also determined by summing extractable and nonextractable radioactivity (presented in parentheses). Summed TRR values were used for all further determinations.

² PBI = Plantback interval.

B. Extraction, Characterization, and Distribution of Residues in Rotational Carrot, Spinach, and Wheat.

The distribution of radioactivity in rotational crop matrices is presented in Tables B.7.9.1.1-6 (carrot root), B.7.9.1.1-7 (carrot tops), B.7.9.1.1-8 (immature spinach), B.7.9.1.1-9 (mature spinach), B.7.9.1.1-10 (wheat forage), B.7.9.1.1-11 (wheat hay), B.7.9.1.1-12 (wheat grain), and B.7.9.1.1-13 (wheat straw). For 30-day PBI carrot roots and tops, immature spinach, and wheat hay and straw, and 365-day PBI carrot tops, immature spinach, and wheat straw, an aliquot of the combined ACN/water and ACN extracts (neutral extract) was also subjected to acid hydrolysis to further characterize residues (hydrolyzed extract).

Table B.7.9.1.1-6. Distribution of Radioactive Residues in Rotational Carrot Root Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹						
Metabolite Fraction ²	30-day PBI				120-day PBI	
	TRR = 0.071 ppm				TRR = 0.026 ppm	
	Neutral extract		Hydrolyzed extract		Neutral extract	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN/water	80.5	0.062	80.5	0.062	80.8	0.021
Pethoxamid	ND ³	--	ND	--	3.8	0.001
RT3 (2.75 minutes)	16.9	0.013	ND	--	ND	--
RT = 3.00 minutes	ND	--	ND	--	15.4	0.004
RT = 3.25 minutes	ND	--	18.2	0.014	ND	--
RT24/RT24H (23.75 minutes)	2.6	0.002	6.5	0.005	ND	--
RT33 (32.75 minutes)	10.4	0.008	ND	--	ND	--
RT36/RT36H (35.75 minutes)	1.3	0.001	5.2	0.004	ND	--
RT38H (38.25 minutes)	ND	--	11.7	0.009	ND	--
RT50 (50.25 minutes)	13.0	0.010	ND	--	ND	--
RT51H (51.25 minutes)	ND	--	9.1	0.007	ND	--
RT52 (51.75 minutes)	10.4	0.008	ND	--	ND	--
RT53 (53.25 minutes)	3.9	0.003	ND	--	ND	--
RT54H (54.25 minutes)	ND	--	20.8	0.016	ND	--
RT55 (54.75 minutes)	3.9	0.003	ND	--	ND	--
RT = 61.07 minutes	ND	--	ND	--	7.7	0.002
RT = 62.62 minutes	ND	--	ND	--	23.1	0.006
RT = 65.05 minutes	ND	--	ND	--	15.4	0.004
Other unknowns ⁴	18.2	0.014	9.1	0.007	15.2	0.004
PES ⁵	19.5	0.062			19.2	0.005
0.1 N HCl hydrolysate	1.4	0.001			ND	--
0.1 N KOH hydrolysate	2.8	0.002			3.8	0.001
6 N HCl hydrolysate	1.4	0.001				
6 N KOH hydrolysate	2.8	0.002				
PES	7.0	0.005			15.4	0.004

¹ ND = Not detectable. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. The 30-day PBI ACN/water extract (neutral extract) was subjected to acid hydrolysis to further characterize residues (hydrolyzed extract). The 30-day PBI extracts were analyzed using HPLC Method CR, and the 120-day PBI extract was analyzed using HPLC Method 6.

² Specific components detected in the hydrolyzed extract(s) are designated by an "H", e.g. RT24H.

³ Analysis of this extract using a different HPLC system (Method 6) identified pethoxamid at 11.3% TRR (0.008 ppm).

⁴ Consisting of: 9 unknowns, each present at ≤3.9% TRR (≤0.003 ppm) in 30-day PBI neutral extract; 4 unknowns, each present at ≤3.9% TRR (≤0.003 ppm) in 30-day PBI hydrolyzed extract; and 4 unknowns, each present at ≤3.8% TRR (≤0.001 ppm) in 120-day PBI extract.

⁵ PES hydrolyses were conducted at ambient temperature.

Table B.7.9.1.1-7. Distribution of Radioactive Residues in Rotational Carrot Tops Following Application of [¹⁴ C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha). ¹										
Metabolite Fraction ²	30-day PBI				120-day PBI		365-day PBI			
	TRR = 0.292 ppm				TRR = 0.104 ppm		TRR = 0.031 ppm			
	Neutral extract		Hydrolyzed extract		Neutral extract		Neutral extract		Hydrolyzed extract	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN/water	86.3	0.252	86.3	0.252	87.5	0.091	87.1	0.027	87.1	0.027
RT = 3.75 minutes	ND	--	ND	--	ND	--	9.4	0.003	ND	--
RT21 (20.75 minutes)	4.1	0.012	ND	--	ND	--	ND	--	ND	--

Table B.7.9.1.1-7. Distribution of Radioactive Residues in Rotational Carrot Tops Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹

Metabolite Fraction ²	30-day PBI				120-day PBI		365-day PBI			
	TRR = 0.292 ppm				TRR = 0.104 ppm		TRR = 0.031 ppm			
	Neutral extract		Hydrolyzed extract		Neutral extract		Neutral extract		Hydrolyzed extract	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
RT24/RT24H (24.25/23.75 minutes)	12.7	0.037	19.5	0.057	ND	--	ND	--	ND	--
RT = 25.75 minutes	6.8	0.020	ND	--	ND	--	ND	--	ND	--
RT = 27.25 minutes	11.6	0.034	ND	--	ND	--	ND	--	ND	--
RT29 (29.25 minutes)	9.2	0.027	ND	--	ND	--	ND	--	ND	--
RT = 30.25 minutes	8.6	0.025	ND	--	ND	--	ND	--	ND	--
RT = 30.78 minutes	ND	--	ND	--	6.7	0.007	ND	--	ND	--
RT31H (31.25 minutes)	ND	--	3.8	0.011	ND	--	ND	--	ND	--
RT33 (32.75 minutes)	9.2	0.027	ND	--	ND	--	ND	--	ND	--
RT = 33.70 minutes	ND	--	ND	--	7.7	0.008	ND	--	ND	--
RT = 35.40 minutes	ND	--	ND	--	5.8	0.003	ND	--	ND	--
RT36/RT36H (36.25/35.75 minutes)	2.4	0.007	14.4	0.042	ND	--	ND	--	ND	--
RT38H (38.25 minutes)	ND	--	17.5	0.051	ND	--	ND	--	ND	--
RT = 42.75 minutes	ND	--	ND	--	ND	--	5.8	0.002	ND	--
RT45H (44.75 minutes)	ND	--	2.1	0.006	ND	--	ND	--	ND	--
RT = 47.25 minutes	ND	--	ND	--	ND	--	11.6	0.004	ND	--
RT = 49.25 minutes	ND	--	ND	--	ND	--	5.5	0.002	ND	--
RT51/RT51H (51.25 minutes)	1.0	0.003	5.1	0.015	ND	--	ND	--	ND	--
RT54/RT54H (54.75 minutes)	1.7	0.005	2.1	0.006	ND	--	ND	--	87.1	0.027
RT = 63.25 minutes	ND	--	ND	--	ND	--	14.8	0.005	ND	--
RT = 65.25 minutes	ND	--	ND	--	ND	--	7.7	0.002	ND	--
Other unknowns ³	18.9	0.055	22.0	0.064	67.3	0.070	32.3	0.010	ND	--
PES ⁴	13.7	0.040			12.5	0.013	12.9	0.004		
0.1 N HCl hydrolysate	0.7	0.002			<0.01	<0.001	ND	--		
0.1 N KOH hydrolysate	1.0	0.003			1.0	0.001				
6 N HCl hydrolysate	0.7	0.002			1.0	0.001				
6 N KOH hydrolysate	3.8	0.011			3.8	0.004				
PES	7.5	0.022			6.7	0.007	12.9	0.004		

¹ ND = Not detectable. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. The 30- and 365-day PBI ACN/water extracts (neutral extracts) were subjected to acid hydrolysis to further characterize residues (hydrolyzed extracts). The 30-day PBI extracts (both) and the 365-day PBI hydrolyzed extract were analyzed using HPLC Method CR, the 120-day PBI extract was analyzed using HPLC Method 6, and the 365-day PBI neutral extract was analyzed using HPLC Method 6A.

² Specific components detected in the hydrolyzed extract(s) are designated by an "H", e.g. RT24H.

³ Consisting of: 10 unknowns, each present at ≤4.5% TRR (≤0.013 ppm) in 30-day PBI neutral extract; 7 unknowns, each present at ≤4.8% TRR (≤0.014 ppm) in 30-day PBI hydrolyzed extract; 32 unknowns, each present at ≤4.8% TRR (≤0.005 ppm) in 120-day PBI extract; and 17 unknowns, each present at ≤4.5% TRR (≤0.001 ppm) in 365-day PBI neutral extract.

⁴ PES hydrolyses were conducted at ambient temperature.

Table B.7.9.1.1-8. Distribution of Radioactive Residues in Rotational Immature Spinach Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹

Metabolite Fraction ²	30-day PBI				120-day PBI		365-day PBI			
	TRR = 0.539 ppm				TRR = 0.335 ppm		TRR = 0.069 ppm			
	Neutral extract		Hydrolyzed extract		Neutral extract		Neutral extract		Hydrolyzed extract	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN/water	72.4 ³	0.390	72.4	0.390	88.7	0.297	85.5	0.059	85.5	0.059
RT3 (2.75 minutes)	6.3	0.034	ND	--	ND	--	ND	--	ND	--
RT = 3.25 minutes	ND	--	9.1	0.049	ND	--	ND	--	ND	--
RT = 16.78 minutes	ND	--	ND	--	6.9	0.023	ND	--	ND	--
RT20 (19.75 minutes)	5.5	0.030	ND	--	ND	--	ND	--	ND	--
RT21 (20.75 minutes)	2.6	0.014	ND	--	ND	--	ND	--	ND	--
RT = 22.25 minutes	5.5	0.030	ND	--	ND	--	ND	--	ND	--
RT24/RT24H (23.75/25.25 minutes)	13.5	0.073	15.2	0.082	ND	--	ND	--	11.6	0.008
RT = 25.25 minutes	6.7	0.036	ND	--	ND	--	ND	--	ND	--
RT28/RT28H (28.25/27.75 minutes)	9.8	0.053	9.8	0.053	ND	--	ND	--	5.8	0.004
RT31H (31.25-32.25 minutes)	ND	--	5.2	0.028	ND	--	ND	--	15.9	0.011
RT33 (32.75 minutes)	3.8	0.021	ND	--	ND	--	ND	--	ND	--
RT34 (34.25 minutes)	2.2	0.012	ND	--	ND	--	ND	--	ND	--
RT36/RT36H (35.75-36.25 minutes)	1.4	0.007	5.2	0.028	ND	--	ND	--	8.7	0.006
RT38H (38.25 minutes)	ND	--	3.0	0.016	ND	--	ND	--	1.4	0.001
RT45H (44.75 minutes)	ND	--	3.2	0.017	ND	--	ND	--	1.4	0.001
RT = 44.93 minutes	ND	--	ND	--	ND	--	5.1	0.004	ND	--
RT51H (51.25 minutes)	ND	--	3.7	0.020	ND	--	ND	--	ND	--
RT54H (55.75 minutes)	ND	--	ND	--	ND	--	ND	--	8.7	0.006
Other unknowns ⁴	15.1	0.080	17.8	0.097	81.9	0.274	80.1	0.056	31.5	0.022
PES ⁵	27.6	0.149			11.3	0.038	14.5	0.010		
0.1 N HCl hydrolysate	1.1	0.006			0.9	0.003	1.4	0.001		
0.1 N KOH hydrolysate	2.4	0.013			1.2	0.004	1.4	0.001		
6 N HCl hydrolysate	1.9	0.010			0.9	0.003	1.4	0.001		
6 N KOH hydrolysate	3.5	0.019			2.1	0.007	2.9	0.002		
6 N HCl reflux hydrolysate	9.1	0.049					5.8	0.004		
6 N KOH reflux hydrolysate	0.9	0.005					ND	--		
PES	8.7	0.047			6.3	0.021	1.4	0.001		

¹ ND = Not detectable. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. The 30- and 365-day PBI ACN/water extracts (neutral extracts) were subjected to acid hydrolysis to further characterize residues (hydrolyzed extracts). The 30-day PBI extracts (both) and the 365-day PBI hydrolyzed extract were analyzed using HPLC Method CR, the 120-day PBI extract was analyzed using HPLC Method 6, and the 365-day PBI neutral extract was analyzed using HPLC Method 6A.

² Specific components detected in the hydrolyzed extract(s) are designated by an "H", e.g. RT24H.

³ 1D-TLC analysis of this extract revealed the presence of MET-30, at <0.001 ppm (<0.2% TRR).

⁴ Consisting of: 10 unknowns, each present at ≤3.7% TRR (≤0.020 ppm) in 30-day PBI neutral extract; 12 unknowns, each present at ≤3.3% TRR (≤0.018 ppm) in 30-day PBI hydrolyzed extract; 46 unknowns, each present at ≤4.8% TRR (≤0.016 ppm) in 120-day PBI extract; 42 unknowns, each present at ≤4.1% TRR (≤0.003 ppm) in 365-day PBI neutral extract; and 12 unknowns, each present at ≤5.8% TRR (≤0.004 ppm).

⁵ PES hydrolyses were conducted at ambient temperature unless otherwise noted.

Table B.7.9.1.1-9. Distribution of Radioactive Residues in Rotational Mature Spinach Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹

Metabolite Fraction	30-day PBI		120-day PBI		365-day PBI	
	TRR = 0.465 ppm		TRR = 0.156 ppm		TRR = 0.069 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN/water	79.8	0.371	87.8	0.137	85.5	0.059
RT3 (2.75 minutes)	6.7	0.031	ND	--	ND	--
RT = 16.17 minutes	ND	--	5.1	0.008	ND	--
RT = 16.63 minutes	ND	--	7.1	0.011	ND	--
RT20+RT21 (19.75 minutes)	9.0	0.042	ND	--	ND	--
RT = 21.28 minutes	ND	--	5.8	0.009	ND	--
RT24 (23.75 minutes)	21.9	0.102	ND	--	ND	--
RT = 27.25 minutes	6.0	0.028	ND	--	ND	--
RT28 (28.25 minutes)	3.9	0.018	ND	--	ND	--
RT = 29.25 minutes	6.5	0.030	ND	--	ND	--
RT33 (32.75 minutes)	4.5	0.021	ND	--	ND	--
RT34 (34.25 minutes)	1.9	0.009	ND	--	ND	--
RT36 (36.25 minutes)	2.4	0.011	ND	--	ND	--
Other unknowns ²	16.9	0.079	69.8	0.109	85.5	0.059
PES ³	20.2	0.094	12.2	0.019	14.5	0.010
0.1 N HCl hydrolysate	1.3	0.006	0.6	0.001	1.4	0.001
0.1 N KOH hydrolysate	2.4	0.011	1.3	0.002	1.4	0.001
6 N HCl hydrolysate	1.7	0.008	0.6	0.001	1.4	0.001
6 N KOH hydrolysate	3.2	0.015	1.9	0.003	2.9	0.002
PES	11.6	0.054	7.7	0.012	7.2	0.005

¹ ND = Not detectable. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. No solvent extracts of mature spinach were subjected to hydrolysis procedures. The 30-day PBI extract was analyzed using HPLC Method CR, the 120-day PBI extract was analyzed using HPLC Method 6, and the 365-day PBI extract was analyzed using HPLC Method 6A.

² Consisting of: 10 unknowns, each present at ≤4.3% TRR (≤0.020 ppm) in 30-day PBI extract; 26 unknowns, each present at ≤4.5% TRR (≤0.007 ppm) in 120-day PBI extract; and 36 unknowns, each present at ≤4.3% TRR (≤0.003 ppm) in 365-day PBI extract.

³ PES hydrolyses were conducted at ambient temperature.

Table B.7.9.1.1-10. Distribution of Radioactive Residues in Rotational Wheat Forage Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹

Metabolite Fraction	30-day PBI		120-day PBI		365-day PBI	
	TRR = 0.232 ppm		TRR = 0.212 ppm		TRR = 0.130 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN/water	72.8	0.169	84.9	0.180	76.2	0.099
RT = 15.78 minutes	ND	--	5.7	0.012	ND	--
RT = 17.07 minutes	ND	--	5.7	0.012	ND	--
RT21 (20.75 minutes)	3.4	0.008	ND	--	ND	--
RT24 (23.75 minutes)	7.8	0.018	ND	--	ND	--
RT = 25.75 minutes	5.2	0.012	ND	--	ND	--
RT = 27.25 minutes	5.6	0.013	ND	--	ND	--
RT29 (29.25 minutes)	12.5	0.029	ND	--	ND	--
RT = 29.40 minutes	ND	--	5.2	0.011	ND	--
RT = 30.98 minutes	ND	--	13.7	0.029	ND	--
RT33 (32.75 minutes)	3.9	0.009	ND	--	ND	--
RT = 39.67 minutes	ND	--	ND	--	4.6	0.006
RT = 43.60 minutes	ND	--	ND	--	4.6	0.006

Table B.7.9.1.1-10. Distribution of Radioactive Residues in Rotational Wheat Forage Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹

Metabolite Fraction	30-day PBI		120-day PBI		365-day PBI	
	TRR = 0.232 ppm		TRR = 0.212 ppm		TRR = 0.130 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
RT = 46.00 minutes	ND	--	ND	--	4.6	0.006
RT51 (50.25 minutes)	2.6	0.006	ND	--	ND	--
RT = 62.85 minutes	ND	--	5.2	0.011	ND	--
Other unknowns ²	31.9	0.074	49.5	0.105	62.3	0.081
PES ³	27.2	0.063	15.1	0.032	23.8	0.031
0.1 N HCl hydrolysate	3.0	0.007	2.4	0.005	2.3	0.003
0.1 N KOH hydrolysate	4.3	0.010	3.3	0.007	3.8	0.005
6 N HCl hydrolysate	2.2	0.005	1.9	0.004	2.3	0.003
6 N KOH hydrolysate	6.5	0.015	3.3	0.007	6.9	0.009
6 N HCl reflux hydrolysate	5.6	0.013				
6 N KOH reflux hydrolysate	1.7	0.004				
PES	3.9	0.009	4.2	0.009	8.5	0.011

¹ ND = Not detectable. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. No solvent extracts of wheat forage were subjected to hydrolysis procedures. The 30-day PBI extract was analyzed using HPLC Method CR, the 120-day PBI extract was analyzed using HPLC Method 6, and the 365-day PBI extract was analyzed using HPLC Method 6A.

² Consisting of: 17 unknowns, each present at ≤4.7% TRR (≤0.011 ppm) in 30-day PBI extract; 19 unknowns, each present at ≤4.7% TRR (≤0.010 ppm) in 120-day PBI extract; and 32 unknowns, each present at ≤3.8% TRR (≤0.005 ppm) in 365-day PBI extract.

³ PES hydrolyses were conducted at ambient temperature unless otherwise noted.

Table B.7.9.1.1-11. Distribution of Radioactive Residues in Rotational Wheat Hay Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹

Metabolite Fraction ²	30-day PBI				120-day PBI		365-day PBI	
	TRR = 0.544 ppm				TRR = 0.269 ppm		TRR = 0.145 ppm	
	Neutral extract		Hydrolyzed extract		Neutral extract		Neutral extract	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN/water	77.8	0.423	77.8	0.423	76.2	0.205	78.6	0.114
RT21 (20.75 minutes)	3.9	0.021	ND	--	ND	--	ND	--
RT24/RT24H (23.75 minutes)	7.2	0.039	13.6	0.074	ND	--	ND	--
RT = 25.75 minutes	5.3	0.029	ND	--	ND	--	ND	--
RT = 26.75 minutes	6.1	0.033	ND	--	ND	--	ND	--
RT28 (28.25 minutes)	3.3	0.018	2.9	0.016	ND	--	ND	--
RT = 28.95 minutes	ND	--	ND	--	7.1	0.019	ND	--
RT29 (29.25 minutes)	11.2	0.061	ND	--	ND	--	ND	--
RT = 30.77 minutes	ND	--	ND	--	12.3	0.033	ND	--
RT31/RT31H (30.75/31.25 minutes)	10.1	0.055	3.3	0.018	ND	--	ND	--
RT33 (32.75 minutes)	5.0	0.027	ND	--	ND	--	ND	--
RT36/RT36H (36.25/35.75 minutes)	2.8	0.015	10.1	0.055	ND	--	ND	--
RT38H (38.25 minutes)	ND	--	16.2	0.088	ND	--	ND	--
RT45H (44.75 minutes)	ND	--	1.7	0.009	ND	--	ND	--
RT51/RT51H (50.25/51.25 minutes)	2.4	0.013	5.5	0.030	ND	--	ND	--
Other unknowns ³	20.9	0.114	24.3	0.133	56.9	0.153	78.6	0.114

Table B.7.9.1.1-11. Distribution of Radioactive Residues in Rotational Wheat Hay Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹

Metabolite Fraction ²	30-day PBI				120-day PBI		365-day PBI	
	TRR = 0.544 ppm				TRR = 0.269 ppm		TRR = 0.145 ppm	
	Neutral extract		Hydrolyzed extract		Neutral extract		Neutral extract	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
PES ⁴	22.2	0.121			23.8	0.064	21.4	0.031
0.1 N HCl hydrolysate	3.5	0.019			2.6	0.007	2.8	0.004
0.1 N KOH hydrolysate	4.0	0.022			3.7	0.010	4.1	0.006
6 N HCl hydrolysate	2.0	0.011			1.5	0.004	2.1	0.003
6 N KOH hydrolysate	5.3	0.029			6.7	0.018	4.8	0.007
6 N HCl reflux hydrolysate					3.0	0.008		
6 N KOH reflux hydrolysate					2.2	0.006		
PES	7.4	0.040			4.1	0.011	7.6	0.011

¹ ND = Not detectable. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. The 30-day PBI ACN/water extract (neutral extract) was subjected to acid hydrolysis to further characterize residues (hydrolyzed extract). The 30-day PBI extracts (both) were analyzed using HPLC Method CR, the 120-day PBI extract was analyzed using HPLC Method 6, and the 365-day PBI neutral extract was analyzed using HPLC Method 6A.

² Specific components detected in the hydrolyzed extract(s) are designated by an "H", e.g. RT24H.

³ Consisting of: 21 unknowns, each present at ≤3.3% TRR (≤0.018 ppm) in 30-day PBI neutral extract; 14 unknowns, each present at ≤3.3% TRR (≤0.018 ppm) in 30-day PBI hydrolyzed extract; 18 unknowns, each present at ≤4.8% TRR (≤0.013 ppm) in 120-day PBI extract; and 34 unknowns, each present at ≤4.6% TRR (≤0.007 ppm) in 365-day PBI neutral extract.

⁴ PES hydrolyses were conducted at ambient temperature unless otherwise noted.

Table B.7.9.1.1-12. Distribution of Radioactive Residues in Rotational Wheat Grain Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹

Metabolite Fraction	30-day PBI		120-day PBI		365-day PBI	
	TRR = 0.058 ppm		TRR = 0.046 ppm		TRR = 0.031 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN/water	31.0	0.018	43.5	0.020	29.0	0.009
RT = 2.75 minutes	5.2	0.003	ND	--		
RT = 3.25 minutes	ND	--	5.2	0.002		
RT21 (20.75 minutes)	5.2	0.003	ND	--		
RT = 21.25 minutes	ND	--	7.0	0.003		
RT24 (24.25 minutes)	3.4	0.002	ND	--		
RT29 (29.25 minutes)	6.9	0.004	ND	--		
RT = 43.25 minutes	ND	--	6.3	0.003		
Other unknowns ²	8.5	0.005	24.8	0.012		
PES ³	69.0	0.040	56.5	0.026	71.0	0.022
0.1 N HCl hydrolysate	3.4	0.002	3.3	0.002	ND	--
0.1 N KOH hydrolysate	3.4	0.002	3.9	0.002	6.5	0.002
6 N HCl hydrolysate	6.9	0.004	12.8	0.006	22.6	0.007

Table B.7.9.1.1-12. Distribution of Radioactive Residues in Rotational Wheat Grain Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹						
Metabolite Fraction	30-day PBI		120-day PBI		365-day PBI	
	TRR = 0.058 ppm		TRR = 0.046 ppm		TRR = 0.031 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
6 N HCl reflux hydrolysate	34.5	0.020	23.3	0.011	22.6	0.007
6 N KOH reflux hydrolysate	8.6	0.005	12.2	0.006	9.7	0.003
PES	12.1	0.007	<0.1	<0.001	9.7	0.003

¹ ND = Not detectable. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. No solvent extracts of wheat grain were subjected to hydrolysis procedures. The 30-day PBI extract was analyzed using HPLC Method CR and 120-day PBI extract was analyzed using HPLC Method 6A.

² Consisting of: 4 unknowns, each present at ≤3.4% TRR (≤0.002 ppm) in 30-day PBI extract; and 29 unknowns, each present at ≤3.0% TRR (≤0.001 ppm) in 120-day PBI extract.

³ PES hydrolyses were conducted at ambient temperature unless otherwise noted.

Table B.7.9.1.1-13. Distribution of Radioactive Residues in Rotational Wheat Straw Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹										
Metabolite Fraction ²	30-day PBI				120-day PBI		365-day PBI			
	TRR = 1.099 ppm				TRR = 0.758 ppm		TRR = 0.321 ppm			
	Neutral extract		Hydrolyzed extract		Neutral extract		Neutral extract		Hydrolyzed extract	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN/water	75.4	0.829	75.4	0.829	78.9	0.598	76.3	0.245	76.3	0.245
RT = 17.75 minutes	5.9	0.065	ND	--	ND	--	ND	--	ND	--
RT21 (20.75 minutes)	3.3	0.036	ND	--	ND	--	ND	--	3.1	0.010
RT24/RT24H (23.75/24.75 minutes)	8.2	0.090	13.6	0.149	ND	--	ND	--	16.2	0.052
RT = 25.75 minutes	5.3	0.058	ND	--	ND	--	ND	--	ND	--
RT = 27.25 minutes	6.6	0.073	ND	--	ND	--	ND	--	ND	--
RT28H (27.75-28.75 minutes)	ND	--	5.4	0.059	ND	--	ND	--	11.2	0.036
RT29 (29.25 minutes)	7.9	0.087	ND	--	ND	--	ND	--	ND	--
RT = 30.62 minutes	ND	--	ND	--	5.8	0.044	ND	--	ND	--
RT = 30.75 minutes	6.5	0.071	ND	--	ND	--	ND	--	ND	--
RT31H (31.75 minutes)	ND	--	3.1	0.034	ND	--	ND	--	2.5	0.008
RT33 (32.75 minutes)	4.5	0.049	ND	--	ND	--	ND	--	ND	--
RT = 34.48 minutes	ND	--	ND	--	ND	--	5.6	0.018	ND	--
RT36/RT36H (35.75/36.75 minutes)	4.5	0.049	8.0	0.088	ND	--	ND	--	11.8	0.038
RT38H (38.25-39.25 minutes)	ND	--	12.9	0.142	ND	--	ND	--	6.5	0.021
RT = 39.86 minutes	ND	--	ND	--	ND	--	6.2	0.020	ND	--
RT45/RT45H (44.75 minutes)	1.8	0.020	1.5	0.017	ND	--	ND	--	1.6	0.005
RT = 46.22 minutes	ND	--	ND	--	ND	--	5.0	0.016	ND	--
RT51H (50.75-52.25 minutes)	0.4	0.004	6.8	0.075	ND	--	ND	--	3.1	0.010
Other unknowns ³	20.8	0.227	24.1	0.265	73.2	0.555	59.4	0.191	20.2	0.065
PES ⁴	24.6	0.270			21.1	0.160	23.7	0.076		
0.1 N HCl hydrolysate	2.5	0.028			2.6	0.020	4.4	0.014		
0.1 N KOH hydrolysate	2.6	0.029			3.4	0.026	6.5	0.021		
6 N HCl hydrolysate	1.3	0.014			1.3	0.010	2.5	0.008		
6 N KOH hydrolysate	4.1	0.045			4.2	0.032	5.0	0.016		

Table B.7.9.1.1-13. Distribution of Radioactive Residues in Rotational Wheat Straw Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹

Metabolite Fraction ²	30-day PBI				120-day PBI		365-day PBI			
	TRR = 1.099 ppm				TRR = 0.758 ppm		TRR = 0.321 ppm			
	Neutral extract		Hydrolyzed extract		Neutral extract		Neutral extract		Hydrolyzed extract	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
6 N HCl reflux hydrolysate	1.4	0.015			1.7	0.013				
6 N KOH reflux hydrolysate	1.9	0.021			2.2	0.017				
PES	10.7	0.118			5.5	0.042	5.3	0.017		

¹ ND = Not detectable. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. The 30- and 365-day PBI ACN/water extracts (neutral extracts) were subjected to acid hydrolysis to further characterize residues (hydrolyzed extracts). The 30-day PBI extracts (both) and the 365-day PBI hydrolyzed extract were analyzed using HPLC Method CR, the 120-day PBI extract was analyzed using HPLC Method 6, and the 365-day PBI neutral extract was analyzed using HPLC Method 6A.

² Specific components detected in the hydrolyzed extract(s) are designated by an "H", e.g. RT24H.

³ Consisting of: 16 unknowns, each present at ≤4.5% TRR (≤0.049 ppm) in 30-day PBI neutral extract; 7 unknowns, each present at ≤8.5% TRR (≤0.093 ppm) in 30-day PBI hydrolyzed extract; 46 unknowns, each present at ≤3.7% TRR (≤0.028 ppm) in 120-day PBI extract; 35 unknowns, each present at ≤4.4% TRR (≤0.014 ppm) in 365-day PBI neutral extract; and 20 unknowns, each present at ≤3.4% TRR (≤0.011 ppm) in 365-day PBI hydrolyzed extract.

⁴ PES hydrolyses were conducted at ambient temperature unless otherwise noted.

C. Storage Stability of Residues

Sample storage conditions and intervals are presented in Table B.7.9.1.1-14. Initial extractions were completed within 16-38 days of sample collection; 30-day PBI carrot was extracted a second time, 299 days after collection. PES hydrolyses were conducted 120-348 days after collection. Analyses of extracts using HPLC Methods 6 or 6A were completed within 30-196 days of extraction, and analyses using HPLC Method CR were completed within 56-431 days of extraction. Hydrolyses of extracts were conducted 56-425 days after initial extraction, and analyses of the hydrolyzed extracts were conducted within 1-2 days.

To support stability of samples during storage, 30-day PBI mature spinach and wheat hay were re-extracted 442 and 401 days, respectively, after collection. The extracts were hydrolyzed and the hydrolyzed extracts were analyzed using HPLC Method CR. The results of the analysis of the hydrolyzed mature spinach extract were compared to those of 30-day PBI immature spinach hydrolyzed extract, in which the extract had been stored for 425 days prior to hydrolysis and analysis; the HPLC profiles were very similar. The results of the analysis of the hydrolyzed wheat hay extract were compared to those of the 30-day PBI wheat hay hydrolyzed extract, in which the extract had been stored for 385 days prior to hydrolysis and analysis; the HPLC profiles were very similar.

The storage stability analyses do not fully support the confined rotational crop study because only two matrices were tested, and the stability of neutral extracts during storage was not evaluated; however, no additional storage stability data will be required. The results of all HPLC Method 6/6A analyses were included in the submission, and these analyses, which were generally conducted within 6 months of sample collection, showed that the metabolite profile in all matrices was multicomponent with no compounds that appeared to co-chromatograph with parent or reference standards.

Table B.7.9.1.1-14. Summary of Storage Conditions.

Matrix	Storage Temperature (°C)	Actual Storage Duration (days)			Interval of Demonstrated Storage Stability (days)
		Collection to extraction/PES hydrolysis ¹	Extraction to hydrolysis of extracts ²	Extraction/ hydrolysis to analysis ³	
Carrot roots	ca. -20 °C	33, 299 ⁴ ; 250	56	30-56	The metabolite profile in 30-day PBI mature spinach and wheat hay, after hydrolysis of extractable radioactivity, was shown to be relatively stable for at least 401 days.
Carrot tops		33-38; 120-250	192-322	1-322	
Immature spinach		16-28; 197-348	292-425	1-425	
Mature spinach		21-35; 186-328	Not applicable	96-431	
Wheat forage		16-22; 197-328	Not applicable	97-411	
Wheat hay		7-31; 182-287	385	1-385	
Wheat grain		23-28; 158-217	Not applicable	146-300	
Wheat straw		23-28; 158-217	241-299	1-300	

¹ When two numbers are presented, the first number represents the interval to solvent extraction and the second number represents the interval to PES hydrolysis.

² Interval from extraction to hydrolysis of extract.

³ Intervals for analyses presented in Tables B.7.9.1.1-6 through B.7.9.1.1-13.

⁴ 30-day PBI carrot root was extracted a second time, 299 days after harvest.

D. Identity of Residues in Rotational Carrot, Spinach, and Wheat

The characterization and identification of radioactivity in rotational carrot, spinach, wheat matrices are summarized in Tables B.7.9.1.1-15 (carrot root), B.7.9.1.1-16 (carrot tops), B.7.9.1.1-17 (immature spinach), B.7.9.1.1-18 (mature spinach), B.7.9.1.1-19 (wheat forage), B.7.9.1.1-20 (wheat hay), B.7.9.1.1-21 (wheat grain), and B.7.9.1.1-22 (wheat straw).

Pethoxamid was only detected in carrot root, at 11.3% TRR (0.008 ppm) in 30-day PBI root and 3.8% TRR (0.001 ppm) in 120-day PBI root. The identification was made in extracts analyzed using HPLC Method 6. Because pethoxamid was not found in 30-day PBI carrot root when the extract was analyzed using HPLC Method CR, the identification should be considered tentative.

1D-TLC analyses of 30-day PBI immature spinach indicated the presence of MET-30 at very low levels (<0.001 ppm); analysis of the extract after hydrolysis showed that MET-30 was no longer present. Although several other extracts were analyzed by 1D-TLC, MET-30 was not conclusively found in any other rotational crop matrix.

Eight hydrolyzed components, labeled as RT24H, RT28H, RT31H, RT36H, RT38H, RT45H, RT51H, and RT54H, were found to comprise a significant portion of the radioactivity in the hydrolyzed extracts of selected rotational crop matrices; these compounds are listed in the summary tables below. Five of the components were isolated from 30-day PBI immature spinach hydrolyzed extract and subjected to LC/MS and LC/MS/MS analyses: RT24H, RT28H, RT31H, RT36H, and RT38H. Based on the analyses, hydrolyzed components RT28H, RT36H, and RT38H were identified, and a structure for RT24H was proposed. For RT24H, the study authors noted that the MS/MS results did not fully support the proposed structure; therefore, the identification is considered to be tentative. Because there did not appear to be any attempt to demonstrate that compounds identified in 30-day PBI immature spinach were the same as those found in other rotational crop matrices (through, for example, co-chromatography of extracts), the identification of these compounds in other matrices is considered tentative.

Additional hydrolysis experiments were conducted with the 30-day PBI wheat straw extract. The extract was hydrolyzed in 1 N HCl, which is known to hydrolyze acetals; this resulted in the loss of the RT45 peak and the formation of an RT28 peak. Pronase hydrolysis yielded unretained components. Hydrogen peroxide treatment produced peaks that were not seen in the acid-hydrolyzed extract. Hydrolysis with meta-chloroperoxybenzoic acid hydrolysis mainly produced polar and unretained components. Base hydrolysis did not change the overall profile compared to that of the neutral extract, except that an additional late-eluting peak was observed in the base hydrolysate. No further investigations were conducted.

Table B.7.9.1.1-15. Summary of Characterization and Identification of Radioactive Residues in Rotational Carrot Root Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹

Metabolite Fraction	30-day PBI				120-day PBI	
	TRR = 0.071 ppm				TRR = 0.026 ppm	
	Neutral extract		Hydrolyzed extract		Neutral extract	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
Pethoxamid	11.3	0.008	ND	--	3.8	0.001
RT24/RT24H	2.6	0.002	6.5	0.005	ND	--
RT36/RT36H	1.3	0.001	5.2	0.004	ND	--
RT38H	ND	--	11.7	0.009	ND	--
RT51H	ND	--	9.1	0.007	ND	--
RT54H	ND	--	20.8	0.016	ND	--
Other unknowns	76.7	0.059	27.3	0.021	76.8	0.020
PES hydrolysates	8.4	0.006	NA	--	3.8	0.001
Total extractable	88.9	0.068	NA	--	84.6	0.022
Total tentatively identified ²	11.3	0.008	23.4	0.018	3.8	0.001
Total characterized	8.4	0.066	NA	--	3.8	0.001
Total unidentified ³	80.5	0.062	57.2	0.044	76.8	0.020
Total bound residues (PES)	7.0	0.005	NA	--	15.4	0.004
% Accountability ⁴	95.9		NA		100.0	

¹ ND = Not detectable. NA = Not applicable. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. The 30-day PBI ACN/water extract (neutral extract) was subjected to acid hydrolysis to further characterize residues (hydrolyzed extract).

² Hydrolyzed components RT24H, RT36H, and RT38H were tentatively identified in 30-day PBI hydrolyzed extract.

³ Unidentified radioactive components released by solvent extraction.

⁴ Total (ppm)/TRR (ppm)×100.

Table B.7.9.1.1-16. Summary of Characterization and Identification of Radioactive Residues in Rotational Carrot Tops Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹										
Metabolite Fraction	30-day PBI				120-day PBI		365-day PBI			
	TRR = 0.292 ppm				TRR = 0.104 ppm		TRR = 0.031 ppm		TRR = 0.031 ppm	
	Neutral extract		Hydrolyzed extract		Neutral extract		Neutral extract		Hydrolyzed extract	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
RT24/RT24H	12.7	0.037	19.5	0.057	ND	--	ND	--	ND	--
RT31H	ND	--	3.8	0.011	ND	--	ND	--	ND	--
RT36/RT36H	2.4	0.007	14.4	0.042	ND	--	ND	--	ND	--
RT38H	ND	--	17.5	0.051	ND	--	ND	--	ND	--
RT45H	ND	--	2.1	0.006	ND	--	ND	--	ND	--
RT51/RT51H	1.0	0.003	5.1	0.015	ND	--	ND	--	ND	--
RT54/RT54H	1.7	0.005	2.1	0.006	ND	--	ND	--	87.1	0.027
Other unknowns	68.4	0.200	22.0	0.064	87.5	0.091	87.1	0.027	ND	--
PES hydrolysates	6.2	0.018	NA	--	5.8	0.006	ND	--	NA	--
Total extractable	92.5	0.270	NA	--	93.3	0.097	87.1	0.027	NA	--
Total tentatively identified ²	NA	--	51.4	0.150	NA	--	NA	--	NA	--
Total characterized	6.2	0.018	NA	--	5.8	0.006	<0.1	--	NA	--
Total unidentified ³	86.2	0.252	35.1	0.102	87.5	0.091	87.1	0.027	87.1	0.027
Total bound residues (PES)	7.5	0.022	NA	--	6.7	0.007	12.9	0.004	NA	--
% Accountability ⁴	100.0		NA		100.0		100.0		NA	

¹ ND = Not detectable. NA = Not applicable. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. The 30- and 365-day PBI ACN/water extracts (neutral extracts) were subjected to acid hydrolysis to further characterize residues (hydrolyzed extracts).

² Hydrolyzed components RT24H, RT36H, and RT38H were tentatively identified in 30-day PBI hydrolyzed extract.

³ Unidentified radioactive components released by solvent extraction.

⁴ Total (ppm)/TRR (ppm)×100.

Table B.7.9.1.1-17. Summary of Characterization and Identification of Radioactive Residues in Rotational Immature Spinach Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹

Metabolite Fraction	30-day PBI				120-day PB		365-day PBI			
	TRR = 0.539 ppm				TRR = 0.335 ppm		TRR = 0.069 ppm			
	Neutral extract		Hydrolyzed extract		Neutral extract		Neutral extract		Hydrolyzed extract	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
MET-30	<0.2	<0.001	ND	--	ND	--	ND	--	ND	--
RT24/RT24H	13.5	0.073	15.2	0.082	ND	--	ND	--	11.6	0.008
RT28/RT28H	9.8	0.053	9.8	0.053	ND	--			5.8	0.004
RT31H	ND	--	5.2	0.028	ND	--			15.9	0.011
RT36/RT36H	1.4	0.007	5.2	0.028	ND	--	ND	--	8.7	0.006
RT38H	ND	--	3.0	0.016	ND	--	ND	--	1.4	0.001
RT45H	ND	--	3.2	0.017	ND	--	ND	--	1.4	0.001
RT51H	ND	--	3.7	0.020	ND	--	ND	--	ND	--
RT54H	ND	--	ND	--	ND	--	ND	--	8.7	0.006
Other unknowns	47.7	0.257	26.9	0.146	88.7	0.297	85.5	0.059	31.5	0.022
PES hydrolysates	18.9	0.102	NA	--	5.1	0.017	12.9	0.009	NA	--
Total extractable	91.3	0.492	NA	--	93.8	0.314	98.4	0.068	NA	--
Total identified/ tentatively identified ²	<0.2	<0.001	33.2	0.179	NA	--	NA	--	27.5	0.019
Total characterized	18.9	0.102	NA	--	5.1	0.017	12.9	0.009	NA	--
Total unidentified ³	72.4	0.390	39.0	0.211	88.7	0.297	85.5	0.059	58.0	0.040
Total bound residues (PES)	8.7	0.047	NA	--	6.3	0.021	1.4	0.001	NA	--
% Accountability ⁴	100.0		NA		100.0		100.0		NA	

¹ ND = Not detectable. NA = Not applicable. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. The 30- and 365-day PBI ACN/water extracts (neutral extracts) were subjected to acid hydrolysis to further characterize residues (hydrolyzed extracts).

² Hydrolyzed components RT28H, RT36H, and RT38H were identified and component RT24H was tentatively identified in 30-day PBI hydrolyzed extract; identification of these metabolites in 365-day PBI hydrolyzed extract was tentative.

³ Unidentified radioactive components released by solvent extraction.

⁵ Total (ppm)/TRR (ppm)×100.

Table B.7.9.1.1-18. Summary of Characterization and Identification of Radioactive Residues in Rotational Mature Spinach Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹

Metabolite Fraction	30-day PBI		120-day PBI		365-day PBI	
	TRR = 0.465 ppm		TRR = 0.156 ppm		TRR = 0.069 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
RT24	21.9	0.102	ND	--	ND	--
RT28	3.9	0.018	ND	--	ND	--
RT36	2.4	0.011	ND	--	ND	--
Other unknowns	42.5	0.240	87.8	0.137	85.5	0.059
PES hydrolysates	8.6	0.040	4.4	0.007	7.1	0.005
Total extractable	88.4	0.411	92.2	0.144	92.6	0.064
Total identified	NA	--	NA	--	NA	--
Total characterized	8.6	0.040	4.4	0.007	7.1	0.005
Total unidentified ²	70.7	0.371	87.8	0.137	85.5	0.059
Total bound residues (PES)	11.6	0.054	7.7	0.012	7.2	0.005
% Accountability ³	100.0		100.0		100.0	

¹ ND = Not detectable. NA = Not applicable. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Unidentified radioactive components released by solvent extraction.

³ Total (ppm)/TRR (ppm)×100

Table B.7.9.1.1-19. Summary of Characterization and Identification of Radioactive Residues in Rotational Wheat Forage Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹

Metabolite Fraction	30-day PBI		120-day PBI		365-day PBI	
	TRR = 0.232 ppm		TRR = 0.212 ppm		TRR = 0.130 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
RT24	7.8	0.018	ND	--	ND	--
RT51	2.6	0.006	ND	--	ND	--
Other unknowns	62.5	0.145	84.9	0.180	76.2	0.099
PES hydrolysates	23.3	0.054	10.9	0.023	15.3	0.020
Total extractable	96.1	0.223	95.8	0.203	91.5	0.119
Total identified	NA	--	NA	--	NA	--
Total characterized	23.3	0.054	10.9	0.023	15.3	0.020
Total unidentified ²	72.9	0.169	84.9	0.180	76.2	0.099
Total bound residues (PES)	3.9	0.009	4.2	0.009	8.5	0.011
% Accountability ³	100.0		100.0		100.0	

¹ ND = Not detectable. NA = Not applicable. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Unidentified radioactive components released by solvent extraction.

³ Total (ppm)/TRR (ppm)×100.

Table B.7.9.1.1-20. Summary of Characterization and Identification of Radioactive Residues in Rotational Wheat Hay Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹

Metabolite Fraction	30-day PBI				120-day PBI		365-day PBI	
	TRR = 0.544 ppm				TRR = 0.269 ppm		TRR = 0.145 ppm	
	Neutral extract		Hydrolyzed extract		Neutral extract		Neutral extract	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
RT24/RT24H	7.2	0.039	13.6	0.074	ND	--	ND	--
RT28/RT28H	3.3	0.018	2.9	0.016	ND	--	ND	--
RT29	11.2	0.061	ND	--	ND	--	ND	--
RT31/RT31H	10.1	0.055	3.3	0.018	ND	--	ND	--
RT36/RT36H	2.8	0.015	10.1	0.055	ND	--	ND	--
RT38H	ND	--	16.2	0.088	ND	--	ND	--
RT45H	ND	--	1.7	0.009	ND	--	ND	--
RT51/RT51H	2.4	0.013	5.5	0.030	ND	--	ND	--
Other unknowns	41.2	0.224	24.3	0.133	76.2	0.205	78.6	0.114
PES hydrolysates	14.8	0.081	NA	--	19.7	0.053	13.8	0.020
Total extractable	92.6	0.504	NA	--	95.9	0.258	92.4	0.134
Total tentatively identified ²	NA	--	42.8	0.233	NA	--	NA	--
Total characterized	14.8	0.081	NA	--	19.7	0.053	13.8	0.020
Total unidentified ³	77.8	0.423	34.9	0.190	76.2	0.205	78.6	0.114
Total bound residues (PES)	7.4	0.040	NA	--	4.1	0.011	7.6	0.011
% Accountability ⁴	100.0		100.0		100.0		100.0	

¹ ND = Not detectable. NA = Not applicable. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. The 30-day PBI ACN/water extract (neutral extract) was subjected to acid hydrolysis to further characterize residues (hydrolyzed extract).

² Hydrolyzed components RT24H, RT28H, RT36H, and RT38H were tentatively identified in 30-day PBI hydrolyzed extract.

³ Unidentified radioactive components released by solvent extraction.

⁴ Total (ppm)/TRR (ppm)×100

Table B.7.9.1.1-21. Summary of Characterization and Identification of Radioactive Residues in Rotational Wheat Grain Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹

Metabolite Fraction	30-day PBI		120-day PBI		365-day PBI	
	TRR = 0.058 ppm		TRR = 0.046 ppm		TRR = 0.031 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
RT24	3.4	0.002	ND	--	NA	--
Other unknowns	25.8	0.015	43.3	0.020	NA	--
PES hydrolysates	56.8	0.033	56.5	0.026	61.3	0.019
Total extractable	87.8	0.051	99.9	0.046	90.4	0.028
Total identified	NA	--	NA	--	NA	--
Total characterized	56.8	0.033	56.5	0.026	61.3	0.019
Total unidentified ²	29.2	0.017	43.3	0.020	NA	--
Total bound residues (PES)	12.1	0.007	<0.1	<0.001	9.7	0.003
% Accountability ³	100.0		100.0		100.0	

¹ ND = Not detectable. NA = Not applicable. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Unidentified radioactive components released by solvent extraction.

³ Total (ppm)/TRR (ppm)×100

Table B.7.9.1.1-22. Summary of Characterization and Identification of Radioactive Residues in Rotational Wheat Straw Following Application of [¹⁴C]Pethoxamid to Soil at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha).¹

Metabolite Fraction	30-day PBI				120-day PBI		365-day PBI			
	TRR = 1.099 ppm				TRR = 0.758 ppm		TRR = 0.321 ppm			
	Neutral extract		Hydrolyzed extract		Neutral extract		Neutral extract		Hydrolyzed extract	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
RT24/RT24H	8.2	0.090	13.6	0.149	ND	--	ND	--	16.2	0.052
RT28H	ND	--	5.4	0.059	ND	--	ND	--	11.2	0.036
RT31H	ND	--	3.1	0.034	ND	--	ND	--	2.5	0.008
RT36/RT36H	4.5	0.049	8.0	0.088	ND	--	ND	--	11.8	0.038
RT38H	ND	--	12.9	0.142	ND	--	ND	--	6.5	0.021
RT45H	1.8	0.020	1.5	0.017	ND	--	ND	--	1.6	0.005
RT51H	ND	--	6.8	0.075	ND	--	ND	--	3.1	0.010
Other unknowns	60.8	0.666	24.1	0.265	78.9	0.598	76.3	0.245	23.3	0.075
PES hydrolysates	13.8	0.152	NA	--	15.4	0.118	18.4	0.059	NA	--
Total extractable	89.2	0.981	NA	--	94.3	0.716	94.7	0.304	NA	--
Total tentatively identified ²	NA	--	39.9	0.438	NA	--	NA	--	45.8	0.147
Total characterized	13.8	0.152	NA	--	15.4	0.118	18.4	0.059	NA	--
Total unidentified ³	75.4	0.829	35.5	0.391	78.9	0.598	76.3	0.245	30.5	0.098
Total bound residues (PES)	10.7	0.118	NA	--	5.5	0.042	5.3	0.017	NA	--
% Accountability ⁴	100.0		NA		100.0		100.0		NA	

¹ ND = Not detectable. NA = Not applicable. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. The 30- and 365-day PBI ACN/water extracts (neutral extracts) were subjected to acid hydrolysis to further characterize residues (hydrolyzed extracts).

² Hydrolyzed components RT24H, RT28H, RT36H, and RT38H were tentatively identified in 30- and 365-day PBI hydrolyzed extracts.

³ Unidentified radioactive components released by solvent extraction.

⁴ Total (ppm)/TRR (ppm)×100

E. Proposed Metabolic Pathway

Based on the results of the study, the study authors proposed that the metabolism of pethoxamid in rotational carrots, spinach, and wheat is similar to that of the primary crops of potato, soybean, and corn (refer to the DERs for MRID 49813527-49813529; B.7.1.1-B.7.1.3). The proposed pathway is presented in Figure B.7.9.1.1-4. The first step is proposed to be the displacement of the chlorine atom by the thiolate anion of glutathione to yield a glutathione conjugate (not found in this study). The glutamic acid and glycine portions of glutathione are then cleaved to form the cysteine conjugate MET-30, which may be metabolized by a number of pathways common to cysteine conjugates, forming many products. The three components identified after hydrolysis of extracts (RT28H, RT36H, and RT38H) were found to be cleaved components of pethoxamid.

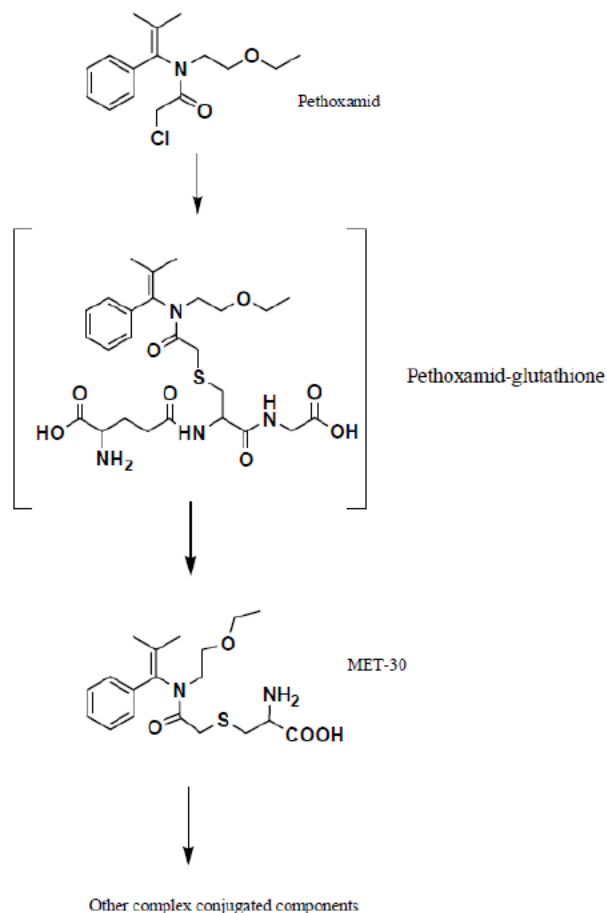
Figure B.7.9.1.1-4. Proposed Metabolic Profile of Pethoxamid in Rotational Crops.


Table B.7.9.1.1-23. Identification of Compounds from Confined Rotational Crop Study (both proposed and found).		
Common Name/Code	Chemical Name	Chemical Structure
Pethoxamid	2-chloro-N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propen-1-yl)acetamide	
MET-30	S-(2-((2-ethoxyethyl)(2-methyl-1-phenylprop-1-en-1-yl)-amino)-2-oxoethyl)cysteine	
RT28H	(2-hydroxy-1-phenyl-propenyl)methyl-ammonium	

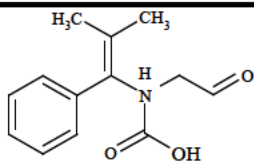
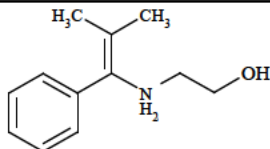
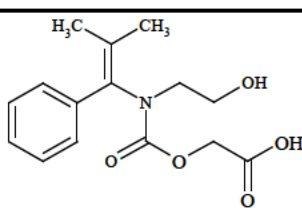
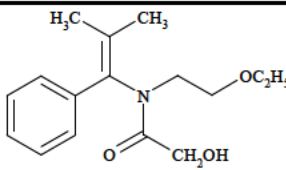
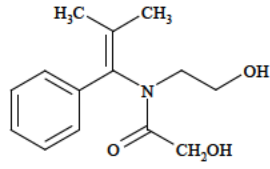
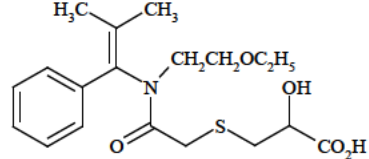
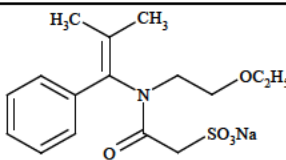
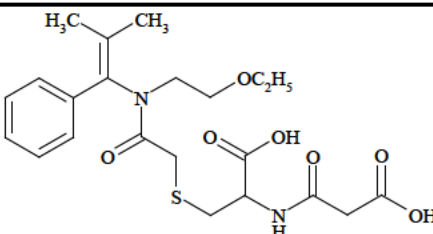
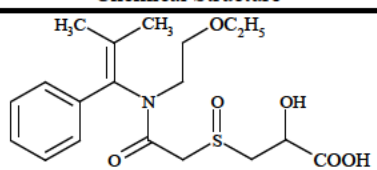
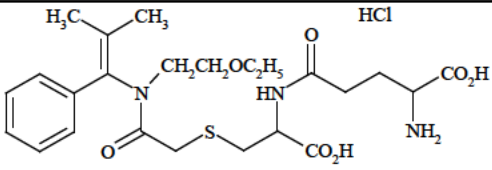
Table B.7.9.1.1-23. Identification of Compounds from Confined Rotational Crop Study (both proposed and found).		
Common Name/Code	Chemical Name	Chemical Structure
RT36H	not provided	
RT38H	(2-hydroxy-ethyl)-(2-methyl-1-phenylpropenyl)-ammonium	
RT24H ¹	[(2-hydroxy-ethyl)-(2-methyl-1-phenylpropenyl)carbamoyloxy]acetic acid	
Additional reference standard compounds used in the study but not identified in any matrix		
MET-2	N-(2-ethoxyethyl)-2-hydroxy-N-(2-methyl-1-phenylprop-1-en-1-yl)-acetamide	
MET-3	2-hydroxy-N-(2-hydroxyethyl)-N-(2-methyl-1-phenylprop-1-en-1-yl)-acetamide	
MET-41	3-((2-((2-ethoxyethyl)(2-methyl-1-phenylprop-1-enyl)amino)-2-oxoethyl)thio)-2-hydroxypropanoic acid	
MET-42	2-[(2-ethoxyethyl)(2-methyl-1-phenyl-1-propen-1-yl)amino]-2-oxoethanesulfonic acid, sodium salt	
MET-47	11-carboxy-6-(2-methyl-1-phenylprop-1-en-1-yl)-7,13-dioxo-3-oxa-9-thia-6,12-diazapentadecan-15-oic acid	

Table B.7.9.1.1-23. Identification of Compounds from Confined Rotational Crop Study (both proposed and found).		
Common Name/Code	Chemical Name	Chemical Structure
MET-50	3-((2-((2-ethoxyethyl)(2-methyl-1-phenylprop-1-en-1-yl)amino)-2-oxoethyl)sulfinyl)-2-hydroxypropanoic acid	
MET-103	16-amino-11-carboxy-6-(2-methyl-1-phenylprop-1-en-1-yl)-7,13-dioxo-3-oxa-9-thia-6,12-diazaheptadecan-17-oic acid hydrochloric acid salt	

¹ The identification of RT24H was tentative based on LC/MS data.

III. CONCLUSIONS

The confined rotational crop study is considered scientifically acceptable. In rotational crops of carrot, spinach, and wheat planted at PBIs of 30, 120, and 365 days following soil application of [¹⁴C]pethoxamid at 1.16-1.17 lb ai/A (1.30-1.31 kg ai/ha), TRR were highest at the 30-day PBI and lowest at the 365-day PBI. Overall, TRR were lowest in carrot root and wheat grain and highest in spinach, wheat hay, and wheat straw.

TRR were >0.01 ppm in all rotational crop matrices except 365-day PBI carrot root. TRR in 30-, 120-, and 365-day PBI matrices, respectively, were: 0.071, 0.026, and 0.007 ppm in carrot root; 0.292, 0.104, and 0.031 ppm in carrot tops; 0.539, 0.335, and 0.069 ppm in immature spinach; 0.465, 0.156, and 0.069 ppm in mature spinach; 0.232, 0.212, and 0.130 ppm in wheat forage; 0.544, 0.269, and 0.145 ppm in wheat hay; 0.058, 0.046, and 0.031 ppm in wheat grain; and 1.099, 0.758, and 0.321 ppm in wheat straw.

Pethoxamid was initially detected in the combined ACN/water extracts of 30- and 120-day PBI carrot root, at 11.3% TRR and 3.8% TRR, respectively, but the identification could not be confirmed. MET-30 was found in 30-day PBI immature spinach at very low levels (<0.2% TRR). The overall level of identification in the initial solvent extracts was low (≤11% TRR). The remainder of the solvent-extractable radioactivity consisted of single or multi-component unknowns, each present at ≤15.9% TRR in hydrolyzed extracts, and at ≤21.9% TRR in neutral extracts. The remainder of the solvent-extractable radioactivity consisted of single or multicomponent unknowns, each present at ≤21.9% TRR.

Following acid hydrolysis of the original extracts, three components, designated RT28H, RT36H, and RT38H, were identified in the acid-hydrolyzed extract of 30-day PBI immature spinach using LC/MS/MS, and a fourth hydrolyzed component, RT24H, was putatively identified in the same manner. These compounds were tentatively identified in the hydrolyzed extracts of other rotational crop matrices by retention time comparison. Compound RT24H was found in 30-day PBI hydrolyzed extracts as follows: 6.5% TRR in carrot root; 19.5% TRR in carrot tops; 15.2% TRR in immature spinach; 13.6% TRR in wheat hay; and 13.6% TRR in wheat straw. RT24H was also found in the hydrolyzed extracts of 365-day PBI immature

spinach (11.6% TRR) and wheat straw (16.2% TRR). RT28H was found in 30-day PBI hydrolyzed extracts as follows: 9.8% TRR in immature spinach; 2.9% TRR in wheat hay; and 5.4% TRR in wheat straw. RT28H was also found in the hydrolyzed extracts of 365-day PBI immature spinach (5.8% TRR) and wheat straw (11.2% TRR). RT36H was found in 30-day PBI hydrolyzed extracts as follows: 5.2% TRR in carrot root; 14.4% TRR in carrot tops; 5.2% TRR in immature spinach; 10.1% TRR in wheat hay, and 8.0% TRR in wheat straw. RT36H was also found in the hydrolyzed extracts of 365-day PBI immature spinach (8.7% TRR) and wheat straw (11.8% TRR). RT38H was found in 30-day PBI hydrolyzed extracts as follows: 11.7% TRR in carrot root; 17.5% TRR in carrot tops; 3.0% TRR in immature spinach; 16.2% TRR in wheat hay; and 12.9% TRR in wheat straw. RT38H was also found in the hydrolyzed extracts of 365-day PBI immature spinach (1.4% TRR) and wheat straw (6.5% TRR). The unidentified compound RT54H comprised a significant amount of radioactivity in 30-day PBI carrot root (20.8% TRR) and 365-day PBI carrot tops (87.1% TRR) and was a minor component in 365-day immature spinach (8.7% TRR). The remainder of the solvent-extractable radioactivity consisted of single or multi-component unknowns, each present at $\leq 15.9\%$ TRR.

Mild and strong acid and base hydrolyses of PES released a total of 56.5-61.3% TRR from wheat grain, and 3.8-18.9% TRR from the other rotational crop matrices; no attempts to further characterize this radioactivity were conducted.

Based on the results of the study, the study authors proposed that the metabolism of pethoxamid in rotational carrots, spinach, and wheat is similar to that of observed in the primary crops of potato, soybean, and corn. The first step is proposed to be the displacement of the chlorine atom by the thiolate anion of glutathione to yield a glutathione conjugate (not found in this study). The glutamic acid and glycine portions of glutathione are then cleaved to form the cysteine conjugate MET-30, which may be metabolized by a number of pathways common to cysteine conjugates, forming many products. The three hydrolyzed components identified after hydrolysis of solvent extracts (RT28H, RT36H, and RT38H) were found to be cleaved components of pethoxamid.

REFERENCES

Document ID: MRID No. 49813529; PMRA Study # xxxxxx, PMRA Review # xxxxxx
Report: Mohajeri, S. and Zane, J. (2015) The Metabolism of [^{14}C]Pethoxamid in Potatoes (One Radiolabel). Study Numbers: 1747 PXA; 2505W; 2505W-1. Unpublished study prepared by PTRL West, Inc., sponsored by Cheminova, Inc., and submitted by FMC Corporation. 259 p.

**B.7.9 Residues in Succeeding Crops
(Annex IIA 6.6; Annex IIIA 8.6)**

B.7.9.1 Metabolism and Distribution Studies on Representative Crops (Confined Accumulation in Rotational Crops)

B.7.9.1.2 Turnip, Spinach, and Wheat; 1998-1999 Study

Document ID: MRID No. 49813553
PMRA No. Not available

Report: Corden, M.T. (2000) TKC-94 Accumulation in Confined Rotational Crops. Study Numbers: 128 PXA; TON 051/002006. Unpublished study prepared by Huntingdon Life Sciences Ltd., sponsored by Cheminova, Inc., and submitted by FMC Corporation. 99 p.

Guidelines: U.S. EPA Residue Chemistry Test Guideline OCSP 860.1850 Confined Accumulation in Rotational Crops (August 1996)
PMRA Regulatory Directive Dir98-02 – Residue Chemistry Guidelines, Section 13 - Confined Accumulation in Rotational Crops
OECD Guideline 502 Metabolism in Rotational Crops (January 2007)

GLP Compliance: No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

Acceptability: The study is considered scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP#452109.

Evaluator: Sheila Piper, Chemist, HED, RAB6 *Sheila Piper*

Secondary Evaluator: Robert Lanthier, PMRA

Note: This Data Evaluation Record (DER) was prepared under contract by CDM/CSS-Dynamac Joint Venture (submitted 2/16/18). The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies. The DER has been reviewed by HED and revised as necessary to reflect current Office of Pesticide Programs (OPP) policies.

EXECUTIVE SUMMARY

The metabolism of pethoxamid was investigated in a confined rotational crop study with [phenyl-¹⁴C]pethoxamid (specific activity 2.45 MBq/mg). The radiolabeled test substance was applied to bare sandy silt loam soil at 1.37 lb ai/A (1.53 kg ai/ha). Rotational crops of turnip, spinach, and wheat were planted at a plantback interval (PBI) of 30 days. Crops were grown in a growth room, and samples of rotational crop commodities were harvested at three intervals: first harvest (immature); second harvest (earliest possible harvest); and third harvest (normal harvest). Samples of turnip roots and tops and spinach leaves were collected at each of the harvest intervals, at 54, 79, and 105 days after planting (DAP) for turnips and 47, 70, and 98 DAP for spinach. For wheat, the entire plant (forage) was collected at the first harvest at 70 DAP, and samples of wheat straw, grain, and chaff were collected at the remaining harvests, 126 and 146 DAP. The study was conducted by Huntingdon Life Sciences Ltd. (Huntingdon, UK).

Total radioactive residues (TRR) in all rotational crops were determined by combustion/LSC. TRR were also calculated by summing extractable and nonextractable radioactivity following extraction with acetonitrile (ACN) and ACN/water, and these values were used for all further determinations. For turnip root and spinach, TRR were highest at the first harvest interval and decreased thereafter. TRR in turnip tops remained approximately the same at each harvest interval. For wheat, residues were generally highest at the final (normal) harvest interval. Overall, TRR were highest in wheat straw and lowest in wheat grain and turnip root.

TRR were >0.01 ppm in all rotational crop matrices. In turnip, TRR at 54, 79, and 105 DAP, respectively, were 0.19, 0.070, and 0.053 ppm in root, and 0.58, 0.62, and 0.50 ppm in tops. TRR in spinach were 0.79, 0.51, and 0.41 ppm at 47, 70, and 98 DAP. TRR were 0.48 ppm in wheat forage at 70 DAP; at 126 and 146 DAP, respectively, TRR were 0.61 and 1.79 ppm in wheat straw, 0.13 and 0.16 ppm in wheat grain, and 0.34 and 0.76 ppm in wheat chaff. All rotational crop matrices were subjected to extraction procedures.

Extraction with ACN and ACN/water released the majority of the radioactivity (55-95% TRR) from rotational crop matrices except wheat grain; solvent extraction released 16-43% TRR from wheat grain. Rotational crop matrices from the final harvest interval for turnip root, spinach, and wheat straw, grain, and chaff were also extracted with water, which released <2% TRR from turnip root and spinach, and 7-13% TRR from wheat matrices. Post-extraction solids (PES) from these crop matrices were subjected to separate hydrolyses with 0.2 M HCl, 0.05 M NaOH, and cellulase/hemicellulase. The enzyme hydrolysis released the most radioactivity: 5-7% TRR from turnip root, spinach, and wheat straw and chaff, and 19% TRR from wheat grain. For wheat straw and grain, the PES after enzyme hydrolysis were subjected to hydrolysis using 0.2 M NaOH, which released 1 and 18% TRR, respectively. Many of the hydrolysates were partitioned with ethyl acetate at neutral and acidic pH, but were not further characterized. Remaining PES accounted for ≤0.053 ppm in turnip root and tops, spinach, wheat forage, and 146-DAP wheat grain, and <6.5% TRR in 146-DAP wheat straw. Remaining PES accounted for 29% TRR (0.18 ppm) in 126-DAP wheat straw, 57% TRR (0.074 ppm) in 126-DAP wheat grain, 45% TRR (0.15 ppm) in 126-DAP wheat chaff, and 32% TRR (0.24 ppm) in 146-DAP wheat chaff. The extraction and hydrolysis procedures adequately extracted the majority of residues from rotational crop matrices of concern. Extraction results were normalized; therefore, accountabilities were 100%.

Residues were quantified in the combined ACN/water extracts of rotational crop matrices by one-dimensional thin-layer chromatography (1D-TLC), using co-chromatography with reference standards to identify metabolites. Evidence of identification of metabolites using two techniques (normal- and reverse-phase 1D-TLC) was only provided for 98-DAP spinach; therefore, all other identifications should be considered tentative. No chromatographic analyses of any hydrolysates, including radioactivity partitioned into ethyl acetate, were conducted. No information was provided pertaining to the storage conditions for samples, extracts, or hydrolysates, and no dates of extraction, hydrolysis, or analysis were provided. Based on the sample harvest dates and the reported experimental completion date, samples may have been stored for up to 10.5 months prior to completion of analysis. Previous study on confined rotation crop (MRID 49813552) showed samples of rotational crop commodities were stored frozen from harvest to initial HPLC analysis for metabolite profiling for ≤196 days; certain samples and

extracts were stored frozen for up to 431 days prior to completion of analyses. Supporting storage stability data were provided which demonstrated that the metabolite profiles in mature spinach and wheat hay were generally stable during at least 401 days of frozen storage. Also, acceptable storage stability data supporting potato foliage for up to 631 days (21 months) and 596 days (20 months) in tubers (MRIDs0 49813529).

A total of 2-54% TRR was identified/tentatively identified in rotational crops. Pethoxamid was not detected in any rotational crop matrix. With some exceptions, the following metabolites were detected in each rotational crop matrix at one or more harvest intervals: MET-30, MET-41, MET-42, MET-48, MET-49 and MET-50. Metabolite MET-42, which was the major soil metabolite, was one of the major metabolites detected in turnip tops (14.2-26.4% TRR, 0.082-0.132 ppm), spinach (7.7-9.1% TRR, 0.032-0.072 ppm), wheat forage (14.2% TRR, 0.068 ppm) and straw (9.9-12.9% TRR, 0.060-0.231 ppm). This metabolite was also detected in turnip roots ($\leq 5.7\%$ TRR; ≤ 0.011 ppm) and wheat grain ($\leq 3.5\%$ TRR, ≤ 0.005 ppm), but was not detected in wheat chaff. Metabolite MET-50 was a major metabolite in turnip root (2.8-8.7% TRR, 0.002-0.017 ppm) and tops (12.1-17.7% TRR, 0.075-0.089 ppm), spinach (4.5-12.2% TRR, 0.018-0.096 ppm), and wheat forage (7.5% TRR, 0.036 ppm), and was also detected at $\leq 6.0\%$ TRR (≤ 0.098 ppm) in wheat straw, grain, and chaff. Metabolite MET-30, another major metabolite, was present in turnip root ($\leq 9.8\%$ TRR, ≤ 0.019 ppm) and tops ($\leq 7.5\%$ TRR, ≤ 0.047 ppm), spinach (3.4-7.5% TRR, 0.025-0.038 ppm), and wheat forage (11.3% TRR, 0.054 ppm) and straw (4.3-6.1% TRR, 0.026-0.109 ppm), but was present at $\leq 2.0\%$ TRR (≤ 0.003 ppm) in wheat grain and was not detected in wheat chaff. Metabolite MET-48 accounted for 7.0-8.6% TRR (0.034-0.055 ppm) in spinach, but was a minor metabolite ($\leq 4.1\%$ TRR, ≤ 0.021 ppm) in turnip root and tops and wheat forage, and was not detected in wheat straw, grain, or chaff. Two additional metabolites were found in all rotational crop commodities: MET-41 at $\leq 5.9\%$ TRR (≤ 0.043 ppm); and MET-49 at $\leq 6.0\%$ TRR (≤ 0.043 ppm). The remainder of the solvent-extractable radioactivity consisted of multiple unknowns, each present at $<10\%$ TRR and/or <0.05 ppm, except for: two unknowns in 79-DAP turnip root (at 10.4% TRR, 0.007 ppm, and 13.6% TRR, 0.010 ppm) and one unknown in 105-DAP turnip root (14.4% TRR, 0.008 ppm); one unknown in 105-DAP turnip tops (11.5% TRR, 0.058 ppm); one unknown each in 47-, 70-, and 98-DAP spinach (11.9-14.9% TRR, 0.050-0.118 ppm); and one unknown in 126-DAP wheat straw (15.8% TRR, 0.096 ppm).

The study report additionally included data pertaining to the characterization of residues in the soil. Soil cores from treated and control containers were taken on the day of application, the day of planting (Day 30), and at the first and third harvest for each crop (Days 77, 84, 100, 128, 135 and 176). Total radioactive residues decreased from 1.24 ppm on the day of application to 0.31 ppm at Day 176. The proportion of solvent-extractable radioactivity decreased with time from 98.3% TRR at the time of application to 30.8% at Day 176. Pethoxamid accounted for 93.8% TRR at application, decreasing to 59.6% TRR at the time of planting, and to 3.8% TRR at Day 176. MET-42 was found to be the major metabolite, increasing from $<0.1\%$ TRR on the day of application to a maximum of 9.3% TRR on Day 100, then decreasing to 5.1% TRR on Day 176. Other observed metabolites were MET-2 ($\leq 4.3\%$ TRR), MET-3 ($\leq 2.3\%$ TRR), MET-28 ($\leq 4.5\%$ TRR), and MET-29 ($\leq 7.7\%$ TRR), plus at least nine other unknowns.

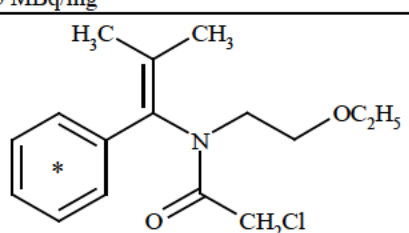
Based on the results of the study, the study author proposed that pethoxamid and metabolites were extensively metabolized in rotated crops. The following metabolites, MET-30, MET-41, MET-48, MET-49, and MET-50, indicate that metabolism proceeds via a glutathione pathway. Soil metabolite MET-42, which was also detected in rotational crops, may have resulted from direct uptake from the soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

The radiolabeled test substance, dissolved in ACN, was isotopically diluted with non-labeled pethoxamid. The solvent was evaporated under nitrogen, and the test substance was mixed with blank formulation (formulation type unspecified) and water. The radiochemical purity was measured in three aliquots using TLC and was found to be 97.6-99.1%.

Table B.7.9.1.2-1. Pethoxamid Nomenclature.	
Common name	Pethoxamid
Identity	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide
CAS registry number	106700-29-2
Company experimental name	TKC-94
Radiolabeled compound	[phenyl- U - ^{14}C]pethoxamid
Lot/Batch #	CFQ10530
Radiochemical purity	97.6-99.1%
Specific activity as received	3.96 MBq/mg; 107 μ Ci/mg
Specific activity of dose	146,723 dpm/ μ g; 2.45 MBq/mg
Position of radiolabel	

2. Test Crops

Table B.7.9.1.2-2. Crop Information.				
Crop/Crop Group	Variety	PBI (days)	Growth Stage at Harvest	Harvested Commodities
Turnip; Root and tuber vegetables	Snowball	30	Immature and mature	Roots and tops
Spinach; Leafy vegetables	Space F1 hybrid (with thiram coating)	30	Immature and mature	Leaves
Spring wheat; Cereal grains and Forage, fodder, and straw of cereal grains	Axona	30	Immature and mature	Whole plant (immature); grain, straw, and chaff (mature)

3. Soil Type

Table B.7.9.1.2-3. Soil Physicochemical Properties.							
Soil Type	pH	Organic Matter (%)	Sand (%)	Silt (%)	Clay (%)	Moisture Holding Capacity (% at 0.001 bar)	Cation Exchange Capacity (meq/100 g)
Sandy silt loam	6.3	2.6	33	53	14	58.8	15.4

B. STUDY DESIGN

Experimental Conditions

Rotational crops were grown in a growth room providing suitable conditions for the growth of the plants: 15-18 °C (15 °C for the first 2 months then gradually increased to 18 °C for the remainder of the study); and 11-hour light/13-hour dark cycle for the first month, then 18-hour light/6-hour dark cycle. Sandy silt loam soil was placed in nine plastic containers to a depth of 25 cm; six containers were used for treatment and three containers were untreated. Fertilizer was applied to each container 3 days prior to test substance application. The formulated test substance was applied to the pots using a mesh grid with 1-cm holes at 1.25-cm centers to allow an even application to the entire surface; 55 µL of test substance solution was applied through each hole. The application rate corresponded to 1.37 lb ai/A (1.53 kg ai/ha). The soil was then cultivated to a depth of approximately 5 cm. Water was applied as necessary to keep the soil moist prior to planting of crops, and weeds were uprooted and allowed to die on the soil surface.

Rotational crops of turnip, spinach, and spring wheat were planted 30 days after soil treatment; two treated pots and one untreated pot were used for each crop. Plants were watered as needed to maintain crop health and to ensure that soil remained relatively moist.

The study use pattern is summarized in Table B.7.9.1.2-4.

Table B.7.9.1.2-4. Use Pattern Information.	
Chemical name	[phenyl-U- ¹⁴ C]pethoxamid
Application method	The formulated test substance was applied to bare soil through a mesh grid to allow even application over entire surface
Application rate	1.37 lb ai/A (1.53 kg ai/ha)
Number of applications	1
PBI (DAT ¹)	30 days

¹ DAT = Days after treatment.

Sampling

Samples of rotational crop commodities were harvested at the three intervals: first harvest (immature); second harvest (earliest possible harvest); and third harvest (normal harvest); the growth stage of each crop at each harvest was not reported. Plant-to-harvest intervals for the first, second, and third harvests were, respectively: 54, 79, and 105 days for turnip; 47, 70, and 98 days for spinach; and 70, 126, and 146 days for wheat. Turnips were pulled from the soil, the root was washed with water to remove soil, and the plants were separated into roots and tops. Spinach and wheat were cut just above the soil surface. For the second and third harvests, wheat

was separated into straw, grain, and chaff. The study report did not include any information regarding the storage of rotational crop matrices prior to or during extraction and analysis. It was stated that crop matrices were homogenized in the presence of dry ice.

TRR Determination

TRR in the extracts and hydrolysates of rotational crop commodities were determined by direct LSC, and TRR in homogenized crop matrices and PES were determined by combustion/LSC. TRR in samples were also determined by summing radioactivity in extract and PES fractions. Summed TRR values were used for all further determinations. The limit of quantitation for TRR determinations was 2x background.

Extraction and Analysis

Samples of all rotational crop matrices were subjected to extraction and analysis. Homogenized rotational crop matrices were extracted sequentially with ACN (3x) and ACN:water (1:1, v:v; 3x) by homogenizing for 5 minutes for each extraction. The extracts were isolated by centrifugation and combined for HPLC and TLC analysis. The residual solids from rotational crop matrices from the final harvest interval for turnip root, spinach, and wheat straw, grain, and chaff were subsequently extracted once with water, and the extracts were isolated by centrifugation. The water extract of 146-DAP wheat straw was partitioned with ethyl acetate (3x), and then the aqueous phase was adjusted to pH 1-2 using HCl and partitioned with ethyl acetate (2x).

PES from final harvest matrices with significant radioactivity (turnip root, spinach, and wheat straw, grain, and chaff) were subjected to separate acid, base, and cellulase/hemicellulase hydrolyses. Acid hydrolyses were conducted using 0.2 M HCl at 37 °C for approximately 48 hours. Base hydrolyses were conducted using 0.05 M NaOH at 37 °C for approximately 48 hours. For cellulase and hemicellulase treatment, PES were mixed in acetate buffer (pH 5), cellulase and hemicellulase enzymes were added, and the mixture was incubated at 37 °C for 48 hours. Hydrolysates were isolated by centrifugation. The enzyme hydrolysate of 98-DAP spinach, 146-DAP wheat straw, and 146-DAP wheat grain was partitioned with ethyl acetate (3x), then the aqueous phase was adjusted to pH 1-2 using HCl and partitioned with ethyl acetate (3x).

For 146-DAP wheat straw and grain, the PES following cellulase/hemicellulase treatment were extracted with water then hydrolyzed with 0.2 M NaOH at 37 °C for 18 hours; for wheat grain, the remaining PES were hydrolyzed with 6 M NaOH at 37 °C for 18 hours. For wheat straw, the water extract of the enzyme PES was combined with the enzyme hydrolysate before that hydrolysate was subjected to ethyl acetate partitioning. The 0.2 M NaOH hydrolysate of Day 176 wheat grain was adjusted to pH 7 (using HCl) and partitioned with ethyl acetate (3x); the aqueous phase was adjusted to pH 1-2 using HCl and partitioned with ethyl acetate.

Representative flowcharts of the extraction procedures for turnip root, spinach, wheat straw, wheat grain, and wheat chaff are presented in Figures B.7.9.1.2-1, B.7.9.1.2-2, B.7.9.1.2-3, B.7.9.1.2-4, and B.7.9.1.2-5, respectively.

Figure B.7.9.1.2-1. Extraction Scheme for Rotational Turnip Root (105 DAP).

Note: Removed figures due to “confidential” was stamped on each figure. Did not hear back from the registrant; therefore, I will remove figures from the DER.

Figure B.7.9.1.2-2. Extraction Scheme for Rotational Spinach (98 DAP).

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Figure B.7.9.1.2-3. Extraction Scheme for Rotational Wheat Straw (146 DAP).

Removed

Figure B.7.9.1.2-4. Extraction Scheme for Rotational Wheat Grain (146 DAP).

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Figure B.7.9.1.2-5. Extraction Scheme for Rotational Wheat Chaff (146 DAP).

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Identification and Characterization

One-dimensional TLC analyses were conducted on normal-phase (Kieselfel 60 F₂₅₄) plates or reverse-phase (LCK_{18F}) plates. Compounds were identified by co-chromatography with reference standards (see Appendix I). Radioactivity was detected and quantified using a bio-imaging analyzer, and non-labeled reference standards were visualized by quenching of the UV indicator. For normal-phase analyses, the following solvent systems were used:

- A: ACN
- C: hexane:ethyl acetate (1:1, v:v)
- F: chloroform:methanol:formic acid:water (70:25:3:3, v:v:v:v)
- K: hexane:acetone (1:1, v:v)
- O: isopropanol:conc. ammonia (8:2, v:v)

The following solvent systems were used for reverse-phase TLC analyses:

- B: ACN:water (4:1, v:v)
- P: ACN:water:formic acid (80:20:0.5, v:v:v)

Based on the reported results, the study author considered 0.1% TRR to be the limit of detection for TLC analyses.

Sample extracts were also reportedly analyzed by HPLC; however, HPLC results were only presented for soil extracts. The HPLC system (Method 2) utilized a reverse-phase column (Zorbax RX-C8) with a gradient mobile phase of 0.015 ammonium formate buffer (pH 2) to ACN. The system was equipped with a UV detector (254 nm), a radiodetector, and a fraction collector.

II. RESULTS AND DISCUSSION

A. Total Radioactive Residues

The TRR in rotational turnip, spinach, and wheat matrices are summarized in Table B.7.9.1.2-5. For turnip root and spinach, TRR were highest at the first harvest interval and decreased thereafter. TRR in turnip tops remained approximately the same at each harvest interval. For wheat, residues were generally highest at the final (normal) harvest interval. Overall, TRR were highest in wheat straw and lowest in wheat grain and turnip root.

The study author also measured radioactivity in the control samples, which were housed in the same growth room as the treated samples. Radioactivity was found in all control samples, at 0.016-0.18 ppm [^{14}C]pethoxamid equivalents. The study author noted that these results indicated the incorporation of $^{14}\text{CO}_2$ into crops by photosynthesis. The production of $^{14}\text{CO}_2$ by incubation of pethoxamid under aerobic conditions has reportedly been demonstrated in an aerobic soil metabolism study (referenced as “TKC-94 Aerobic soil metabolism. 1465/72. Covance, Harrogate, UK”).

Matrix	Harvest Interval [days after planting]	Residues ¹ (ppm [^{14}C]pethoxamid equivalents)	
		Treated samples	Control samples
Turnip root	Immature [54]	0.19	0.026
	Earliest possible [79]	0.070	0.026
	Normal harvest [105]	0.053	0.021 ²
Turnip tops	Immature [54]	0.58	0.026
	Earliest possible [79]	0.62	0.029
	Normal harvest [105]	0.50	0.044 ²
Spinach	Immature [47]	0.79	0.068
	Earliest possible [70]	0.51	0.016
	Normal harvest [98]	0.41	0.026 ²
Wheat forage	Immature [70]	0.48	0.034
Wheat straw	Earliest possible [126]	0.61	0.073
	Normal harvest [146]	1.79	0.14
Wheat grain	Earliest possible [126]	0.13	0.077
	Normal harvest [146]	0.16	0.12
Wheat chaff	Earliest possible [126]	0.34	0.16
	Normal harvest [146]	0.76	0.18

¹ Unless otherwise noted, calculated by summing extractable and nonextractable radioactivity. These values were used for all further determinations.

² Determined by combustion/LSC.

B. Extraction, Characterization, and Distribution of Residues in Rotational Turnip, Spinach, and Wheat.

The distribution of radioactivity in rotational crop matrices is presented in Tables B.7.9.1.2-6 (turnip root), B.7.9.1.2-7 (turnip tops), B.7.9.1.2-8 (spinach), B.7.9.1.2-9 (wheat forage and straw), and B.7.9.1.2-10 (wheat grain and chaff). The study report included minimal detail regarding levels of radioactivity found at each extraction step (except for crop matrices for which extraction flowcharts were provided); summary tables of identified/unidentified chromatographic

peaks and radioactivity in hydrolysates were provided. Raw data supporting chromatographic analyses were only provided for one matrix (47-DAP spinach) as an example.

Table B.7.9.1.2-6. Distribution of Radioactive Residues in Rotational Turnip Root From a 30-day PBI Following Application of [¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹						
Metabolite Fraction	54 DAP First harvest		79 DAP Second harvest		105 DAP Third harvest	
	TRR = 0.19 ppm		TRR = 0.070 ppm		TRR = 0.053 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN + ACN/water	75.2	0.14	66.2	0.046	76.0	0.040
MET-30	9.8	0.019	1.8	0.001	ND	--
MET-41	2.1	0.004	3.2	0.002	5.9	0.003
MET-42	5.7	0.011	ND	--	ND	--
MET-48	1.1	0.002	ND	--	ND	--
MET-49	ND	--	ND	--	3.2	0.002
MET-50	8.7	0.017	2.8	0.002	5.4	0.003
P1 (origin)	4.1	0.008	10.4	0.007	8.6	0.005
P3	2.3	0.004	ND	--	4.6	0.002
P5	3.1	0.006	3.2	0.002	1.7	0.001
P6	8.5	0.016	7.7	0.005	1.2	0.001
P8	2.0	0.004	2.5	0.002	7.1	0.004
P9	ND	--	4.0	0.003	ND	--
P11	3.0	0.006	13.6	0.010	9.7	0.005
P14	3.9	0.007	2.9	0.002	14.4	0.008
P17	5.8	0.011	3.3	0.002	7.2	0.004
P18	3.9	0.007	1.4	0.001	ND	--
P19	6.2	0.012	4.4	0.003	4.4	0.002
Other unknowns ²	5.0	0.010	5.0	0.004	2.6	0.001
Water					1.3	0.001
PES ³	24.8	0.047	33.8	0.024	22.7	0.012
1. 0.2 M HCl hydrolysate					2.1	0.001
1. Acid hydrolysate PES					20.6	0.011
2. 0.05 M NaOH hydrolysate					2.9	0.002
2. Base hydrolysate PES					19.8	0.010
3. Cellulase/hemicellulase hydrolysate					4.8	0.003
3. Enzyme PES					17.9	0.009

¹ ND = Not detected. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. Extracts were analyzed using normal-phase TLC, solvent system F.

² Corresponds to areas on radiochromatogram which sum above zero with no discrete area of radioactivity.

³ Separate subsamples of 105-DAP PES were subjected to acid, base, and enzyme hydrolyses.

Table B.7.9.1.2-7. Distribution of Radioactive Residues in Rotational Turnip Tops From a 30-day PBI Following Application of [¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹						
Metabolite Fraction	54 DAP First harvest		79 DAP Second harvest		105 DAP Third harvest	
	TRR = 0.58 ppm		TRR = 0.62 ppm		TRR = 0.50 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN + ACN/water	95.2	0.55	91.5	0.57	94.0	0.47
MET-30	4.5	0.026	7.5	0.047	ND	--
MET-41	2.9	0.017	1.2	0.007	1.2	0.006
MET-42	14.2	0.082	21.0	0.130	26.4	0.132

Table B.7.9.1.2-7. Distribution of Radioactive Residues in Rotational Turnip Tops From a 30-day PBI Following Application of [¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹						
Metabolite Fraction	54 DAP First harvest		79 DAP Second harvest		105 DAP Third harvest	
	TRR = 0.58 ppm		TRR = 0.62 ppm		TRR = 0.50 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
MET-48	ND	--	ND	--	4.1	0.021
MET-49	ND	--	6.0	0.037	4.8	0.024
MET-50	15.3	0.089	12.1	0.075	17.7	0.089
P1 (origin)	2.3	0.013	1.1	0.007	2.3	0.012
P3	5.7	0.033	4.1	0.025	2.8	0.014
P5	7.1	0.041	8.7	0.054	11.5	0.058
P6	8.3	0.048	5.5	0.034	5.9	0.030
P8	3.7	0.021	3.9	0.024	ND	--
P11	7.8	0.045	9.9	0.061	9.7	0.049
P14	4.8	0.028	2.8	0.017	2.4	0.012
P17	7.9	0.046	4.5	0.028	3.0	0.015
P18	3.0	0.017	1.8	0.011	0.8	0.004
P19	2.3	0.013	1.3	0.008	1.4	0.007
Other unknowns ²	5.4	0.031	0.1	0.001	ND	--
PES	4.9	0.028	8.5	0.053	6.0	0.030

¹ ND = Not detected. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. Extracts were analyzed using normal-phase TLC, solvent system F.

² Corresponds to areas on radiochromatogram which sum above zero with no discrete area of radioactivity.

Table B.7.9.1.2-8. Distribution of Radioactive Residues in Rotational Spinach From a 30-day PBI Following Application of [¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹						
Metabolite Fraction	47 DAP First harvest		70 DAP Second harvest		98 DAP Third harvest	
	TRR = 0.79 ppm		TRR = 0.51 ppm		TRR = 0.41 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN + ACN/water	93.8	0.74	94.7	0.48	87.7	0.36
MET-30	3.4	0.027	7.5	0.038	6.0	0.025
MET-41	1.7	0.013	3.1	0.016	2.0	0.008
MET-42	9.1	0.072	7.7	0.039	7.9	0.032
MET-48	7.0	0.055	8.6	0.044	8.2	0.034
MET-49	5.5	0.043	4.6	0.023	2.1	0.009
MET-50	12.2	0.096	10.3	0.053	4.5	0.018
P1 (origin)	2.7	0.021	4.0	0.020	5.5	0.023
P2	1.8	0.014	5.5	0.028	ND	--
P3	4.0	0.032	1.7	0.009	6.0	0.025
P5	4.2	0.033	11.9	0.061	12.3	0.050
P6	5.3	0.042	7.1	0.036	4.7	0.019
P8	7.0	0.055	3.1	0.016	5.4	0.022
P11	5.8	0.046	7.7	0.039	8.8	0.036
P14	3.0	0.024	4.2	0.021	5.8	0.024
P16	ND	--	2.1	0.011	2.6	0.011
P17	4.3	0.034	4.5	0.023	4.8	0.020
P19	14.9	0.118	1.2	0.006	1.1	0.005
Other unknowns ²	1.9	0.015	ND	--	ND	--
Water					1.8	0.007
PES ³	6.3	0.050	5.3	0.027	10.5	0.043

Table B.7.9.1.2-8. Distribution of Radioactive Residues in Rotational Spinach From a 30-day PBI Following Application of [¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹

Metabolite Fraction	47 DAP First harvest		70 DAP Second harvest		98 DAP Third harvest	
	TRR = 0.79 ppm		TRR = 0.51 ppm		TRR = 0.41 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
1. 0.2 M HCl hydrolysate					3.5	0.014
1. Acid hydrolysate PES					7.0	0.029
2. 0.05 M NaOH hydrolysate					3.9	0.016
2. Base hydrolysate PES					6.6	0.027
3. Cellulase/hemicellulase hydrolysate					5.6	0.023
Ethyl acetate					0.9 ⁴	0.004
Aqueous					2.2	0.009
3. Enzyme PES					4.9	0.020

¹ ND = Not detected. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. Extracts were analyzed using normal-phase TLC, solvent system F.

² Corresponds to areas on radiochromatogram which sum above zero with no discrete area of radioactivity.

³ Separate subsamples of 98-DAP PES were subjected to acid, base, and enzyme hydrolyses.

⁴ Comprised of 0.4% TRR released by partitioning at neutral pH and 0.5% TRR released by partitioning at pH 1-2.

Table B.7.9.1.2-9. Distribution of Radioactive Residues in Rotational Wheat Forage and Straw From a 30-day PBI Following Application of [¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹

Metabolite Fraction	Wheat forage		Wheat straw			
	70 DAP First harvest		126 DAP Second harvest		146 DAP Third harvest	
	TRR = 0.48 ppm		TRR = 0.61 ppm		TRR = 1.79 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN + ACN/water	90.5	0.43	71.1	0.43	68.9	1.23
MET-30	11.3	0.054	4.3	0.026	6.1	0.109
MET-41	3.6	0.017	2.8	0.017	2.4	0.043
MET-42	14.2	0.068	9.9	0.060	12.9	0.231
MET-48	3.6	0.017	ND	--	ND	--
MET-49	2.6	0.012	ND	--	1.3	0.023
MET-50	7.5	0.036	4.8	0.029	5.5	0.098
P1 (origin)	5.1	0.024	1.2	0.007	3.3	0.059
P3	2.2	0.011	ND	--	ND	--
P5	ND	--	3.2	0.020	4.5	0.081
P6	6.5	0.031	ND	--	2.0	0.036
P8	ND	--	ND	--	2.6	0.047
P9	4.6	0.022	ND	--	ND	--
P11	8.0	0.038	6.8	0.041	6.9	0.124
P14	1.3	0.006	2.6	0.016	3.8	0.068
P16	3.2	0.015	ND	--	ND	--
P17	3.8	0.018	6.9	0.042	6.7	0.120
P18	2.9	0.014	7.1	0.043	ND	<0.002
P19	5.8	0.028	15.8	0.096	7.2	0.129
P20	3.2	0.015	ND	--	ND	--
Other unknowns ²	1.1	0.005	5.7	0.035	3.7	0.066
Water					12.5	0.224
Ethyl acetate					7.6 ³	0.136
Aqueous					2.7	0.048

Table B.7.9.1.2-9. Distribution of Radioactive Residues in Rotational Wheat Forage and Straw From a 30-day PBI Following Application of [¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹

Metabolite Fraction	Wheat forage		Wheat straw			
	70 DAP First harvest		126 DAP Second harvest		146 DAP Third harvest	
	TRR = 0.48 ppm		TRR = 0.61 ppm		TRR = 1.79 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
PES ⁴	9.6	0.046	28.9	0.18	18.6	0.333
1. 0.2 M HCl hydrolysate					5.3	0.095
1. Acid hydrolysate PES					13.3	0.238
2. 0.05 M NaOH hydrolysate					7.9	0.141
2. Base hydrolysate PES					10.7	0.192
3. Cellulase/hemicellulase hydrolysate ⁵					7.1	0.127
Ethyl acetate					7.2 ⁶	0.129
Aqueous					2.1	0.038
3. Enzyme PES					11.5	0.206
Water ⁵					3.6	0.064
0.2 M NaOH hydrolysate					1.4	0.025
PES					6.5	0.116

¹ ND = Not detected. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. Extracts were analyzed using normal-phase TLC, solvent system F.

² Corresponds to areas on radiochromatogram which sum above zero with no discrete area of radioactivity.

³ Comprised of 6.2% TRR released by partitioning at neutral pH and 1.4% TRR released by partitioning at pH 1-2.

⁴ Separate subsamples of 146-DAP PES were subjected to acid, base, and enzyme hydrolyses.

⁵ The water extract of enzyme PES was combined with the enzyme hydrolysate prior to partitioning.

⁶ Comprised of 6.0% TRR released by partitioning at neutral pH and 1.2% TRR released by partitioning at pH 1-2.

Table B.7.9.1.2-10. Distribution of Radioactive Residues in Rotational Wheat Grain and Chaff From a 30-day PBI Following Application of [¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹

Metabolite Fraction	Wheat grain				Wheat chaff			
	126 DAP Second harvest		146 DAP Third harvest		126 DAP Second harvest		146 DAP Third harvest	
	TRR = 0.13 ppm		TRR = 0.16 ppm		TRR = 0.34 ppm		TRR = 0.76 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN + ACN/water	43.4	0.056	15.6	0.025	55.1	0.19	56.9	0.43
MET-30	2.0	0.003	ND	--	ND	--	ND	--
MET-41	1.3	0.002	0.6	0.001	ND	--	0.7	0.005
MET-42	3.5	0.005	ND	--	ND	--	ND	--
MET-49	0.9	0.001	ND	--	1.1	0.004	2.4	0.018
MET-50	4.6	0.006	1.7	0.003	4.1	0.014	6.0	0.046
P1 (origin)	5.5	0.007	5.4	0.009	4.7	0.016	3.4	0.026
P2	1.4	0.002	0.6	0.001	ND	--	ND	--
P5	1.8	0.002	ND	--	ND	--	2.9	0.022
P6	1.1	0.001	ND	--	1.3	0.004	1.2	0.009
P8	ND	--	ND	--	1.1	0.004	5.1	0.039
P11	10.4	0.014	2.8	0.004	1.7	0.006	6.3	0.048
P14	1.4	0.002	0.7	0.001	4.8	0.016	5.0	0.038
P16	1.0	0.001	0.8	0.001	5.8	0.020	2.1	0.016
P17	0.5	0.001	1.0	0.002	5.7	0.019	3.4	0.026
P18	1.5	0.002	ND	--	3.2	0.011	3.0	0.023
P19	3.0	0.004	1.4	0.002	13.1	0.045	7.5	0.057
Other unknowns ²	3.5	0.005	0.6	0.001	8.5	0.029	7.9	0.060

Table B.7.9.1.2-10. Distribution of Radioactive Residues in Rotational Wheat Grain and Chaff From a 30-day PBI Following Application of [¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹								
Metabolite Fraction	Wheat grain				Wheat chaff			
	126 DAP Second harvest		146 DAP Third harvest		126 DAP Second harvest		146 DAP Third harvest	
	TRR = 0.13 ppm		TRR = 0.16 ppm		TRR = 0.34 ppm		TRR = 0.76 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Water			9.6	0.015			7.0	0.053
PES ³	56.7	0.074	74.8	0.12	44.9	0.15	36.1	0.27
1. 0.2 M HCl hydrolysate			6.3	0.010			4.0	0.030
1. Acid hydrolysate PES			68.5	0.110			32.1	0.244
2. 0.05 M NaOH hydrolysate			15.4	0.025			6.9	0.052
2. Base hydrolysate PES			59.4	0.095			29.2	0.222
3. Cellulase/hemicellulase hydrolysate			19.4	0.031			4.3	0.033
Ethyl acetate			1.6 ⁴	0.003				
Aqueous			16.4	0.026				
3. Enzyme PES			55.4	0.089			31.8	0.24
Water			3.7	0.006				
0.2 M NaOH hydrolysate			18.2	0.029				
Ethyl acetate			5.6 ⁵	0.009				
Aqueous			2.5	0.004				
6 M NaOH hydrolysate			2.7	0.004				
PES			30.8	0.049				

¹ ND = Not detected. Shading indicates that the extraction step and/or characterization analysis was not conducted for the fraction in question. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues. Extracts were analyzed using normal-phase TLC, solvent system F.

² Corresponds to areas on radiochromatogram which sum above zero with no discrete area of radioactivity.

³ Separate subsamples of 146-DAP PES were subjected to acid, base, and enzyme hydrolyses.

⁴ Comprised of 0.5% TRR released by partitioning at neutral pH and 1.1% TRR released by partitioning at pH 1-2.

⁵ Comprised of 2.4% TRR released by partitioning at neutral pH and 3.2% TRR released by partitioning at pH 1-2.

C. Storage Stability of Residues

Sample storage conditions and intervals are presented in Table B.7.9.1.2-11. No information was provided pertaining to the storage conditions for samples, extracts, or hydrolysates, and no dates of extraction, hydrolysis, or analysis were provided. No supporting storage stability data were included in the submission. Based on the sample harvest dates and the reported experimental completion date, samples may have been stored up to 10.5 months prior to completion of analysis. Previous study on confined rotation crop (MRID 49813552) showed samples of rotational crop commodities were stored frozen from harvest to initial HPLC analysis for metabolite profiling for ≤196 days; certain samples and extracts were stored frozen for up to 431 days prior to completion of analyses. Also, acceptable storage stability data supporting potato foliage for up to 631 days (21 months) and 596 days (20 months) in tubers (MRID 49813529).

Table B.7.9.1.2-11. Summary of Storage Conditions.			
Matrix	Storage Temperature (°C)	Actual Storage Duration	Interval of Demonstrated Storage Stability
Rotational turnip root and tops	Not reported	Not reported; ≤10.3 months ¹	The metabolite profile in 30-day PBI mature spinach and wheat hay, after hydrolysis of extractable radioactivity, was shown to be relatively stable for at least 401 days. Residues of pethoxamid are stable during frozen storage for up to 20 months in potato foliage and tubers ²
Rotational spinach		Not reported; ≤10.5 months ¹	
Rotational wheat forage, straw, grain, and chaff		Not reported; ≤9.7 months ¹	

¹ Based on harvest dates and experimental completion date.

² Refer to the DERs for MRIDs 49813529 and 49813552

D. Identity of Residues in Rotational Turnip, Spinach, and Wheat

The characterization and identification of radioactivity in rotational crop matrices is summarized in Tables B.7.9.1.2-12 (turnip root), B.7.9.1.2-13 (turnip tops), B.7.9.1.2-14 (spinach), B.7.9.1.2-15 (wheat forage and straw), and B.7.9.1.2-16 (wheat grain and chaff).

All extracts were analyzed by 1D-TLC. It was stated that most extracts were analyzed by both normal-phase and reverse-phase TLC; however, chromatograms showing identification of metabolites using both normal-phase and reverse-phase TLC analyses were only provided for 98-DAP spinach. Therefore, the identification of metabolites (see Table B.7.9.1.2-17) in all matrices except 98-DAP spinach should be considered tentative as identification was not confirmed using a second technique.

Table B.7.9.1.2-12. Summary of Characterization and Identification of Radioactive Residues in Rotational Turnip Root From a 30-day PBI Following Application of [phenyl-U-¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹						
Compound	54 DAP First harvest		79 DAP Second harvest		105 DAP Third harvest	
	TRR = 0.19 ppm		TRR = 0.070 ppm		TRR = 0.053 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
MET-30	9.8	0.019	1.8	0.001	ND	--
MET-41	2.1	0.004	3.2	0.002	5.9	0.003
MET-42	5.7	0.011	ND	--	ND	--
MET-48	1.1	0.002	ND	--	ND	--
MET-49	ND	--	ND	--	3.2	0.002
MET-50	8.7	0.017	2.8	0.002	5.4	0.003
Minor unknowns ²	47.8	0.091	58.4	0.041	61.5	0.033
Aqueous extractable	NA	--	NA	--	1.3	0.001
Enzyme extractable	NA	--	NA	--	4.8	0.003
Total extractable	75.2	0.14	66.2	0.046	82.1	0.044
Total identified	27.4	0.053	7.8	0.005	14.5	0.008
Total characterized	NA	--	NA	--	4.8	0.003

Table B.7.9.1.2-12. Summary of Characterization and Identification of Radioactive Residues in Rotational Turnip Root From a 30-day PBI Following Application of [phenyl-U-¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹

Compound	54 DAP First harvest		79 DAP Second harvest		105 DAP Third harvest	
	TRR = 0.19 ppm		TRR = 0.070 ppm		TRR = 0.053 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Total unidentified	47.8	0.091	58.4	0.041	62.8	0.034
Total bound residues (PES)	24.8	0.047	33.8	0.024	17.9	0.009
% Accountability ³	100.0		100.0		100.0	

¹ ND = Not detected. NA = Not applicable. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Single or multicomponent unknowns with each component present at <10% TRR, except for two unknowns in 79-DAP root (P1: 10.4% TRR, 0.007 ppm; P11: 13.6% TRR, 0.010 ppm) and one unknown in 105-DAP root (P14: 14.4% TRR, 0.008 ppm).

³ Total (ppm)/TRR (ppm)×100

Table B.7.9.1.2-13. Summary of Characterization and Identification of Radioactive Residues in Rotational Turnip Tops From a 30-day PBI Following Application of [phenyl-U-¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹

Compound	54 DAP First harvest		79 DAP Second harvest		105 DAP Third harvest	
	TRR = 0.58 ppm		TRR = 0.62 ppm		TRR = 0.50 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
MET-30	4.5	0.026	7.5	0.047	ND	--
MET-41	2.9	0.017	1.2	0.007	1.2	0.006
MET-42	14.2	0.082	21.0	0.130	26.4	0.132
MET-48	ND	--	ND	--	4.1	0.021
MET-49	ND	--	6.0	0.037	4.8	0.024
MET-50	15.3	0.089	12.1	0.075	17.7	0.089
Minor unknowns ²	58.3	0.336	43.7	0.270	39.83	0.201
Total extractable	95.2	0.55	91.5	0.57	94.0	0.47
Total identified	36.9	0.214	47.8	0.296	54.2	0.272
Total unidentified	58.3	0.336	43.7	0.270	39.8	0.201
Total bound residues (PES)	4.9	0.028	8.5	0.053	6.0	0.030
% Accountability ³	100.1		100.0		100.0	

¹ ND = Not detected. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Single or multicomponent unknowns with each component present at <10% TRR, except for one unknown in 105-DAP tops (P5: 11.5% TRR, 0.058 ppm).

³ Total (ppm)/TRR (ppm)×100

Table B.7.9.1.2-14. Summary of Characterization and Identification of Radioactive Residues in Rotational Spinach From a 30-day PBI Following Application of [phenyl-U-¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹

Compound	47 DAP First harvest		70 DAP Second harvest		98 DAP Third harvest	
	TRR = 0.79 ppm		TRR = 0.51 ppm		TRR = 0.41 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
MET-30	3.4	0.027	7.5	0.038	6.0	0.025
MET-41	1.7	0.013	3.1	0.016	2.0	0.008
MET-42	9.1	0.072	7.7	0.039	7.9	0.032
MET-48	7.0	0.055	8.6	0.044	8.2	0.034
MET-49	5.5	0.043	4.6	0.023	2.1	0.009

Table B.7.9.1.2-14. Summary of Characterization and Identification of Radioactive Residues in Rotational Spinach From a 30-day PBI Following Application of [phenyl-U-¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹

Compound	47 DAP First harvest		70 DAP Second harvest		98 DAP Third harvest	
	TRR = 0.79 ppm		TRR = 0.51 ppm		TRR = 0.41 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
MET-50	12.2	0.096	10.3	0.053	4.5	0.018
Minor unknowns ²	54.9	0.434	48.8	0.249	63.8	0.262
Aqueous extractable	NA	--	NA	--	1.8	0.007
Enzyme extractable	NA	--	NA	--	5.6	0.023
Total extractable	93.8	0.74	94.7	0.48	95.1	0.39
Total identified	38.9	0.306	41.8	0.213	30.7	0.126
Total characterized	NA	--	NA	--	5.6	0.023
Total unidentified	54.9	0.434	48.8	0.249	65.6	0.269
Total bound residues (PES)	6.3	0.050	5.3	0.027	4.9	0.020
% Accountability ³	100.1		100.0		100.0	

¹ ND = Not detected. NA = Not applicable. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Single or multicomponent unknowns with each component present at <10% TRR, with exception of one unknown in 47-DAP spinach (P19: 14.9% TRR, 0.118 ppm), one unknown in 70-DAP spinach (P5: 11.9% TRR, 0.061 ppm), and one unknown in 98-DAP spinach (P5: 12.3% TRR, 0.050 ppm).

³ Total (ppm)/TRR (ppm)×100

Table B.7.9.1.2-15. Summary of Characterization and Identification of Radioactive Residues in Rotational Wheat Forage and Straw From a 30-day PBI Following Application of [phenyl-U-¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹

Compound	Wheat forage		Wheat straw			
	70 DAP First harvest		126 DAP Second harvest		146 DAP Third harvest	
	TRR = 0.48 ppm		TRR = 0.61 ppm		TRR = 1.79 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
MET-30	11.3	0.054	4.3	0.026	6.1	0.109
MET-41	3.6	0.017	2.8	0.017	2.4	0.043
MET-42	14.2	0.068	9.9	0.060	12.9	0.231
MET-48	3.6	0.017	ND	--	ND	<0.002
MET-49	2.6	0.012	ND	--	1.3	0.023
MET-50	7.5	0.036	4.8	0.029	5.5	0.098
Minor unknowns ²	47.7	0.227	49.3	0.300	40.7	0.730
Aqueous extractable	NA	--	NA	--	12.5	0.224
Enzyme extractable	NA	--	NA	--	7.1	0.127
Post enzyme extracts/hydrolysates	NA	--	NA	--	5.0	0.090
Total extractable	90.5	0.43	71.1	0.43	93.5	1.66
Total identified	42.8	0.204	21.8	0.132	28.2	0.504
Total characterized	NA	--	NA	--	7.1	0.127
Total unidentified	47.7	0.227	49.3	0.300	53.2	0.954
Total bound residues (PES)	9.6	0.046	28.9	0.18	6.5	0.116
% Accountability ³	100.1		100.0		100.0	

¹ ND = Not detected. NA = Not applicable. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Single or multicomponent unknowns with each component present at <10% TRR, except for one unknown in 126-DAP wheat straw (P19: 15.8% TRR, 0.096 ppm).

³ Total (ppm)/TRR (ppm)×100

Table B.7.9.1.2-16. Summary of Characterization and Identification of Radioactive Residues in Rotational Wheat Grain and Chaff Following Application of [phenyl-U-¹⁴C]Pethoxamid to Soil at 1.37 lb ai/A (1.53 kg ai/ha).¹

Compound	Wheat grain				Wheat chaff			
	126 DAP Second harvest		146 DAP Third harvest		126 DAP Second harvest		146 DAP Third harvest	
	TRR = 0.13 ppm		TRR = 0.16 ppm		TRR = 0.34 ppm		TRR = 0.76 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
MET-30	2.0	0.003	ND	--	ND	--	ND	--
MET-41	1.3	0.002	0.6	0.001	ND	--	0.7	0.005
MET-42	3.5	0.005	ND	--	ND	--	ND	--
MET-49	0.9	0.001	ND	--	1.1	0.004	2.4	0.018
MET-50	4.6	0.006	1.7	0.003	4.1	0.014	6.0	0.046
Minor unknowns ²	31.1	0.041	13.3	0.021	49.9	0.170	47.8	0.364
Aqueous extractable	NA	--	9.6	0.015	NA	--	7.0	0.053
Enzyme extractable	NA	--	19.4	0.031	NA	--	4.3	0.033
Post enzyme extracts/hydrolysates	NA	--	24.6	0.039	NA	--	NA	--
Total extractable	43.4	0.056	69.2	0.110	55.1	0.19	68.2	0.516
Total identified	12.3	0.017	2.3	0.004	5.2	0.018	9.1	0.069
Total characterized	NA	--	19.4	0.031	NA	--	4.3	0.033
Total unidentified	31.1	0.041	47.5	0.075	49.9	0.170	54.8	0.417
Total bound residues (PES)	56.7	0.074	30.8	0.049	44.9	0.15	31.8	0.242
% Accountability ³	100.1		100.0		100.0		100.0	

¹ ND = Not detected. NA = Not applicable. Ppm are reported in parent equivalents, and TRR values were calculated by summing extractable and nonextractable residues.

² Single or multicomponent unknowns with each component present at <10% TRR, except for one unknown in 126-DAP wheat grain (P11: 10.4% TRR, 0.014 ppm) and one unknown in 126-DAP wheat chaff (P19: 13.1% TRR, 0.045 ppm).

³ Total (ppm)/TRR (ppm)×100

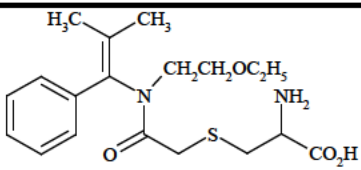
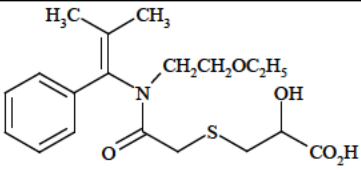
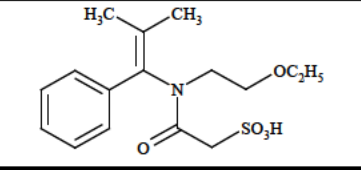
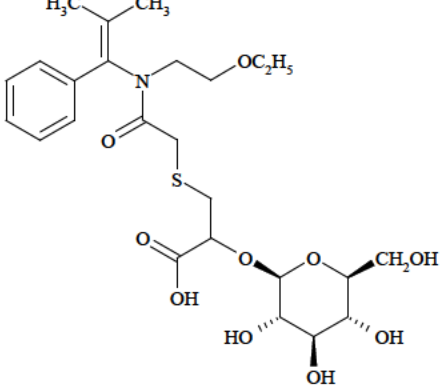
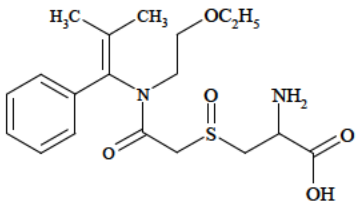
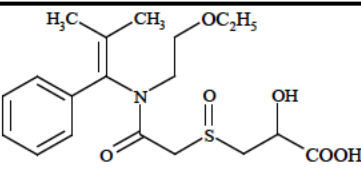
The study report additionally included data pertaining to the characterization of residues in the soil. Soil cores from treated and control containers were taken on the day of application, the day of planting (Study Day 30), and at the first and third harvest for each crop (Days 77, 84, 100, 128, 135 and 176). Total radioactive residues decreased from 1.24 ppm on the day of application to 0.31 ppm at Study Day 176; no radioactivity was found in soil cores taken from control containers. The proportion of extractable radioactivity (using ACN and ACN/water) decreased with time from 98.3% TRR at the time of application to 30.8% at Study Day 176. HPLC and TLC analyses indicated that pethoxamid accounted for 93.8% TRR at application, decreasing to 59.6% TRR at the time of planting, and to 3.8% TRR at Study Day 176. MET-42 (TKC-94-sulfonic acid) was found to be the major metabolite, increasing from <0.1% TRR on the day of application to a maximum of 9.3% TRR on Study Day 100, then decreasing to 5.1% TRR on Study Day 176. Other observed metabolites were MET-2 (≤4.3% TRR), MET-3 (≤2.3% TRR TLC only), MET-28 (≤4.5% TRR), and MET-29 (≤7.7% TRR), plus at least nine other unknowns.

E. Proposed Metabolic Pathway

The study author proposed that pethoxamid and metabolites were extensively metabolized in rotated crops. The major metabolites were MET-30 (TKC-94-cysteine), MET-41 (TKC-94-S-lactic acid), MET-48 (TKC-94-S-lactic acid glucoside), MET-49 (TKC-94-SO-cysteine), and MET-50 (TKC-94-SO-lactic acid). The observed metabolites indicate that metabolism proceeds

via a glutathione pathway. MET-42 was also detected in rotational crops, which may have resulted from direct uptake from the soil. The proposed pathway (including the proposed metabolism of pethoxamid in soil) is presented in Figure B.7.9.1.2-6.

Figure B.7.9.1.2-6. Proposed Metabolic Profile of Pethoxamid in Soil and Rotational Crops.

Table B.7.9.1.2-17. Identification of Compounds from Confined Rotational Crop Study.		
Common Name/Code	Chemical Name	Chemical Structure
MET-30 (TKC-94-cysteine)	S-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]-D,L-cysteine	
MET-41 (TKC-94-S-lactic acid)	3-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethylthio]-D,L-lactic acid	
MET-42 (TKC-94-sulfonic acid)	N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-sulfoacetamide	
MET-48 (TKC-94-S-lactic acid glucoside)	3-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethylthio]-2-(α-D-glucopyranosyloxy)propionic acid	
MET-49 (TKC-94-SO-cysteine)	S-[N-(2-ethoxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]-D,L-cysteine-S-oxide	
MET-50 (TKC-94-SO-lactic acid)	3-((2-((2-ethoxyethyl)(2-methyl-1-phenylprop-1-en-1-yl)amino)-2-oxoethyl)sulfinyl)-2-hydroxypropanoic acid	

III. CONCLUSIONS

The confined rotational crop study is considered scientifically acceptable. Rotational crops of turnip, spinach, and wheat were planted at a 30-day PBI following application of [¹⁴C]pethoxamid at 1.37 lb ai/A (1.53 kg ai/ha). Samples of rotational crop commodities were harvested at three intervals: first harvest (immature); second harvest (earliest possible harvest); and third harvest (normal harvest). For turnip root and spinach, TRR were highest at the first harvest interval and decreased thereafter. TRR in turnip tops remained approximately the same at each harvest interval. For wheat, residues were generally highest at the final (normal) harvest interval. Overall, TRR were highest in wheat straw and lowest in wheat grain and turnip root.

TRR were >0.01 ppm in all rotational crop matrices. In turnip, TRR at 54, 79, and 105 DAP, respectively, were 0.19, 0.070, and 0.053 ppm in root, and 0.58, 0.62, and 0.50 ppm in tops. TRR in spinach were 0.79, 0.51, and 0.41 ppm at 47, 70, and 98 DAP. TRR were 0.48 ppm in wheat forage at 70 DAP; at 126 and 146 DAP, respectively, TRR were 0.61 and 1.79 ppm in wheat straw, 0.13 and 0.16 ppm in wheat grain, and 0.34 and 0.76 ppm in wheat chaff. All rotational crop matrices were subjected to extraction procedures.

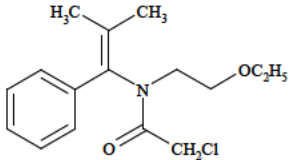
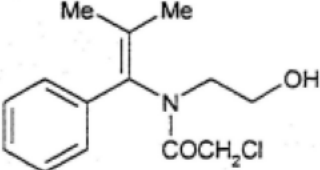
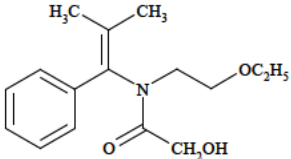
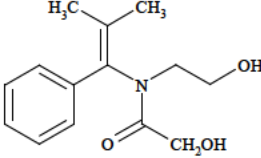
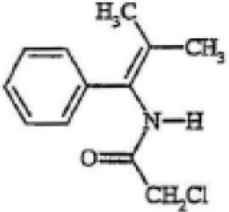
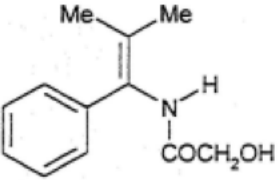
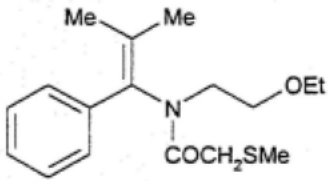
A total of 2-54% TRR was identified/tentatively identified in rotational crops. Pethoxamid was not detected in any rotational crop matrix. With some exceptions, the following metabolites were detected in each rotational crop matrix at one or more harvest intervals: MET-30, MET-41, MET-42, MET-48, MET-49 and MET-50. Metabolite MET-42, which was the major soil metabolite, was one of the major metabolites detected in turnip tops (14.2-26.4% TRR), spinach (7.7-9.1% TRR), wheat forage (14.2% TRR) and straw (9.9-12.9% TRR). This metabolite was also detected in turnip roots ($\leq 5.7\%$ TRR) and wheat grain ($\leq 3.5\%$ TRR), but was not detected in wheat chaff. Metabolite MET-50, another major metabolite, was present in turnip root (2.8-8.7% TRR) and tops (12.1-17.7% TRR), spinach (4.5-12.2% TRR), and wheat forage (7.5% TRR), and was also detected at $\leq 6.0\%$ TRR in wheat straw, grain, and chaff. Metabolite MET-30 was also a major metabolite in most matrices, including turnip root ($\leq 9.8\%$ TRR) and tops ($\leq 7.5\%$ TRR, ≤ 0.047 ppm), spinach (3.4-7.5% TRR), and wheat forage (11.3% TRR) and straw (4.3-6.1% TRR), but was present at $\leq 2.0\%$ TRR in wheat grain and was not detected in wheat chaff. Metabolite MET-48 accounted for 7.0-8.6% TRR in spinach, but was a minor metabolite ($\leq 4.1\%$ TRR) in turnip root and tops and wheat forage, and was not detected in wheat straw, grain, or chaff. Two additional metabolites were found in all rotational crop commodities: MET-41 at $\leq 5.9\%$ TRR; and MET-49 at $\leq 6.0\%$ TRR. The remainder of the solvent-extractable radioactivity consisted of multiple unknowns, each present at $<10\%$ TRR and/or <0.05 ppm, except for: two unknowns in 79-DAP turnip root (at 10.4% TRR and 13.6% TRR) and one unknown in 105-DAP turnip root (14.4% TRR); one unknown in 105-DAP turnip tops (11.5% TRR); one unknown each in 47-, 70-, and 98-DAP spinach (11.9-14.9% TRR, 0.050-0.118 ppm); and one unknown in 126-DAP wheat straw (15.8% TRR, 0.096 ppm).

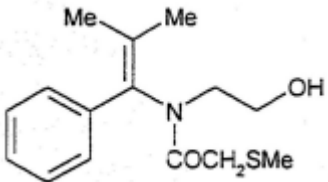
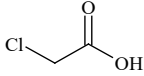
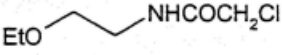
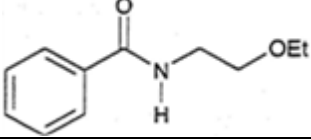
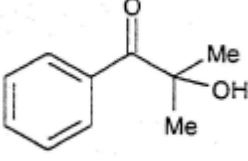
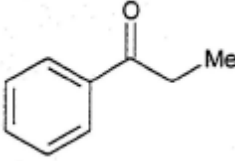
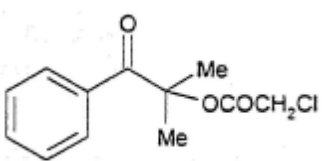
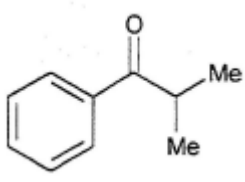
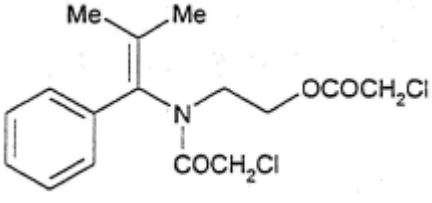
Based on the results of the study, the study author proposed that pethoxamid and metabolites were extensively metabolized in rotated crops. The following metabolites, MET-30, MET-41, MET-48, MET-49, and MET-50, indicate that metabolism proceeds via a glutathione pathway. Soil metabolite MET-42, which was also detected in rotational crops, may have resulted from direct uptake from the soil.

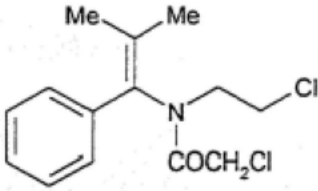
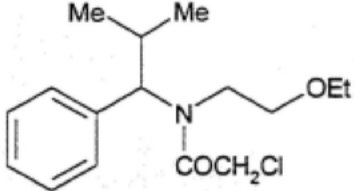
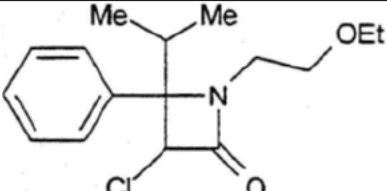
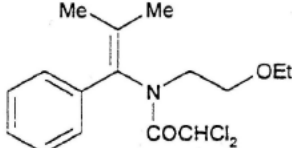
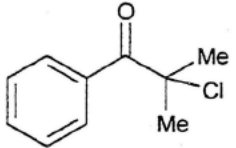
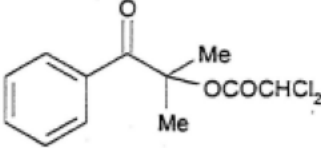
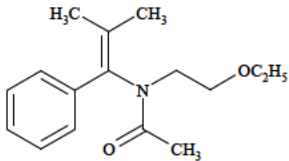
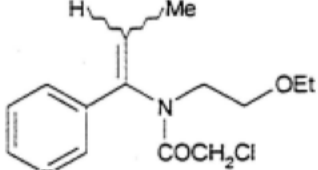
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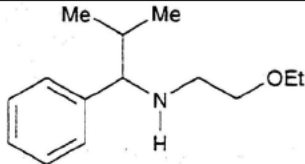
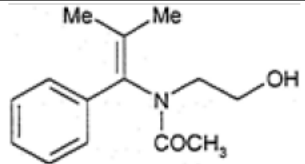
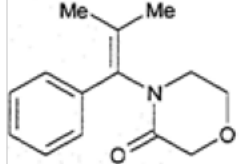
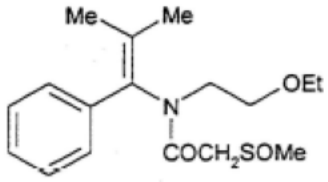
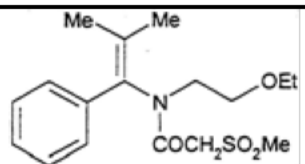
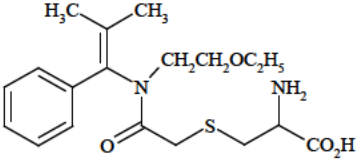
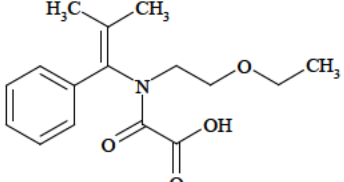
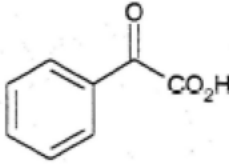
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- Document ID:** MRID No. 49813552; PMRA Study # xxxxxx, PMRA Review # xxxxxx
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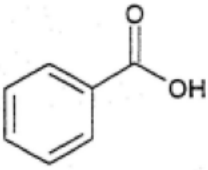
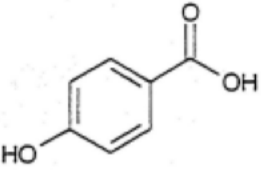
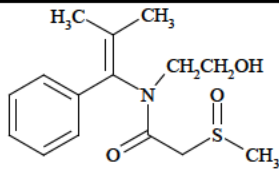
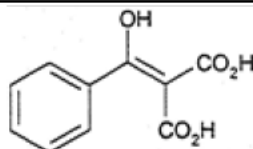
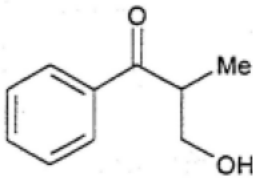
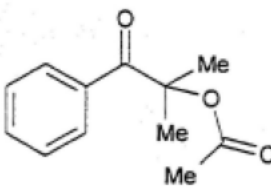
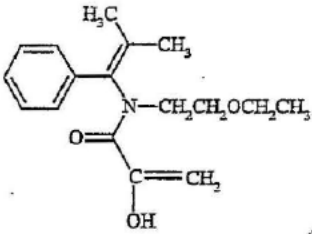
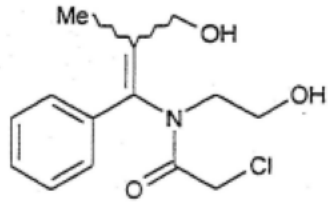
Appendix I. Reference Standards Used in the Study.

Name	Chemical name	Structure
Pethoxamid	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propen-1-yl)acetamide	
MET-1	2-chloro- <i>N</i> -(2-hydroxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-2	<i>N</i> -(2-ethoxyethyl)-2-hydroxy- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-3	2-hydroxy- <i>N</i> -(2-hydroxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-4	2-chloro- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-5	2-hydroxy- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-6	<i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)-2-(methylthio)acetamide	

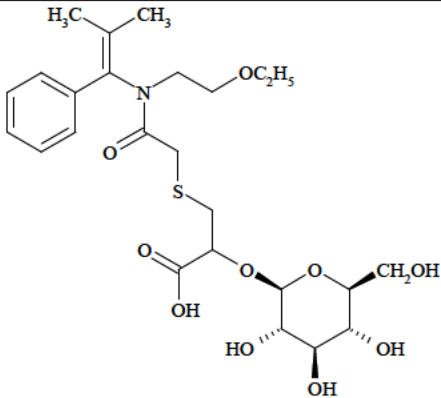
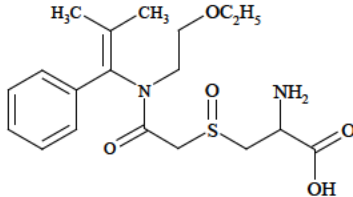
Name	Chemical name	Structure
MET-7	N-(2-hydroxyethyl)-N-(2-methyl-1-phenyl-1-propenyl)-2-(methylthio)acetamide	
MET-8	chloroacetic acid	
MET-9	2-chloro-N-(2-ethoxyethyl)acetamide	
MET-10	N-(2-ethoxyethyl)benzamide	
MET-11	2-hydroxy-2-methylpropiophenone	
MET-12	propiophenone	
MET-13	2-(2-chloroacetoxy)-2-methylpropiophenone	
MET-14	isobutyrophenone	
MET-15	2-chloro-N-[2-(2-chloroacetoxy)ethyl]-N-(2-methyl-1-phenyl-1-propenyl)acetamide	

Name	Chemical name	Structure
MET-16	2-chloro- <i>N</i> -(2-chloroethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-17	2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -[(2-methyl-1-phenyl)propyl]acetamide	
MET-18	3-chloro- <i>N</i> -(2-ethoxyethyl)-4-isopropyl-4-phenyl-2-azetidinone	
MET-19	2,2-dichloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-20	2-chloro-2-methylpropiophenone	
MET-21	2-(2,2-dichloroacetoxy)-2-methylpropiophenone	
MET-22	<i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-23	(<i>E,Z</i>)-2-chloro- <i>N</i> -(2-ethoxyethyl)- <i>N</i> -(1-phenyl-1-propenyl)acetamide	

Name	Chemical name	Structure
MET-24	<i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl)propylamine	
MET-25	2-ethoxyethylamine	
MET-26	<i>N</i> -(2-hydroxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-27	<i>N</i> -(2-methyl-1-phenyl-1-propenyl)morpholine-3-one	
MET-28	<i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)-2-(methylsulfinyl)acetamide	
MET-29	<i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)-2-(methylsulfonyl)acetamide	
MET-30	<i>S</i> -[<i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]-D,L-cysteine	
MET-31	<i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)oxamic acid	
MET-32	phenylglyoxylic acid	

Name	Chemical name	Structure
MET-33	benzoic acid	
MET-34	4-hydroxybenzoic acid	
MET-35	<i>N</i> -(2-hydroxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)-2-(methylsulfinyl)acetamide	
MET-36	2-carboxy-3-hydroxycinnamic acid	
MET-37	3-hydroxy-2-methylpropiophenone	
MET-38	2-acetoxy-2-methylpropiophenone	
MET-39	2-hydroxy- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)propenoylamide	
MET-40	(<i>E,Z</i>)-2-chloro- <i>N</i> -(2-hydroxyethyl)- <i>N</i> -(3-hydroxy-2-methyl-1-phenyl-1-propenyl)acetamide	

Name	Chemical name	Structure
MET-41	3-[<i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)carbamoyl methylthio]-D,L-lactic acid	
MET-42	<i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)-2-sulfoacetamide	
MET-43	<i>N</i> -(2-ethoxyethyl)-2-mercapto- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-44	bis S-[<i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]	
MET-45	2-hydroxy- <i>N</i> -[2-(2-hydroxyethoxy)-ethyl]- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-46	<i>N</i> -[2-(2-hydroxyethoxy)ethyl]- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)acetamide	
MET-47	<i>N</i> -(2-carboxyacetyl)-S-[<i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]-D,L-cysteine	

Name	Chemical name	Structure
MET-48	3-[<i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)carbamoylmethylthio)-2-(α -D-glucopyranosyloxy)propionic acid	
MET-49	S-[<i>N</i> -(2-ethoxyethyl)- <i>N</i> -(2-methyl-1-phenyl-1-propenyl)carbamoylmethyl]-D,L-cysteine-S-oxide	
MET-50	3-((2-((2-ethoxyethyl)(2-methyl-1-phenylprop-1-en-1-yl)amino)-2-oxoethyl)sulfinyl)-2-hydroxypropanoic acid	