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Document Type	OECD Annex Point(s) Planned (Registration docs only)	PTR Number	Report / File Number(s)	Date Issued (dd-MMM-yy)
Regulatory	IIA 6.2	10001126-5051-3 (PTR Id 72581)	050057	02-MAR-10

Document Title

A Nature of the Residue Study with 14C-Quizalofop-P Ethyl Ester Applied to AAD-1 Maize 2008 (Event 474)

Author(s) and ID(s)

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Reviewer(s) and ID(s)

Materials being tested:

Active Ingredient(s) / Structural Gene(s) / Protein(s), etc.	Formulation Nos.	Product Name(s) (DAS only)
		14C-quizalofop-P ethyl ester (PH- and QU-labels)

Lab Notebook(s)	Information Release No.	GLP Substance Nos (TSN, AGR)	Other Batch/Lot Number(s)

Key Terms including compound numbers (ex: XDE-123)

Trial Category (s) (Efficacy or Research docs only) Bayer Codes (target crops and pests)

List 1 or blank	List continued or blank	
List 1 or blank	List continued or blank	
List 1 or blank	List continued or blank	

Data Requirement(s) (Guideline Numbers)

Global Data Matrix? ☐

State or Province of Study	Country of Study	Protocol Number(s)	Internal Study/Trial Number(s)	External Study/Trial Number(s)	Study Completion Date (dd-MMM-yy)
					2-Mar-10
					GLP Status
					GLP

Analytical Methods Only:

Method Type	Analyte(s)
Choose one or blank	

Method Technique(s)	Matrix

SUMMARY

(In accordance with 40 CFR part 152, this summary is available for public release after registration)

STUDY TITLE

A Nature of the Residue Study with 14C-Quizalofop-P Ethyl Ester Applied to AAD-1 Maize 2008 (Event 474)

DATA REQUIREMENTS

OECD Guidance Document 501 for Metabolism in Crops (Issued 8 January 2007)

AUTHORS

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STUDY COMPLETED ON

02 March 2010

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LABORATORY STUDY ID

080057

A Nature of the Residue Study with 14C-Quizalofop-P Ethyl Ester Applied to AAD-1 Maize 2008 (Event 474)

SUMMARY

[^{14}C]-quizalofop-P ethyl ester ((RS)-2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]propionic acid ethyl ester) was foliar-applied to plots of AAD-1 maize (applied at growth stage V6) at the maximum seasonal rate of 92 g a.i./ha. The maize was grown outdoors to maturity at Research For Hire. Plot maintenance simulated typical cultural practices.

Immature plants were collected 7 August 2008, 36 days after the foliar application. Mature grain, cobs, and fodder were collected 3 September 2008, 63 days after the application. At maturity, the mature grain contained 0.009 mg/kg and 0.010 mg/kg quizalofop acid equivalents in the PH-label and QU-labeled samples, respectively. The mature cobs contained 0.006 mg/kg quizalofop acid equivalents in both the PH-label and QU-labeled samples. The mature fodder contained 0.384 mg/kg and 0.415 mg/kg quizalofop acid equivalents in the PH-label and QU-labeled samples, respectively. The cobs were not analyzed further, due to the very low levels of radioactivity. A portion of the grain was sequentially extracted with neutral solvent then an acid reflux, and starch was isolated from a separate portion. A portion of the fodder was sequentially analyzed beginning with a neutral extraction, then an acid reflux followed by an organic rinse and the non-extractable residue was subjected to bound residue determinations such as pectin, acid-detergent fiber, lignin, and cellulose isolation.

Less than 0.001 mg ae/kg was extracted from grain using either neutral organic solvent or acid. Approximately 50% of the TRR in grain (0.004 mg ae/kg) was associated with starch. In the mature fodder, quizalofop-P ethyl ester was not detected and quizalofop acid was detected at 1% TRR (≤0.002 mg ae/kg). Quizalofop was extensively metabolized and incorporated into natural plant constituents such as lignin, cellulose, and starch.

STUDY TITLE

A Nature of the Residue Study with 14C-Quizalofop-P Ethyl Ester Applied to AAD-1 Maize 2008 (Event 474)

DATA REQUIREMENTS

OECD Guidance Document 501 for Metabolism in Crops (Issued 8 January 2007)

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080057

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: quizalofop

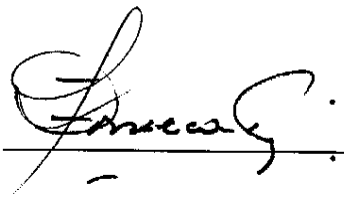
Title: A Nature of the Residue Study with 14C-Quizalofop-P Ethyl Ester Applied to AAD-1 Maize 2008 (Event 474)

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA sec. 10(g).

Company: Dow AgroSciences LLC

Company Agent: D. Fonseca

Title: Regulatory Manager

Signature: 

Date: February 18, 2010

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STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Title: A Nature of the Residue Study with 14C-Quizalofop-P Ethyl Ester Applied to AAD-1 Maize 2008 (Event 474)

Study Initiation Date: 25-MAR-2008

This report represents data generated after the effective date of the EPA FIFRA Good Laboratory Practice Standards.

United States Environmental Protection Agency
Title 40 Code of Federal Regulations Part 160
FEDERAL REGISTER, August 17, 1989

Organisation for Economic Co-Operation and Development
ISBN 92-64-12367-9, Paris 1982

All aspects of this study were conducted in accordance with the requirements for Good Laboratory Practice Standards, 40 CFR 160 with the exceptions noted in the in-life report: weather data and equipment used for plot maintenance was calibrated prior to use but not GLP verified.

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February 18, 2010
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02 March 2010
Study Completion Date

Dow AgroSciences Quality Assurance Unit
Good Laboratory Practice Statement Page

Study ID: 080057

Title: A Nature of Residue Study with 14C-Quizalofop-P ethyl ester Applied to AAD-1 Maize 2008 (Event 474)

Study Initiation Date: 25 March 2008 **Study Completion Date:** 2 March 2010

GLP Quality Assurance Inspections

Date of GLP Inspection(s)	Date Reported to the Study Director and to Management	Phases of the Study which received a GLP Inspection by the Quality Assurance Unit
24 March 2008	25 March 2008	Protocol Review
24 June 2008	23 October 2008	Procedures for the Determination of Ammonium Acetate Extractable Ca, Mg Na & K in Soil (NUT.02.12), Organic Matter-Walkley Black Method (NUT.02.09.06)
11 August 2008	23 October 2008	Raw Data audit, Agvise Lab Numbers 08-383
15 October 2008	15 October 2008	Extraction and LSC of Treated Fodder Samples
22, 23, 25 February, 1 March 2010	1 March 2010	Report & Raw Data Review, Test Substance Container Verification

QUALITY ASSURANCE STATEMENT:

The Quality Assurance Unit has reviewed the final study report and has determined that the report reflects the raw data generated during the conduct of this study.

AUDITS FROM RESEARCH FOR HIRE ARE FOUND ON R058024-4 OF THE APPENDED REPORT

AUDITS FROM ABC LABORATOREIS ARE FOUND ON PAGE 3 OF 10 OF THE APPENDED REPORT

C. L. Dudley — 2 March 2010

C. L. Dudley
Dow AgroSciences, Quality Assurance

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A Nature of the Residue Study with 14C-Quizalofop-P Ethyl Ester Applied to AAD-1 Maize 2008 (Event 474)

1.0 SUMMARY ABSTRACT

[^{14}C]-quizalofop-P ethyl ester ((RS)-2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]propionic acid ethyl ester) was foliar-applied to AAD-1 maize (applied at growth stage V6) at the maximum seasonal rate of 92 g a.i./ha. Separate plots were treated with the PH-label and QU-label of quizalofop. The maize was grown outdoors to maturity at Research For Hire. Plot maintenance simulated typical cultural practices.

Immature plants were collected 7 August 2008, 36 days after the foliar application. Mature grain, cobs, and fodder were collected 3 September 2008, 63 days after the application. At maturity, the mature grain contained 0.009 mg/kg and 0.010 mg/kg quizalofop acid equivalents in the PH-label and QU-labeled samples, respectively. The mature cobs contained 0.006 mg/kg quizalofop acid equivalents in both the PH-label and QU-labeled samples. The mature fodder contained 0.384 mg/kg and 0.415 mg/kg quizalofop acid equivalents in the PH-label and QU-labeled samples, respectively. The cobs were not analyzed further, due to the very low levels of radioactivity. A portion of the grain was sequentially extracted with neutral solvent then an acid reflux, and starch was isolated from a separate portion. A portion of the fodder was sequentially analyzed beginning with a neutral extraction, then an acid reflux followed by an organic rinse and the non-extractable residue was subjected to bound residue determinations such as pectin, acid-detergent fiber, lignin, and cellulose isolation.

Less than 0.001 mg ae/kg was extracted from grain using either neutral organic solvent or acid. Approximately 50% of the TRR in grain (0.004 mg a.e./kg) was associated with starch. In the mature fodder, quizalofop-P ethyl ester was not detected and quizalofop acid was detected at 1% TRR (≤0.002 mg a.e./kg). One metabolite was observed in the fodder at 10.4% of the TRR in the PH-label only (0.040 mg a.e./kg). Quizalofop was extensively metabolized and incorporated into natural plant constituents such as lignin, cellulose, and starch. This study was repeated in 2009 using a similar AAD-1 event-construct.

2.0 INTRODUCTION

2.1. Objective of Study and Guidelines Followed

2.1.1. Purpose of Study

The purpose of this study was to characterize the radioactive residue in immature and mature AAD-1 genetically modified corn following application of ^{14}C-quizalofop at the maximum seasonal rate. Two separate plots were treated with one foliar spray application per radiolabel. The ^{14}C-quizalofop was formulated as an emulsifiable concentrate (EC) for the foliar spray applications, the current proposed commercial formulation, and applied at a target rate of 92 g ai/ha.

2.1.2. Relevant History and Background Information

Quizalofop ((RS)-2-[4-(6-chloroquinoxalin-2-yloxyphenoxy]propanoic acid), is an herbicide developed for post-emegence control of annual and perennial grass weeds (monocotyledons) in a variety of root and oilseed crops. The molecular target of quizalofop is acetyl coenzyme A carboxylase. Quizalofop is toxic to non-genetically modified corn.

Quizalofop is absorbed from the leaf surface and translocated throughout the plant. The ester is hydrolyzed in the plant to the free acid which is the active form. Previous studies (*1*) demonstrated that quizalofop is readily degraded in sterile soil (DT_{50} <1 day) and degradation was accelerated by soil microorganisms. The ethyl ester of quizalofop is stable in neutral and acidic media but is unstable in alkaline media. In broad-leaved plants, absorption and translocation is very limited and most of the applied herbicide remains as quizalofop acid on and/or in treated leaves. The metabolism of 14C-quizalofop-ethyl was studied in soybean and cotton plants (*2*). This study indicated that metabolism was similar, with quizalofop-ethyl rapidly metabolized to the acid (quizalofop) which was further metabolized to the phenol

metabolites 6-chloroquinoxalin-2-ol and 2-(4-hydroxyphenoxy)propanoic acid, and likely glucose conjugates of these phenols.

2.1.3. Guidelines

This study was conducted to fulfill requirements for nature of the residue in plant as outlined in the OECD Guidance Document 501 for Metabolism in Crops (Issued 8 January 2007). This study was conducted in compliance with Good Laboratory Practices standards, 40 CFR Part 160.

2.1.4. Guideline Deviations

None

2.2. Justification of Study Application Rate

The maximum seasonal application rate for quizalofop is 92 g a.i./ha (85 g a.e./ha). This study was conducted at an approximate 1X rate.

3.0 MATERIALS AND METHODS

3.1. Test, Reference, and Control Substances

3.1.1. Test Substance

Two radiolabeled forms of the test substance of the technical product were obtained from the Dow AgroSciences Specialty Synthesis Group. See Figure 1 for structures, radiolabel positions, and nomenclature. Physico-chemical properties of quizalofop ester and acid can be found in Table 1.

3.1.2. Reference Substances

Non-radiolabeled quizalofop ester and quizalofop acid standards were acquired from the Test Substance Coordinator at Dow AgroSciences for use as reference standards for chromatographic and mass spectral comparison. Non-radiolabeled quizalofop ester was also used to dilute the specific activity of the application solution. All reference standards were received in solid form. Solubilities and storage stability data for metabolite standards are not available at this time. Structures, purity, ID numbers, chemical names and abbreviations for reference substances are presented in Figure 2. Reference standards were prepared in acetonitrile at a concentration of approximately 1 mg/mL. Prepared standard solutions were stored refrigerated when not in use.

The chromatogram in Figure 3 presents the UV retention times of the test and reference materials.

3.1.3. Control Substance

An emulsifiable concentrate formulation blank that was comparable to the formulation that is currently being developed for commercial use was added to test material spray solutions. Details of the formulation blank are provided in Figure 2.

3.2. Test Site

The in-life phase of this study was conducted at Research For Hire (RFH). The address of the RFH is: 1696 South Leggett Street, Porterville, California 93257, USA.

3.3. Test System

Genetically modified AAD-1 corn (event-construct pDAS1740-474) was obtained for use in this study. The modification makes the corn resistant to both 2,4-D and “fop” grass herbicides including quizalofop and haloxyfop. Corn is representative of the pulses & oilseeds group. The soil type, transplanting, and maintenance may be found in the in-life report (Appendix A).

The experimental design is detailed in Table 2.

3.4. Preparation and Application of Test Substance

3.4.1. Preparation of Application Solutions

The ^{14}C-phenyl-labeled quizalofop, nominally 0.5 mCi, was received from Specialty Synthesis in approximately 1.8 mL of ethyl acetate. The ^{14}C-quinoxaline-labeled quizalofop, nominally 0.7 mCi, was received from Specialty Synthesis in approximately 2.0 mL of ethyl acetate. Each sample was diluted to 5-mL using acetonitrile, in a volumetric flask. Aliquots (0.025 mL) were diluted in 10-mL volumetric flasks, using acetonitrile to dilute to volume. Aliquots of the dilutions were analyzed by LSC to determine the actual amount of radioactive test substance received as well as the purity (Figure 4). The specific activity of the ^{14}C-quizalofop, was adjusted by combining non-radiolabeled quizalofop ester (10 mg/mL, 0.75 mL for the PH-label, and 0.76 for the QU-label) separately with the remainder of the ^{14}C-quizalofop test substance solutions. The original radioactive solution vial was rinsed with 2 x 1.0 mL acetonitrile, to quantitatively transfer the radioactivity.

The majority of the individual test substance samples (7.3 mL and 7.0 mL for the PH- and QU-label, respectively) were transferred to separate vials for application. The application aliquots were blown to dryness under a stream of nitrogen, and shipped to Research For Hire (RFH). Upon receipt at RFH, each of the test substance solution aliquots were dissolved in 0.12 g of formulation blank E2469-23. Each test substance solution was shaken, swirled, and sonicated until all solids went into solution then stored in a refrigerator.

Each application solution was prepared separately, on the day of application. Details of each preparation can be found in the in-life report (Appendix A). In general, the application solution was thawed, rinsed multiple times with water and brought to a known volume with water, then transferred to an application container. Aliquots were taken to determine the concentration of the spray solution and confirm homogeneity.

3.4.2. Application Procedures

Details of each application can be found in the in-life report (Appendix A).

The applications were made using an R&D wand sprayer pressurized with CO_2, in which the container was covered with aluminum foil, evenly spraying in two passes per row of corn (growth stage V6). The spray solution container was then rinsed with 30 mL of water, swirled, then sprayed evenly onto the plot in the same manner.

3.4.3. Significant Events

Table 3 lists the significant events for this study. More detailed information on the in-life phase of the study can be found in the in-life report (Appendix A).

3.5. Sample Collection

Details of each harvest can be found in the in-life report (Appendix A), while harvest dates are listed in Table 3.

Immature plant samples, R4 growth stage (milky inner fluid in kernels) were harvested on 7 August 2008. The plants were cut approximately 5 cm above the soil surface, then cut into approximately 20 cm segments to fit into bags, weighed, and frozen pending shipment to ABC Laboratories.

The mature crop was harvested on 3 September 2008, at BBCH 89 (fully ripe: kernels hard and shiny, about 65% dry matter). The cobs were removed from the stalks then the stalks cut approximately 8 cm above the soil surface. The grain and cobs were separated by hand. Each sample was individually placed in tared bags and weighed. The samples were frozen pending frozen shipment to ABC Laboratories.

3.6. Sample Milling

Details of the milling procedures can be found in the ABC Laboratories report (Appendix B). In general, samples were broken into smaller pieces while frozen using hammer and/or a Robot Coupe with dry ice. The milling of the treated grain, cobs, and fodder was completed using a Straub grinding mill with dry ice.

3.7. Measurement of Total Radioactive Residue (TRR)

Details of the oxidative combustion procedures can be found in the ABC Laboratories report (Appendix B). Aliquots (5 x approximately 0.2 g) of the milled samples were analyzed by oxidative combustion as described in Appendix B to determine the radioactive residues in the samples. Raw data from the combustion assay of the treated mature samples can be found in Appendix B. Forage samples were combusted in a similar fashion at Dow AgroSciences due to an apparent error in the recording of the combustion weights at ABC Laboratories.

3.8. Sample Extraction, Analysis, Characterization, and Identification

In general, the milled samples were analyzed by the sequence of extractions described in Table 4 and Figure 5. Details of each step are provided below.

3.8.1. Neutral Organic Solvent Extraction (EX1)

Approximately 20 g of homogenized forage and grain, or 10 g fodder, were extracted with approx. 75 mL of 80/20 acetonitrile/water, as described in Figure 5 and Table 4. The mixture was blended using a Polytron homogenizer for approx. 5 minutes at ≥10,000 rpm. The mixture was then shaken on a horizontal shaker for approx. 30 minutes (low speed). After vacuum filtering, the solids were transferred back into the original jar. The extraction procedure was repeated two more times but without Polytron homogenization, pooling the extracts. The volume of the extract was measured and triplicate aliquots were analyzed by liquid scintillation counting as described in Section 3.9.2. Additional aliquots of the extract were cleaned-up using

the Strata-X SPE procedure described in Section 3.9.3, then analyzed by HPLC as described in 3.9.4.

3.8.2. Acid Extraction (EX2)

The tissue remaining after the neutral extraction (3.8.1) was further extracted with approx. 50 or 75 mL of 1 N HCl, as described in Figure 5 and Table 4. The mixture was heated to approximately 50 ºC while shaking for one hour, then shaken at room temperature on a horizontal shaker for approximately 60 minutes (low speed). After vacuum filtering (or centrifuging and decanting), the solids were transferred back into the original jar. The tissue was extracted one or two more times with 80/20 acetonitrile/water, shaking 30 minutes without heat, and pooling the extracts. The volume of the combined extract was measured and triplicate aliquots were analyzed by liquid scintillation counting as described in 3.9.2. Additional aliquots of the extracts were cleaned-up using the SPE procedure described in Section 3.9.3, then analyzed by HPLC as described in 3.9.4.

3.8.3. Determination of the Non-Extractable Residue (NER)

The tissue remaining after the second sequential extraction (Section 3.8.2) was air-dried and weighed. Triplicate aliquots were analyzed by oxidative combustion (Section 3.9.1) to determine the amount of non-extractable radioactive residue.

3.8.4. Bound Residue Determination of the Non-Extractable Residue (NER)

The general bound residue characterization scheme used was a modification of the IUPAC Technical Report (*3*).

3.8.4.1. Pectin Solubilization

The pectin substances in the NER were solubilized using ethylenediaminetetraacetic acid (EDTA), 50 mM in a 50 mM pH 4.5 buffer (*4*) as described in Figure 6 and the second page of Table 4. An aliquot (*ca*. 1 g) of the non-extractable tissue (Section 3.8.3) was sonicated or Polytron homogenized at 10,000 rpm for approximately two minutes, then heated (approx. 70 ºC) with 50-100 mL of the buffered EDTA while stirring for approximately 5 hours. After

cooling then vacuum filtering, the solids were transferred back into the original jar. The volume of the extract was measured and triplicate aliquots were analyzed by liquid scintillation counting as described in 3.9.2.

3.8.4.2. Lignin Extraction

The lignin was removed from the solids remaining after the pectin solubilization using a procedure adapted by Hatfield (*5*). First, the solids were transferred to a flask and covered with water (40 mL). Sodium chlorite (1.25 g $NaClO_2$) and glacial acetic acid (150 µL of 17.4 M CH_3COOH) were added to each solid sample, stirred, and heated in a hot water bath (approx. 70 ºC) for one hour. Additional $NaClO_2$ (0.4 g) and acetic acid (150 µL) were added to each sample, mixed thoroughly, and incubated for another hour. After centrifugation, the solids were vacuum filtered and washed several times with water. The total amount of radioactivity in the liquid fraction, which included dissolved lignin, was determined by LSC analysis. After air-drying overnight, the remaining solids were weighed and used in the ADF Isolation procedure, below. This procedure is also described in described in Figure 6 and the second page of Table 4.

3.8.4.3. Acid-Detergent Fiber (ADF) Isolation

The ADF fraction was isolated from the solids remaining after the lignin extraction step, using a procedure adapted by Van Soest (*6*) and is also described in described in Figure 6 and the second page of Table 4. The pellet from the lignin extraction step (Section 3.8.4.2) was refluxed with stirring for approximately one hour in acid detergent solution (20 g hexadecyltrimethyl-ammonium bromide in 1 L 2.0 N H_2SO_4). Following the reflux period, the solids were removed by vacuum filtration through a tared sintered glass filter. The resulting filter cake was washed with water, then acetone. After drying in a 100 ºC oven overnight, the remaining solids (this is the ADF fraction) were weighed and combusted as described in Section 3.9.1. The ADF fraction consists of cellulose and including radioactivity encapsulated by cellulose. The total amount of radioactivity in the liquid fraction, which included hemicellulose and dissolved plant proteins, was determined by LSC analysis.

3.8.4.4. Starch Isolation

The procedure for isolating starch was adapted from Wargo *et al* (7). One replicate of each of the following was used for the isolation procedures: fresh, unextracted grain (replicates C & D) and non-extractable residue remaining after exhaustive extraction of grain (replicates A & B). The tissue (5 g) was weighed into a centrifuge jar and covered with 100 mL dimethyl sulfoxide (DMSO)/water (90/10, v/v) and blended at 10,000 rpm for 5-10 minutes using a Polytron homogenizer. The mixtures were shaken overnight on a horizontal shaker (low speed). The samples were centrifuged 30 minutes at 600*g* and the supernatant decanted. Anhydrous ethanol was used to precipitate the starch from the supernatant. The starch was filtered and washed several times with anhydrous ethanol. The volume of the combined supernatant and washes was recorded and aliquots analyzed by LSC. The non-extractable residue was dried under warm air and submitted for oxidative combustion analysis. The isolated starch was air-dried in a hood and aliquots submitted for combustion analysis.

3.8.5. Sample Storage Conditions

Samples, including milled tissue, extracts, and post-extracted samples, were stored in freezers at *ca*. -20 ºC when not in the process of analysis.

3.9. Instrumental Methods

3.9.1. Oxidative Combustion

The amount of total ^{14}C activity in samples (particularly post-extracted tissue) was determined by combusting aliquots of the samples in an oxygen atmosphere to give $^{14}CO_2$ which was trapped in an alkaline trapping reagent. The ^{14}C activity was then measured by LSC.

3.9.2. Liquid Scintillation Counting (LSC)

The liquid scintillation counters automatically converted the radioactivity counting rate in counts per minute (cpm) to disintegrations per minute (dpm) using an external standard to correct for sample quenching. The instrument was calibrated at least every six months with a set of ten

quenched standards. Each day of use, the instrument was normalized and its performance was checked with respect to background cpm value, unquenched standard cpm value, and quenched standard dpm value for a range of quenched standards. The scintillation counters used were Packard Tri-Carb (Packard Instrument Co.). The dpm value for an extraction sample was determined by LSC after diluting an appropriate aliquot of the sample with scintillation cocktail and counting for at least five minutes.

3.9.3. Strata-X Solid Phase Extraction (SPE)

The general clean-up procedure for the neutral organic extracts (forage and fodder) was with a Waters C_{18} SPE cartridge (500 mg) or Strata-X SPE (QU-label forage). The samples were prepared by concentrating (40 ºC waterbath, 10 psi nitrogen) to remove the majority of the organic solvent, and diluting with water. The SPE cartridges were conditioned with acetonitrile (5 mL) followed by water (2 x 5 mL). The prepared sample was applied to the conditioned SPE, eluted at approx. 2 mL/min, collecting the eluate. The sample vial was rinsed with water (5 mL, forage) or 1% acetic acid in water (5 mL, fodder), transferred to the SPE cartridge, and eluted at approx. 2 mL/min, pooling with the load eluate. For the QU-forage and fodder samples only, the SPE was dried under full vacuum for 20 seconds. The load/wash volume was measured by weight. The SPE was eluted with acetonitrile/water (80/20, v/v) in three aliquots (4 mL, 4 mL, 2 mL), pooling the elution aliquots.

The elution samples were concentrated to near dryness in a Turbovap (40 ºC water bath and 10 psi nitrogen). The elution samples were reconstituted in 250 µL of acetonitrile, sonicated, and diluted with 750 µL of water. The load/wash samples were concentrated in a Turbovap (40 ºC water bath and 10 psi nitrogen) to <2 mL (fodder) and the volume measured, or for forage samples concentrated to dryness and reconstituted in 1.0 mL acetonitrile/water (80/20, v/v). Triplicate aliquots of each load/wash, concentrated load/wash, and reconstituted elution sample were analyzed by LSC. The concentrated load/wash and elution samples were also analyzed by HPLC.

The acid extracts (forage and fodder) were similarly prepared for HPLC, except that for the forage samples only a Strata-X SPE was used (500 mg, Phenomenex part number 8B-S100-HDG) and the SPE was dried after each conditioning solvent and for 10 seconds after the load eluted (forage only) and for 20 seconds after the water was eluted. The C_{18} SPE used for the fodder samples was rinsed with 1% acetic acid in water and combined with the load. In addition, the load/wash samples were concentrated to 0.9-2 mL, and neutralized prior to HPLC.

3.9.4. High Performance Liquid Chromatography

The primary HPLC system (ARC-3) used for this study consisted of an Agilent 1200 Series autoinjector, degasser, and binary pump, an 1200 Series variable wavelength detector, and an ν.ARC Radio-LC System on-line radioactivity detector (AIM Research Co., Hockessin, Delaware, USA). The ν.ARC sample cell was 0.8 mL, and the efficiency was approximately 75%. All components were controlled by ARC Data System software on a Dell Optiplex computer.

The primary reversed phase HPLC method used for sample analysis is presented in Table 5. The typical HPLC column used was a Synergi 4 µm Hydro-RP, 150 x 4.6 mm (Phenomenex). The sum of the radioactivity accounted for in each sample analyzed was compared to LSC data from each sample and used to determine chromatographic recovery.

Typical retention times for quizalofop acid and ester are shown in Table 5. A typical UV chromatogram showing the retention times for quizalofop acid and ester is provided in Figure 3.

3.10. Method Verification and Data Handling

3.10.1. Detection Limits

The formulas used to estimate the reliability of the radioactive counting data were obtained from Currie (*8*).

$$\text{Limit of Detection (LOD)}_{(\text{dpm})} = \frac{2.71 + \left(4.65\sqrt{\text{bkg dpm} \times \text{count time}}\right)}{\text{count time}}$$

$$\text{LOD}_{(\text{ppm})} = \frac{\text{LOD}_{\text{dpm}}}{\text{Sample Weight}_{\text{g}} \times \text{Specific Activity}_{\text{dpm/µg}}}$$

$$\text{Limit of Quantitation (LOQ)}_{(\text{dpm})} = \frac{50\left(1 + \sqrt{1 + \frac{\text{bkg dpm} \times \text{count time}}{12.5}}\right)}{\text{count time}}$$

$$\text{LOQ}_{(\text{ppm})} = \frac{\text{LOQ}_{\text{dpm}}}{\text{Sample Weight}_{\text{g}} \times \text{Specific Activity}_{\text{dpm/µg}}}$$

Example: For the combustions, background was typically 55 dpm, typical aliquot weight was 0.2 g, and count time was 5 minutes).

$$\text{LOD, tissue}_{(\text{dpm})} = \frac{2.71 + \left(4.65\sqrt{55\ \text{dpm} \times 5\ \text{min}}\right)}{5\ \text{min}} = 16\ \text{dpm over background}$$

$$\text{LOD, tissue}_{(\text{ppm})} = \frac{16\ \text{dpm}}{0.2\ \text{g} \times 76{,}145\ \text{dpm/µg}} = 0.0010\ \text{µg/g (0.0008 µg/g for QU-label)}$$

$$\text{LOQ, tissue}_{(\text{dpm})} = \frac{50 \times \left(1 + \sqrt{1 + \left(\frac{55\ \text{dpm} \times 5\ \text{min}}{12.5}\right)}\right)}{5\ \text{min}} = 58\ \text{dpm over background}$$

$$\text{LOQ, tissue}_{(\text{ppm})} = \frac{58\ \text{dpm}}{0.2\ \text{g} \times 76{,}145\ \text{dpm/µg}} = 0.0038\ \text{µg/g (0.0029 µg/g for QU-label)}$$

3.10.2. Statistical Methods

Statistical analyses included calculations of means and standard deviations for the interpretation and summarization of results. Means, standard deviations, and Q-tests were calculated using Microsoft Excel™. More decimal places than are shown in tables were used to calculate values presented in this report. Therefore, minor differences due to rounding may be found when calculating values from data in tables presented here.

3.10.3. Sample Calculations

Sample Calculations may be found in Appendix C.

3.10.4. Material Balance

No material balance determinations were made. However, individual recovery results are reported for each sample analysis step.

3.10.5. Reference Values

HPLC retention times for quizalofop acid and ester may be found in Table 5.

4.0 RESULTS AND DISCUSSION

4.1. In-Life Summary

Average radiochemical purity of the test substances prior to application were determined to be 99.6% and 98.8% for the ^{14}C-PH-label quizalofop and ^{14}C-QU-label quizalofop, respectively. Representative HPLC chromatograms are provided in Figure 4. The specific activity was calculated to be 76,145 dpm/µg (12.79 mCi/mmol) for the applied ^{14}C-PH-label quizalofop. For the ^{14}C-QU-label quizalofop the specific activity was calculated to be 100,384 dpm/µg (16.86 mCi/mmol).

On 19 June 2008, every corn plant was tested for the presence of the AAD-1 gene, using a strip test kit provided by DAS. Only one untreated control plant was found to be negative for the gene; it was removed from the control plot. The remainder of the plants was thinned according to standard agricultural practices.

As shown in Table 3, the applications were made on 2 July 2008, when the plants were at the V6 growth stage. The PH-label plot received 12.7 mg, equivalent to 91 g ai/ha or 99% of the target amount of formulated quizalofop (Table 6). The QU-label plot received 14.2 mg, equivalent to 102 g a.i./ha or 110% of the target amount of formulated quizalofop (Table 6). Both plots were applied at a seasonal 1X rate.

Radiochemical purity and stability of the formulated PH-label application solution averaged 99.0%, (98.8-99.1%), for the pre- and post-application retainer samples. Radiochemical purity and stability of the formulated QU-label application solution averaged 98.7%, (98.2-99.4%). Example chromatograms are provided in Figure 7.

The report of the in-life phase of this study is presented in Appendix A, including weights of the harvested crop samples.

4.2. Distribution of Total Tissue Residue

Table 7 presents the distribution of the total tissue residues within the crop harvests. In general, samples from the QU-label plot contained higher residue levels than those from the PH-label plot. For example, forage samples contained 0.069 and 0.212 mg ae/kg, in the PH- and QU-label samples, respectively. Mature grain and cobs contained ≤0.01 mg ae/kg irrespective of radiolabel. Fodder contained 0.384 and 0.415 mg ae/kg for the PH- and QU-label, respectively. Sample weights are provided in the in-life report (Appendix A). Due to the low TRR levels, cob samples were not analyzed further.

4.3. Characterization and Identification of Residues

4.3.1. Neutral Organic Extraction (EX1)

Aliquots of forage, grain, and fodder were initially extracted with neutral organic solvent (80:20 acetonitrile:water) as described in Section 3.8.1 summarized in Table 8. In general, 50-65% of the TRR was extracted from the forage and fodder using this procedure. Much lower levels of the mature grain were extracted, less than 10% of the TRR. Due to the low levels of radioactivity, the grain samples were not analyzed further.

An aliquot of each forage and fodder neutral organic extract was purified and concentrated using a SPE as described in Section 3.9.3 and analyzed by HPLC. SPE recoveries are summarized in Table 9. In general, SPE recoveries were greater than 90%, however, approximately 15-25% of the radioactivity was recovered in the load/wash. Therefore, both phases were analyzed by HPLC. Concentration recoveries for the load/wash fractions were acceptable (72-87% for forage, and 90-102% for fodder).

Sample chromatograms are provided in Figure 8 and Figure 9 for the forage, and Figure 10 and Figure 11 for the fodder. As shown in these figures, the load/wash samples contained multiple components eluting near the solvent front, while the eluent fractions contained numerous, low-level peaks eluting from near the solvent front until almost 30 minutes. HPLC recoveries were 62-121%, which were considered acceptable because of the low amounts of radioactivity injected and the multiple peaks.

HPLC results are summarized in Table 10. Following a single application of quizalofop to AAD-1 corn, the neutral organic extracts of immature and mature samples contained multiple, low-level metabolites. In the forage samples less than 1% of the TRR eluted in the region of quizalofop ethyl ester and less than 2% of the TRR eluted in the region of quizalofop acid. The polar unknowns totaled an average of approximately 14-17% of the TRR, which was ≤0.030 mg a.e./kg. Unknowns eluting at approximately 12 and 15 minutes were tracked although barely noticeable above the hump of non-resolved radioactivity. These two unknowns

each accounted for an average of <5% of the forage TRR (≤0.010 mg ae/kg), demonstrating the low levels of any individual component.

There was no quizalofop ester detected in the fodder extracts, and an average of less than 1% of the TRR eluted in the region of quizalofop ester. The polar unknowns totaled an average of <8% of the TRR, which was ≤0.030 mg ae/kg. Unknowns eluting at approximately 12 and 15 minutes each accounted for an average of <7% of the TRR (≤0.027 mg ae/kg).

4.3.2. Acid Extraction (EX2)

The pellet remaining after the neutral extraction was next extracted with 1 N HCl, then rinsed, as described in Sections 3.8.2 and summarized in Table 8. In the forage, an average of an additional 11-14% of the TRR was extracted with this procedure. An average of less than 8% of the TRR was extracted from the grain. The grain extracts were not analyzed further due to the low levels of radioactivity (<0.001 mg ae/kg). The acid extracts of the mature fodder contained an average of 16-18% of the TRR. The forage and fodder extracts were prepared for HPLC using the SPE procedure described in Section 3.9.3. SPE recoveries are summarized in Table 9. In general, SPE recoveries were greater than 90%, however, approximately 15-25% of the radioactivity was recovered in the load/wash. Therefore, both phases were analyzed by HPLC. Concentration recoveries for the load/wash fractions were acceptable (85-111% for forage, and 87-104% for fodder).

Sample chromatograms are provided in Figure 12 for the forage, and Figure 13 and Figure 14 for the fodder. As shown in these figures, the acid extracts contained multiple components eluting near the solvent front, and numerous, low-level peaks eluting from near the solvent front until about 18 minutes. HPLC recoveries were 43-152%, which were considered acceptable because of the very low amounts of radioactivity injected and the multiple peaks.

HPLC results are summarized in Table 10. Following a single application of quizalofop to AAD-1 corn, the acid organic extracts of immature and mature samples contained multiple, polar, low-level metabolites. No radioactivity eluted in the region of quizalofop ethyl ester or

quizalofop acid. The polar unknowns totaled an average of approximately less than 2% of the TRR in the forage (≤0.030 mg ae/kg) and 7-9% of the TRR in fodder (≤0.033 mg ae/kg).

4.3.3. Extraction Summary

Table 8 and Table 10 summarizes the amount of the TRR that was extractable and the HPLC results, respectively. From the forage and fodder, 60-80% of the TRR was extracted and analyzed by HPLC. Greater than 80% of the radioactivity in the grain was unextractable, although less than 0.010 mg ae/kg. There were no significant metabolites observed in either the forage or fodder. One metabolite was observed in the fodder at 10.4% of the TRR in the PH-label only (0.040 µg/g), but is most likely multiple components that were not resolved by HPLC. Quizalofop ethyl ester was detected only in the forage samples at less than 1% of the TRR (≤0.001 mg ae/kg), and quizalofop acid was detected in both the forage and fodder samples at less than 2% of the TRR (≤0.002 mg ae/kg).

4.3.4. Bound Residues

The bound residues were evaluated as described in Section 3.8.4, and the results are shown in Table 11. The majority of the non-extractable forage and grain residue was ADF-soluble, consisting of primarily hemicellulose and dissolved plant proteins. In the fodder, radioactivity was associated with all of the fractions, with the highest amounts in the lignin. The amount of radioactivity in any one fraction was ≤0.033 mg ae/kg, even when the lower procedural recoveries were normalized to 100%. These data demonstrate the extensive incorporation/encapsulation of the radioactivity following application of quizalofop to AAD-1 corn.

4.3.5. Starch Isolation

Starch was isolated from the grain as described in Section 3.8.4.4, and the results are shown in Table 12. As shown in Table 12, 50-55% of the TRR in the PH-label grain was associated with starch, while approximately 44% of the TRR in the QU-label grain was associated with starch. Very little of the radioactivity was non-extractable with DMSO (≤0.003 mg ae/kg) and the

balance was extractable but did not precipitate as starch (≤0.005 mg ae/kg). Recoveries were high, averaging 113-138%.

4.4. Sample Storage Stability

All samples and extracts were stored frozen at approximately -20 ºC when not in use. Initial analyses of rinses and extracts occurred within 8 weeks.

4.5. Metabolic Pathway

The proposed metabolic pathway is presented in Figure 15. As shown in the diagram, the metabolism of quizalofop-P-ethyl ester proceeds from the ester to the acid, the active form. Metabolism continues through incorporation of the radiolabeled carbon into natural plant constituents, such as lignin and cellulose.

Quizalofop is phytotoxic to non-genetically modified corn. In broad-leaved plants, absorption and translocation is very limited and most of the applied herbicide remains quizalofop acid on and/or in treated leaves (*1*). Therefore, the metabolism in AAD-1 corn is much more extensive in AAD-1 corn than in broad-leaved plants.

5.0 CONCLUSIONS

A foliar application of quizalofop at the maximum proposed seasonal application rate resulted in immature forage containing 0.069 and 0.212 mg ae/kg, in the PH- and QU-label, respectively. The grain and cobs collected at maturity contained ≤0.010 mg ae/kg, while the fodder contained 0.384 and 0.415 mg ae/kg in the PH- and QU-label, respectively.

Approximately 80% of the TRR in forage and fodder tissue was neutral and acid extractable. When chromatographed, multiple, low-level peaks of a broad range of polarity were detected, all

of which were individually ≤0.04 mg ae/kg. There were no significant metabolites observed in either the forage or fodder. Quizalofop ethyl ester was detected only in the forage samples at less than 1% of the TRR (≤0.001 mg ae/kg), and quizalofop acid was detected in both the forage and fodder samples at less than 2% of the TRR (≤0.002 mg ae/kg). The non-extractable radioactivity in forage and fodder tissue was found to be incorporated into natural plant constituents such as lignin, hemicellulose, and cellulose.

Although the grain contained very low levels of radioactivity, aliquots were analyzed. The majority of the radioactivity, approximately 80% of the TRR, was non-extractable with neutral or acid solvents. None of the residue was analyzed by HPLC, due to the very low levels extracted. The non-extractable residue was characterized as naturally incorporated into protein and hemicellulose (approximately 60% of the TRR, 0.005 mg ae/kg), and by a separate procedure, incorporated into starch (44-55% of the TRR, approximately 0.004 mg ae/kg).

In summary, the radioactive residue was distributed amongst multiple components including natural plant products. Quizalofop acid is the only residue requiring detection in the MOR studies conducted on AAD-1 corn, and a hydrolysis step is not necessary in the analytical method for complete detection of quizalofop acid.

This study was repeated in 2009 using a similar AAD-1 event-construct (*9*).

6.0 RETENTION OF RECORDS

Original raw data, as defined by 40 CFR 160, the signed protocol original, amendments, deviations, and the signed original of the final report are retained in the archives of Dow AgroSciences located at 9330 Zionsville Road, Indianapolis, Indiana 46268-1054.

7.0 REFERENCES

1. Metabolic Pathways of Agrochemicals Part 1:Herbicides and Plant Growth Regulators; Roberts, T. R. editor-in-chief; The Royal Society of Chemistry: Cambridge, UK, 1998.

2. Koeppe, M. K., Anderson, J. J., and Shalaby, L. M. Metabolism of [14C]Quizalofop-ethyl in Soybean and Cotton Plants. *J. Agric. Food Chem.* **1990** (38) 1085-1091.

3. Skidmore, M. W., Paulson, G. D., Kuiper, H. A., Ohlin, B., Reynolds, S. Bound Xenobiotic Residues in Food Commodities of Plant and Animal Origin. *Pure & Appl. Chem.*, **1998**, 70 (7), 1423-1447.

4. Langebartels, C., Harms, H. Analysis for Nonextractable (Bound) Residues of Pentachlorophenol in Plant Cells Using a Cell Wall Fractionation Procedure. *Ecotox. Environ. Safety*, **1985**, 10, 268-279.

5. Hatfield, R. D. Carbohydrate Composition of Alfalfa Cell Walls Isolated from Stem Sections Differing in Maturity. *J. Agric. Food Chem.* **1992**, 40, 424-430.

6. Van Soest, P. J. Use of Detergents in the Analysis of Fibrous Feeds. II. A Rapid Method for the Determination of Fiber and Lignin. *J. AOAC Int*. **1963**, 46, 829-835.

7. Wargo, J. P.; Honeycutt, R. C.; Adler, I. L. Characterization of Bound Residues of Nitrofen in Cereal Grains. *J. Agric. Food Chem.* **1975**, 23 (6), 1096.

8. Currie, L. A. Limits for Qualitative Detection and Quantitative Determination-Application to Radiochemistry. *Anal. Chem*. **1968**, 40 (3), 586-593.

9. Liu, D. and Smith, K. P. A Nature of the Residue Study with [^{14}C]-Quizalofop Applied to AAD-1 Corn (Event 278). Dow AgroSciences Study ID 090059, in-progress.

Table 1. Physico-chemical properties of quizalofop-P acid and quizalofop-P-ethyl ester

Parameter	Values for acid	Values for ethyl ester
Water solubility	0.3 mg/L (20 ºC)	0.4 mg/L (20 ºC)
Vapor Pressure	0.866 Pa (20 ºC)	1.1×10^{-7} Pa (20 ºC)
pK_a	3.1	non-ionized
Log K_{ow}	1.9×10^{4} (23 ± 1 ºC)	4.66 (23 ± 1 ºC)

From Metabolic Pathways of Agrochemicals Part 1:Herbicides and Plant Growth Regulators; Roberts, T. R. editor-in-chief; The Royal Society of Chemistry: Cambridge, UK, 1998.

Table 2. Experimental Design

Parameter	Description – Foliar Plot
Test Site	Research For Hire (RFH) 1696 South Leggett Street, Porterville, California 93257, USA
Soil type	sandy clay loam
Crop type	AAD-1 corn, pDAS 1740-474
Application formulation	emulsifiable concentrate (EC)
Application timing and target rate (crop stage)	92 g ai/ha *at least* 7 days after V4 *and no later than* V6
Immature harvests	forage (R4)
Mature harvest	grain, cobs, and fodder
Other details	none

Table 3. Significant Events

Event	Date	Days after First Application	Days after Harvest
Planting	28 May 2008	Not Applicable	Not Applicable
Foliar Application (V6)	2 July 2008	0	Not Applicable
Immature Harvest – Forage (R4)	7 August 2008	36	Not Applicable
Milling Completed	15 August 2008	Not Applicable	8
Combustion Analysis	20 August 2008	Not Applicable	13
Initiate Extraction	16 September 2008	Not Applicable	40
HPLC characterization init.	19 September 2008	Not Applicable	43
Mature Harvest - Grain	3 September 2008	63	Not Applicable
Milling Completed	18 September 2008	Not Applicable	15
Combustion Analysis	6 October 2008	Not Applicable	33
Initiate Extraction	9 October 2008	Not Applicable	36
HPLC characterization init.	23 October 2008	Not Applicable	50
Mature Harvest - Cobs	3 September 2008	63	Not Applicable
Milling Completed	17 September 2008	Not Applicable	14
Combustion Analysis	6 October 2008	Not Applicable	33
Not extracted	Not Applicable	Not Applicable	Not Applicable
Mature Harvest - Fodder	3 September 2008	63	Not Applicable
Milling Completed	1 October 2008	Not Applicable	28
Combustion Analysis	6 October 2008	Not Applicable	33
Initiate Extraction	15 October 2008	Not Applicable	42
HPLC characterization init.	27 October 2008	Not Applicable	54

Table 4. Typical Sample Analysis Procedure for Forage, Grain, and Fodder Samples Except Where Noted (See Also Figure 6)

Parameter		Description
Neutral Extraction (EX1) *	Solvent	Acetonitrile/water (80/20, v/v)[1]
	Procedure*	Weigh approximately 20 g milled tissue (10 g for fodder) Add extraction solvent (approx. 75 mL) Polytron homogenize (~5 min @ ≥10K rpm) Shake on horizontal shaker (low speed) ~30 min Vacuum filter Repeat 2 more times using approx. 50 mL solvent (75 mL for fodder), without Polytron Record volume of the extract Transfer tissue back into original jar
	Method of analyses	LSC of triplicate aliquots of the extract
Acid Extraction (EX2)	Solvent	1 N HCl
	Procedure	Add extraction solvent (approx. 50 mL, 75 mL for fodder) Heat to approximately 50 ºC while shaking on an orbital shaker, then shaking on horizontal shaker (low speed) for ~60 min Vacuum filter Extract 2 more times (only once more for fodder) with 80/20 acetonitrile/water[2] (v/v), shaking 30 minutes without heat Record volume of the extract
	Method of analyses	LSC of triplicate aliquots of the extract
Extracted Tissue Combustion	Solvent	NA
	Procedure	Obtain weight of remaining tissue sample
	Method of analyses	Oxidative combustion of triplicate aliquots

[1] PH-label Forage extractions inadvertently used 90/10 methanol/water for the first pass (2nd and 3rd passes used 80/20 acetonitrile/water)

[2] PH-label Grain replicate A was inadvertently extracted with 80/20 acetone/water for the 2nd pass.

Table 4, Cont. Typical Sample Analysis Procedure for Forage, Grain, and Fodder Samples Except Where Noted (See Also Figure 6)

Bound Residue Analysis

Pectin Extraction	Solvent	ethylenediaminetetraacetic acid (EDTA), 50 mM in 50 mM pH 4.5 buffer
	Procedure	Reference 4
		Add 50-100 mL solvent per g post-extracted tissue Sonicate or Polytron homogenize (10-11 K rpm) 2 minutes Heat (68 to ~80 ºC) & stir approx. 5 hours Cool Vacuum filter, record volume
	Method of analyses	LSC of triplicate aliquots of the filtrate Continue analysis of tissue with Lignin Extraction
Lignin Extraction	Solvent	Sodium chlorite
	Procedure	Reference 5
		Transfer solids from step above to a flask Cover with 40 mL water Add sodium chlorite (1.25 g) and glacial acetic acid (150 µL) to flask and mix well Heat to 70 ºC for 1 hour Add additional sodium chlorite (0.4 g) and glacial acetic acid (150 µL), and mix well Heat to 70 ºC for 1 hour Centrifuge, vacuum filter Wash solids several times with water
	Method of analyses	LSC of triplicate aliquots of the filtrate (some by combustion assay prior to LSC) Continue analysis of tissue with ADF Isolation
ADF Isolation	Solvent	Hexadecyltrimethylammonium bromide (20 g) in 1 L 2.0 N H_2SO_4
	Procedure	Reference 6
		Add 50 mL acid-detergent per g tissue Reflux approx. 1 hour Vacuum filter Rinse solids with water and acetone Record volume of filtrate Dry solids (ADF) in 100 ºC oven overnight
	Method of analyses	LSC of triplicate aliquots of the filtrate Combust ADF

Table 5. HPLC Conditions for Quizalofop AAD-1 Corn Nature of the Residue Study

Program:	ν.ARC System (LC-ARC-3)	
Column	Synergi 4µm Hydro-RP, 150 x 4.6 mm	
Flow Rate	1.0 mL/min	
Radioactivity Detection	Agent (Cocktail)	StopFlow AD
	Ratio	1.0
	Efficiency	approximately 75%
	Stop-Flow Mode	DynamicFlow
	DynamicFlow Start	0.00 min
	DynamicFlow Stop	50.00 min
	Peak Width	25.00
	LC Factor	100.00
	Background Threshold	approx. 14 cpm
UV Detection	254 nm	
Solvent A	0.1% acetic acid in water	
Solvent B	0.1% acetic acid in 80/20 acetonitrile/methanol	
Time (min)	Solvent Elution	
0.0	90/10 A/B initial conditions, begin linear gradient	
30.0	5/95 A/B, linear gradient, begin 5 minute hold	
35.0	5/95 A/B, linear gradient to original conditions	
40.0	90/10 A/B, re-equilibration	
50.0	90/10 A/B, end run	

HPLC Retention Time (min)[a]	Compound
24.125	quizalofop acid
29.164	quizalofop ethyl ester

[a] All reference values are approximate, and may vary slightly due to temperature, column age, matrix, sample size, etc.

Table 6. Test Substance Applied to AAD-1 Corn for ^{14}C-Quizalofop NOR Study

Application	Actual (dpm/0.10 mL)	Volume (mL)[a]	Amount Applied (mg a.i.)[b]	Application Rate (g a.i./ha)[c]	Target Application Rate (g a.i./ha)[d]	% of Target
PH-label	647,905	148.7	12.7	91	92	99
QU-label	953,850	148.7	14.2	102	92	110

[a] Volume actually applied, after aliquots removed for storage stability and LSC, if applicable.

[b] Amount applied = $\frac{\text{amount (dpm/mL)} \times \text{volume (mL)}}{\text{specific activity (dpm/µg)} \times 1000\ \mu g / mg}$, where specific activity is 76,145 dpm/µg for the PH label and 100,384 dpm/µg for the QU label.

[c] Application rate = $\frac{\text{Amount Applied (mg)}}{1000\ \text{mg/g} \times \text{plot size (ha)}}$, where plot size was 1.39 x 10^{-4} ha.

[d] Target does not include any overages.

Table 7. Total Radioactive Residues in Plant Samples Collected for Quizalofop Nature of Residue in AAD-1 Corn Study

Sample	dpm/g	mg ae/kg (ppm)
PH-label		
forage (immature plants)	5,720	0.069
mature grain	702	0.009
mature cobs	502	0.006
mature fodder	31,653	0.384
QU-label		
forage (immature plants)	23,043	0.212
mature grain	1,088	0.010
mature cobs	622	0.006
mature fodder	42,028	0.415

Table 8. Fractionation of the Residues in Quizalofop-Treated AAD-1 Corn (Average of Duplicates)

	Neutral Organic Extraction		Acid Extraction		Post-Extracted Tissue		Recovery
Fraction	%TRR	mg ae/kg	%TRR	mg ae/kg	%TRR	mg ae/kg	%TRR
PH-label							
Forage	65%	0.045	14%	0.010	17%	0.012	96%
Grain	9.0%	<0.001	7.8%	<0.001	87%	0.007	104%
Fodder	60%	0.231	18%	0.071	21%	0.080	99%
QU-label							
Forage	64%	0.136	11%	0.024	22%	0.047	97%
Grain	8.8%	<0.001	6.7%	<0.001	79%	0.008	94%
Fodder	51%	0.211	16%	0.066	25%	0.102	91%

Table 9. SPE Recoveries for Forage and Fodder Extracts, from the Quizalofop NOR in AAD-1 Corn Study, 2008 (Average of Duplicates)

		Amount Extracted[1]		SPE Load/Wash		SPE Eluent		SPE Recovery
Fraction		%TRR	mg ae/kg	%TRR	mg ae/kg	%TRR	mg ae/kg	%
PH-label								
Forage	neutral[2]	65%	0.045	16	0.011	43	0.030	91%
	acid[3]	14%	0.010	5.2	0.004	4.6	0.003	70%
Fodder	neutral	60%	0.231	15	0.058	45	0.171	99%
	acid	18%	0.071	8.8	0.034	8.7	0.034	95%
QU-label								
Forage	neutral[2]	64%	0.136	12	0.026	49	0.104	95%
	acid	11%	0.024	4.5	0.009	4.5	0.010	81%
Fodder	neutral	51%	0.211	7.6	0.031	40	0.166	94%
	acid	16%	0.066	6.4	0.027	8.0	0.033	90%

[1] From Table 8.
[2] C_{18} SPE used for PH-forage, Strata-X SPE used for QU-forage
[3] Strata-X SPE used for the forage acid extracts. Otherwise, a C_{18} SPE was used.

Table 10. Quizalofop and Metabolite Levels In Extracts of AAD-1 Treated Forage and Fodder (Average of Duplicates)

Sample ID	extract	QPEE		Quizalofop acid		polar unknown(s)		unknown(s) 1 (5.7 min RT)		unknown 2 (12 min RT)		unknown 3 (15 min RT)	
		TRR	mg ae/kg	TRR	mg ae/kg	TRR	mg ae/kg	TRR	mg ae/kg	TRR	mg ae/kg	TRR	mg ae/kg
PH-label Forage	neutral	0.5%	<0.001	1.3%	<0.001	16.4%	0.011	ND	ND	3.1%	0.002	4.3%	0.003
	acid	ND	ND	ND	ND	1.7%	0.001	ND	ND	ND	ND	ND	ND
	total	0.5%	<0.001	1.3%	<0.001	18.1%	0.013	ND	ND	3.1%	0.002	4.3%	0.003
QU-label Forage	neutral	0.6%	0.001	0.5%	<0.001	14.3%	0.030	ND	ND	4.9%	0.010	4.5%	0.010
	acid	ND	ND	ND	ND	1.0%	0.002	ND	ND	0.3%	<0.001	ND	ND
	total	0.6%	0.001	0.5%	<0.001	15.3%	0.033	ND	ND	5.1%	0.011	4.5%	0.010
PH-label Fodder	neutral	ND	ND	0.4%	0.001	7.7%	0.030	9.9%	0.038	2.0%	0.008	5.7%	0.022
	acid	ND	ND	ND	ND	8.5%	0.033	0.5%	0.002	ND	ND	0.3%	0.001
	total	ND	ND	0.4%	0.001	16.2%	0.062	10.4%	0.040	2.0%	0.008	6.0%	0.023
QU-label Fodder	neutral	ND	ND	0.6%	0.002	6.4%	0.026	ND	ND	6.6%	0.027	5.4%	0.022
	acid	ND	ND	ND	ND	7.3%	0.030	ND	ND	0.9%	0.004	0.4%	0.002
	total	ND	ND	0.6%	0.002	13.6%	0.057	ND	ND	7.5%	0.031	5.8%	0.024

Table 11. Fractionation of the Bound Residues in Quizalofop Treated AAD-1 Corn, 2008

Sample ID	Bound Residue[a]		Pectin (EDTA Soluble)		Lignin ($NaClO_2$ Soluble)		Acid-Detergent Soluble		ADF (Solids)		Recovery
	%TRR	mg ae/kg	%TRR	mg ae/kg	%TRR	mg ae/kg	%TRR	mg ae/kg	%TRR	mg ae/kg	%
PH-label											
Forage	17%	0.012	2.4%	0.002	1.5%	0.001	4.8%	0.003	2.6%	0.002	67%
Grain	87%	0.007	30%	0.003	12%	0.001	58%	0.005	3.7%	<0.001	120%
Fodder	21%	0.080	2.8%	0.011	6.4%	0.025	4.0%	0.015	2.5%	0.009	75%
		normalized	3.7%	0.014	8.6%	0.033	5.3%	0.020	3.3%	0.013	100%
QU-label											
Forage	22%	0.047	1.3%	0.003	5.7%	0.012	6.7%	0.014	3.7%	0.008	79%
Grain	79%	0.008	27%	0.003	7.7%	<0.001	62%	0.006	2.4%	<0.001	125%
Fodder	25%	0.102	2.9%	0.012	6.0%	0.025	5.7%	0.024	4.0%	0.017	76%
		normalized	3.9%	0.016	7.9%	0.033	7.5%	0.031	5.3%	0.022	100%

[a] Values from Table 8.

Table 12. Determination of the Radioactive Residue in Grain Associated with Starch (Average of Duplicates)

Sample ID	Non-Extractable		Extractable (non-starch)		Starch		
	% TRR	mg ae/kg	% TRR	mg ae/kg	% TRR	mg ae/kg	Recovery
PH-label Grain	19.4	0.002	54.5	0.005	54.9	0.005	128.7
Post-Extracted[a] PH-label Grain	12.2	0.001	56.1	0.005	51.4	0.004	137.8
QU-label Grain	29.3	0.003	39.5	0.004	44.4	0.004	113.2
Post-Extracted[a] QU-label Grain	13.6	0.001	33.7	0.003	44.1	0.004	116.4

[a] Non-extractable residue remaining after neutral organic and acid extractions, see Table 8.

Figure 1. Chemical Nomenclature and Structures of ^{14}C-Quizalofop

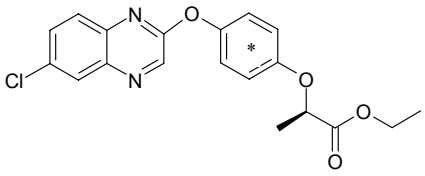
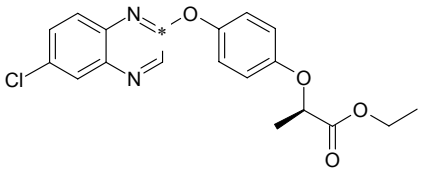
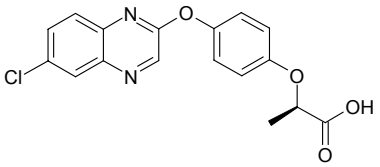
	Test Substance	Structure
Common Name	**14C-PH-quizalfop**	 * denotes ^{14}C
Synonyms	Quizalofop-ethyl PH-UL-14C	
Chemical Name	(R)-2-[4-(6-chloro-quinoxalin-2-yloxy)-phenoxy]-propanoic acid ethyl ester-Ph-UL-14C	
Inventory Number	INV2075	
FA & PC Reference	074-012	
SPS Reference	36891-19	
Specific Activity	25.5 mCi/mmol (68.4 µCi/mg)	
Radiochemical purity	98.2%	
GLP analysis	Yes, 8/2/2007	
Common Name	**14C-QU-quizalofop**	 * denotes ^{14}C
Synonyms	Quizalofop-ethyl quinoxaline label-14C	
Chemical Name	(R)-2-[4-(6-chloro-quinoxalin-2-yloxy-2-14C)-phenoxy]-propanoic acid ethyl ester	
Inventory Number	INV2081	
FA & PC Reference	074-017	
SPS Reference	36891-60	
Specific Activity	32.8 mCi/mmol (88.0 µCi/mg)	
Radiochemical purity	97.2%	
GLP analysis	Yes, 8/3/2007	

Figure 2. Chemical Nomenclature and Structures of Quizalofop Reference Standards and Formulation Blank

	Reference Substances	Structure
Common Name	quizalofop	
Synonyms	quizalofop acid, Quizalofop-P acid, Quizalofop-P	
CAS Nomenclature	(+/-)-2-[4-[(6-chloro-2-quinoxalinyl)oxy]phenoxy]propanoic acid	
IUPAC Nomenclature	(RS)-2-[4-[(6-chloroquinoxalin-2-yloxy)phenoxy]propanoic acid	
CAS Number	76578-12-6, 94051-08-8	
Molecular Formula	$C_{17}H_{13}ClN_2O_4$	
SMILES Code	Clc1ccc2c(c1)ncc(n2)Oc1ccc(cc1)OC(C(=O)O)C	
Molecular Weight	344.8 g/mole	
Inventory Number	TSN106172	
Description	solid	
Purity	96%	

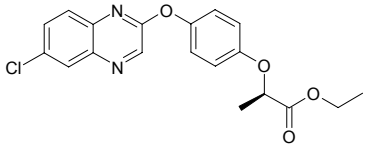
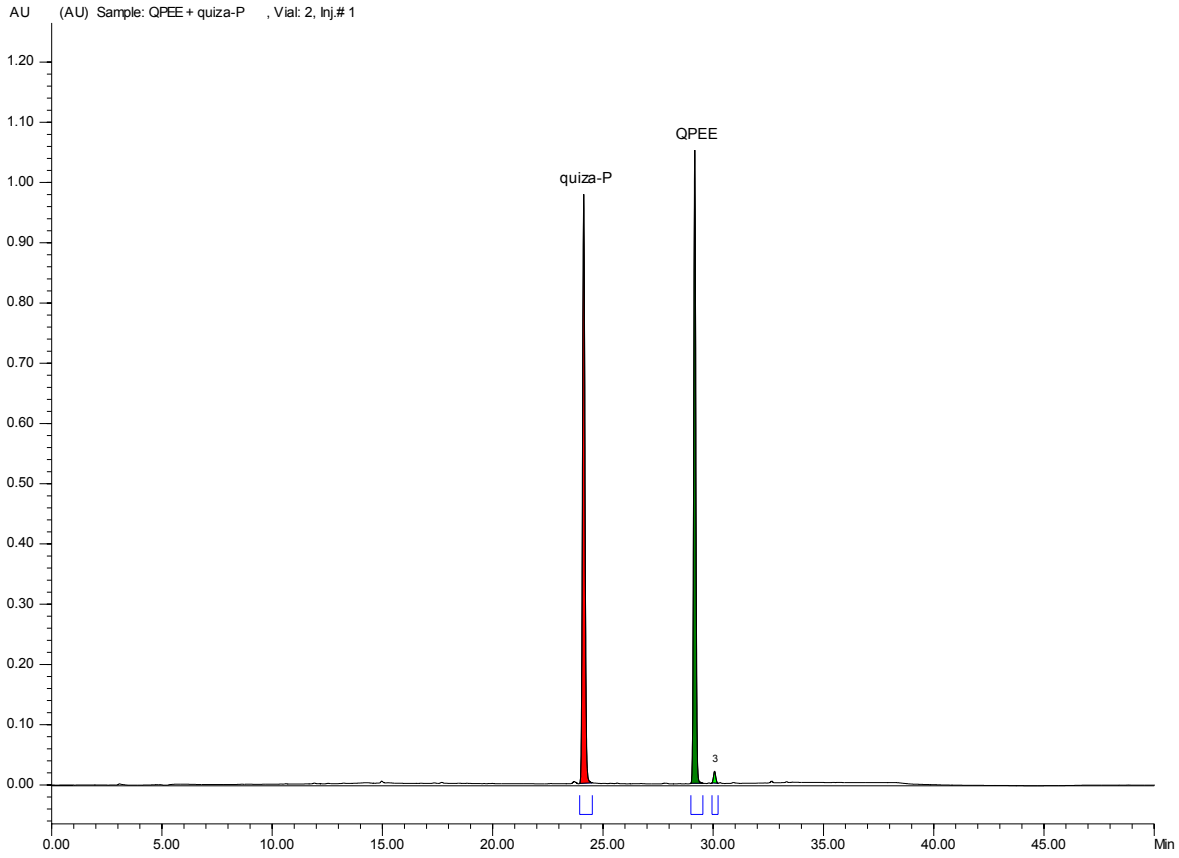
Common Name	quizalofop ethyl ester	
Synonyms	Quizalofop-P ethyl, QPEE	
IUPAC Nomenclature	Ethyl-(R)- 2-[4-[(6-chloroquinoxalin-2-yloxy)phenoxy]propanoic acid	
CAS Number	100646-51-3	
Molecular Formula	$C_{19}H_{17}ClN_2O_4$	
Molecular Weight	372.8 g/mole	
Inventory Number	TSN106317	
Description	solid	
Purity	99%	

Figure 2, Cont. Chemical Nomenclature and Structures of Quizalofop Reference Standards and Formulation Blank

	Formulation Blank		
Common Name	E2469-23 Formulation Blank		
Synonyms	quizalofop formulation blank		
Description	Component	Role	% W/W
	Agrimol Lipo-D	emulsifier	6
	Aromatic 200	solvent	47
	NMP (N-methyl pyrrolidinone)	solvent	47
			100
	E2469-23	formulation blank	89.7
	quizalofop ethyl ester	Active ingredient	10.3
			100.0

Figure 3. Chromatogram of Quizalofop Acid and Quizalofop Ethyl Ester Reference Standards



Retention Time (min)	Reference
24.125	quizalofop acid
29.164	quizalofop-P-ethyl ester

Figure 4. Chromatogram of ^{14}C-quizalofop ethyl ester, top: PH-label (dilution A), bottom: QU-label (dilution A)

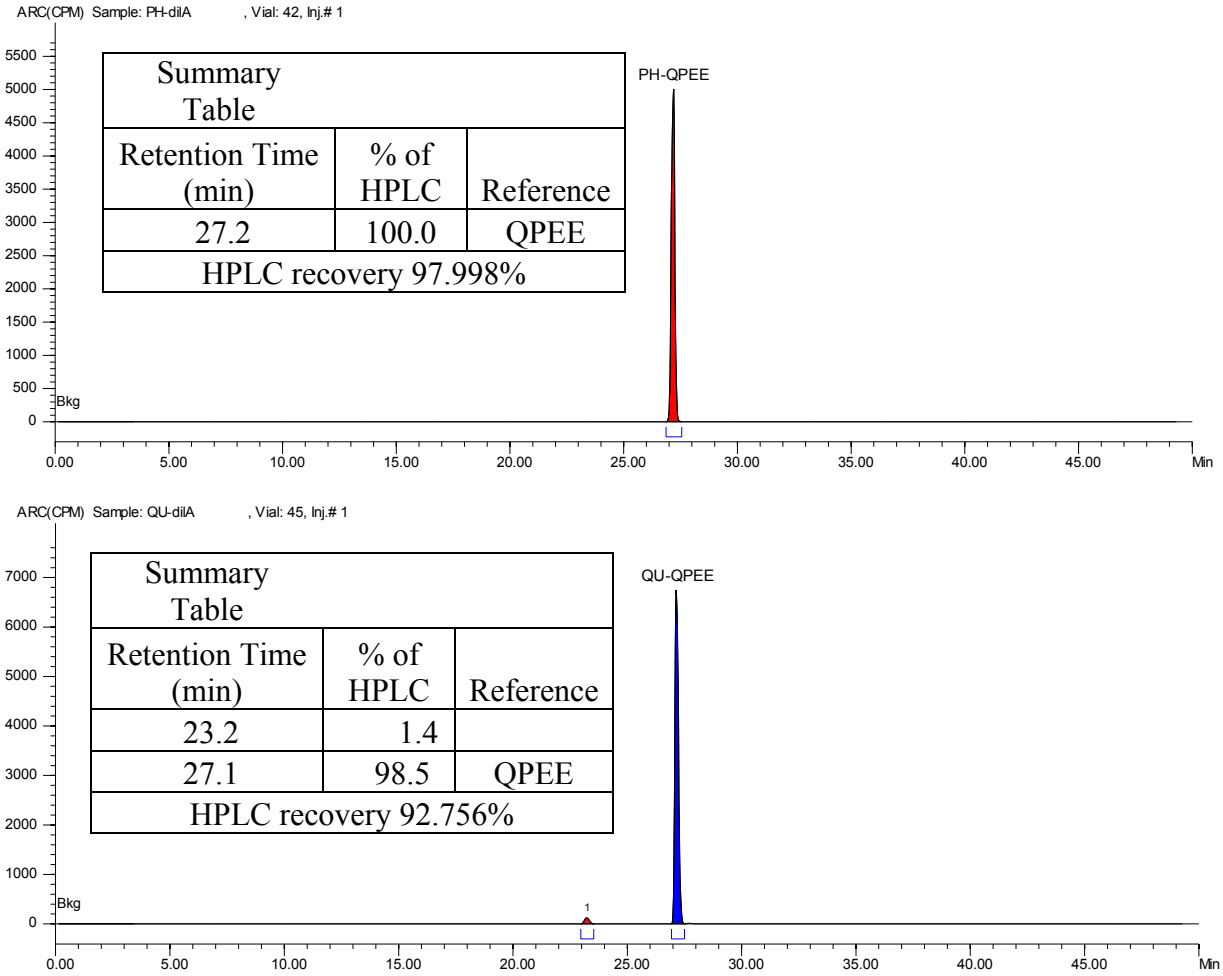


Figure 5. Schematic Flowchart for the Analysis of Forage, Grain, and Fodder Fractions (See Also Table 4)

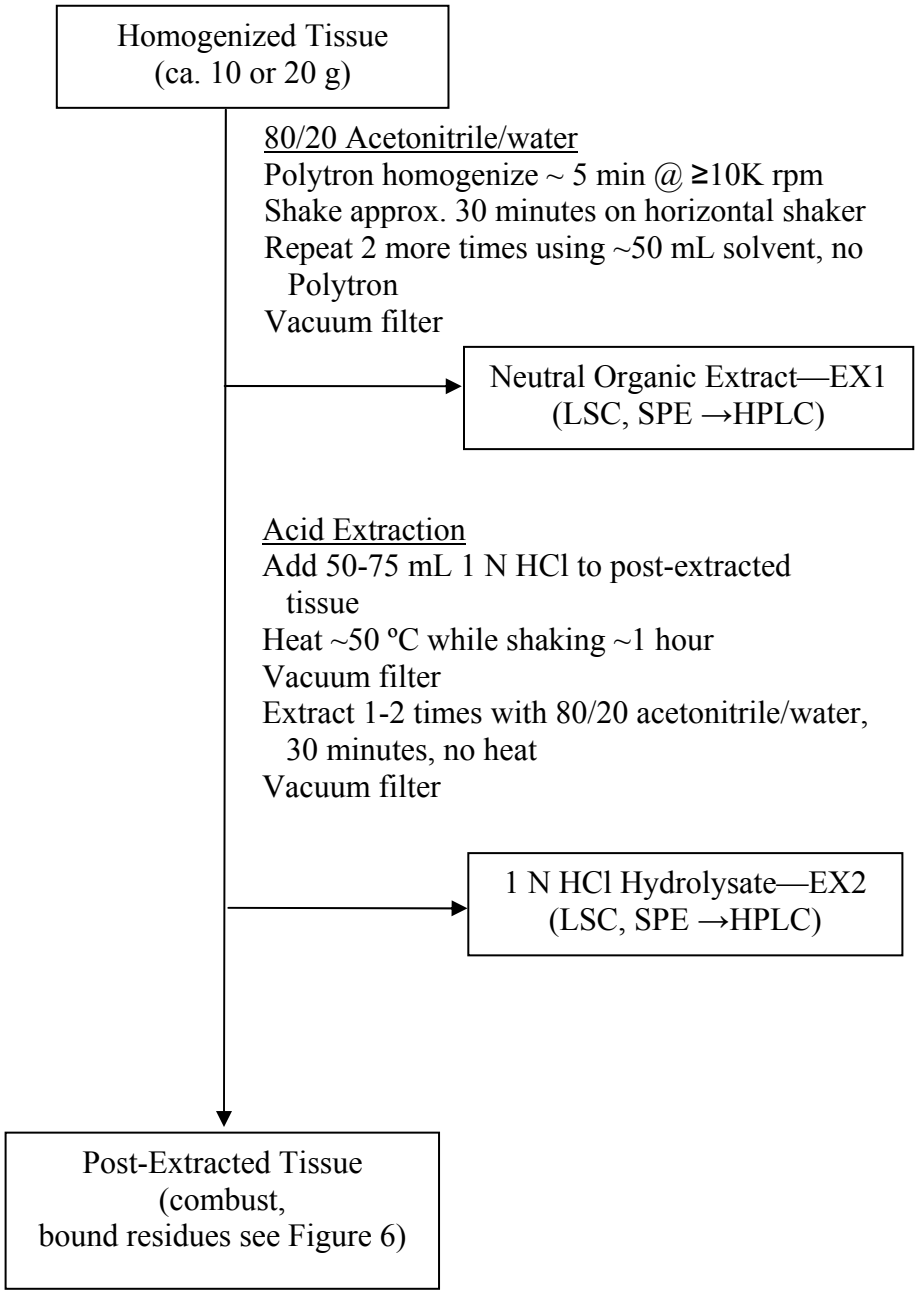


Figure 6. Schematic Flowchart for the Analysis of Bound Residues in Forage, Grain, and Fodder Samples

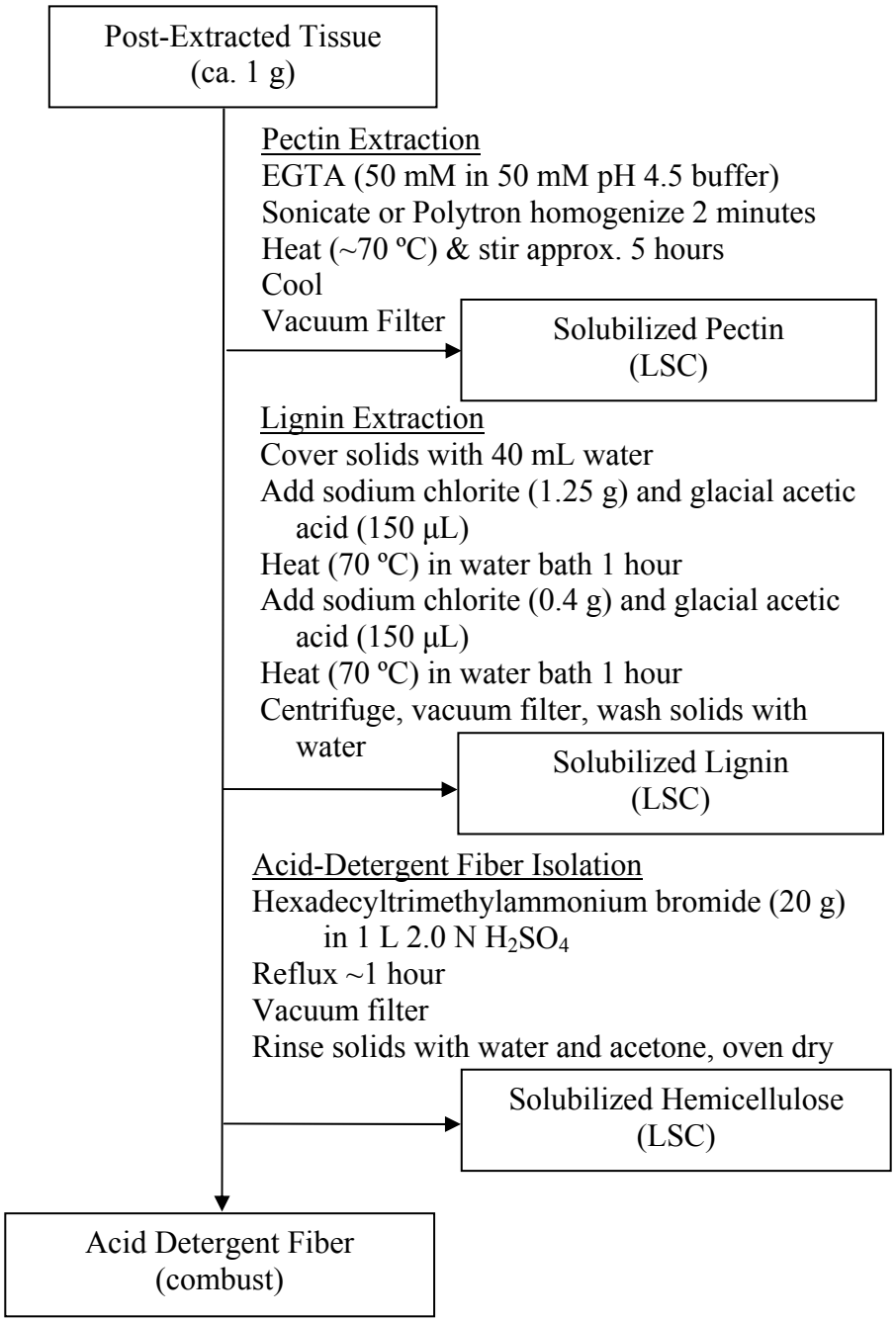
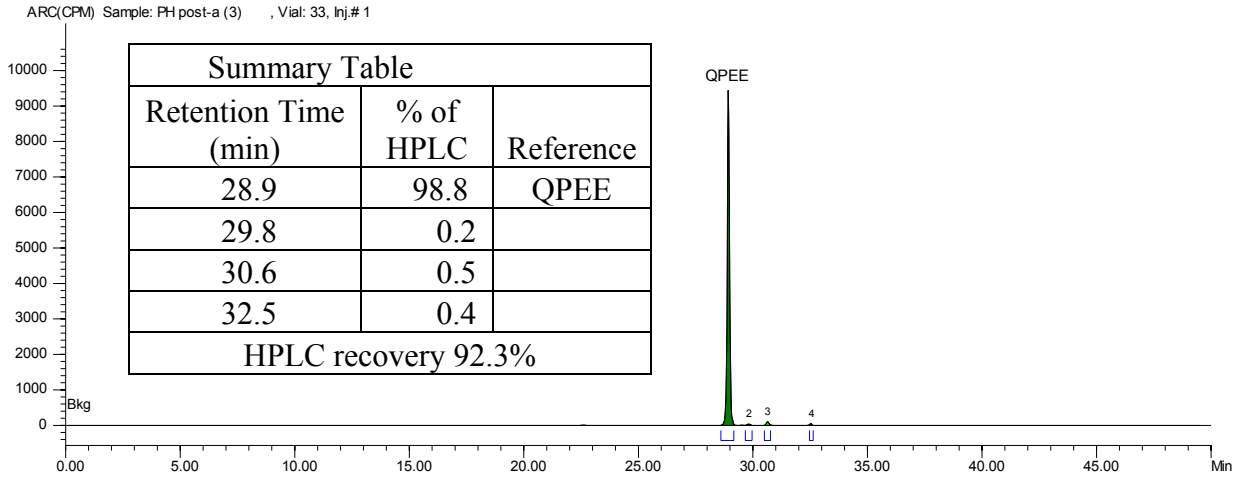


Figure 7. HPLC Chromatograms Indicating Purity of the ^{14}C-Quizalofop After Application, Top – PH-Label, Bottom – QU-label (both post-application replicate A)



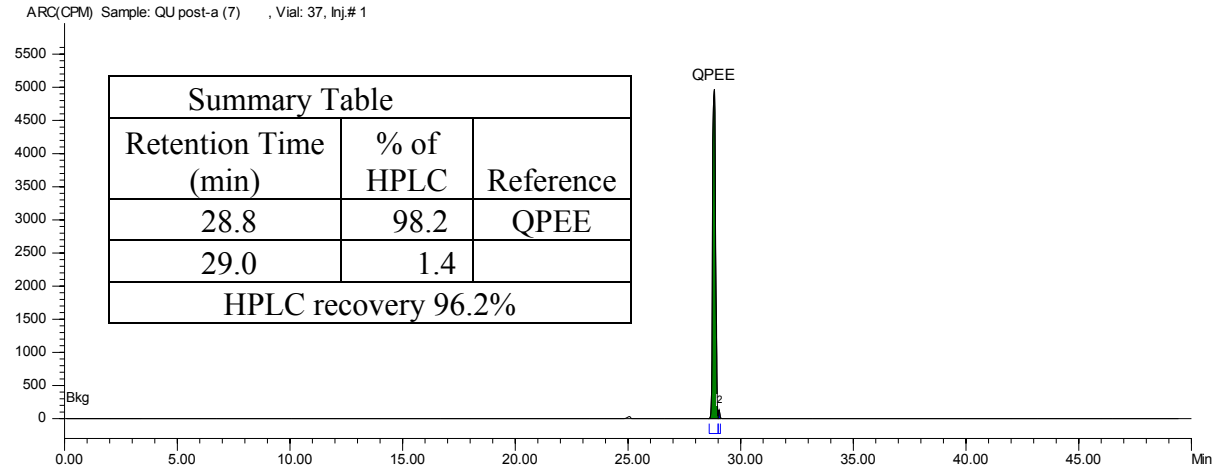
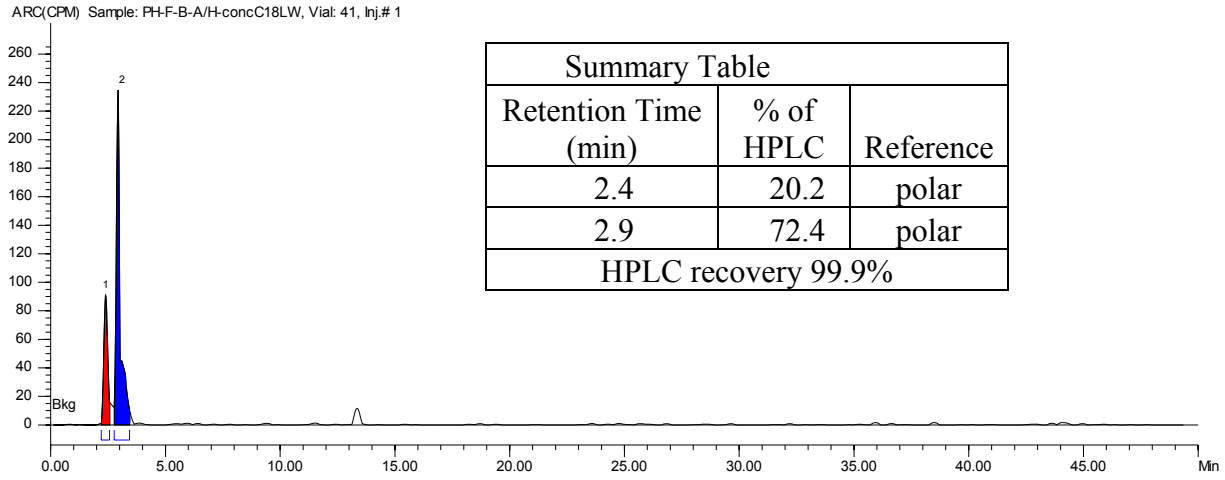
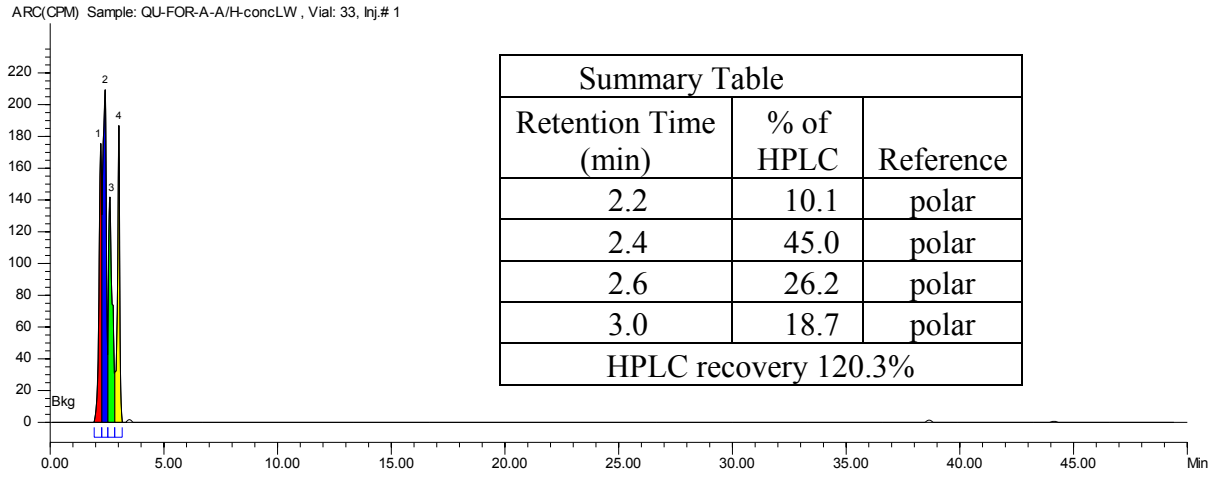


Figure 8. HPLC Chromatograms of the Forage Neutral Organic Extracts, Concentrated SPE Load/Wash, Top – PH-label; Bottom – QU-label (both Replicate A)

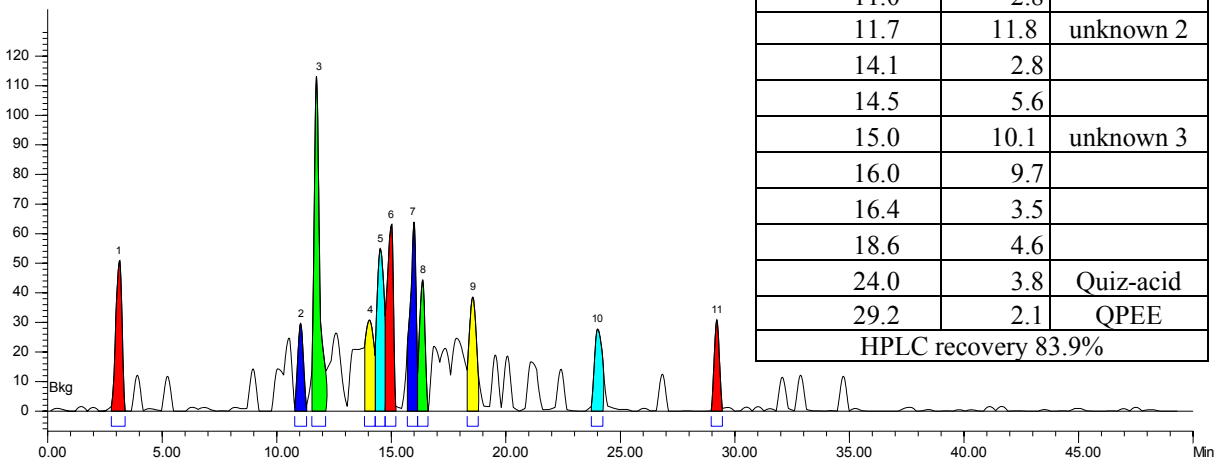


Summary Table		
Retention Time (min)	% of HPLC	Reference
2.4	20.2	polar
2.9	72.4	polar
HPLC recovery 99.9%		

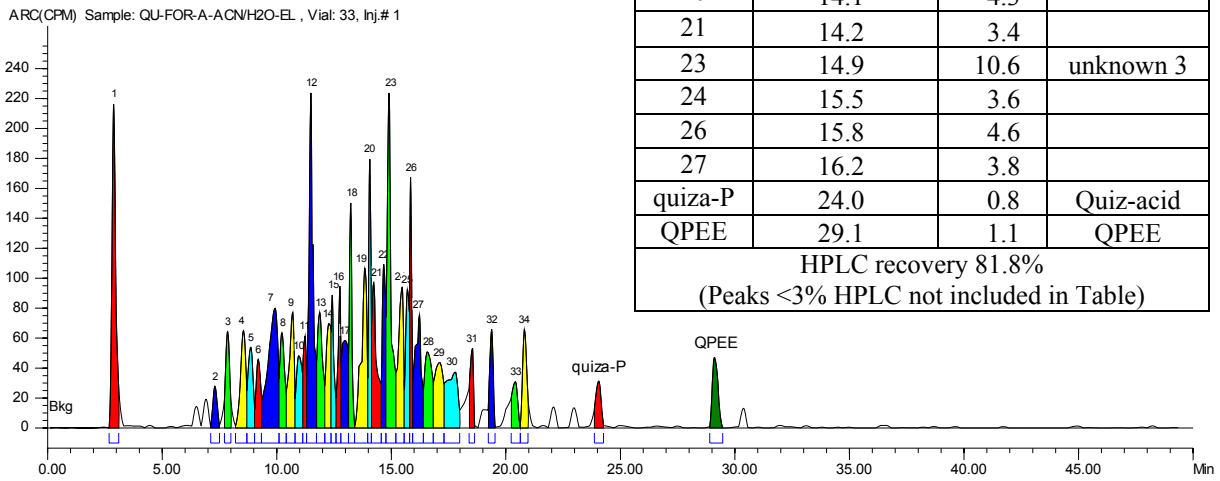


Summary Table		
Retention Time (min)	% of HPLC	Reference
2.2	10.1	polar
2.4	45.0	polar
2.6	26.2	polar
3.0	18.7	polar
HPLC recovery 120.3%		

Figure 9. HPLC Chromatograms of the Forage Neutral Organic Extracts, Concentrated SPE Eluents, Top – PH-label; Bottom – QU-label (both Replicate A)

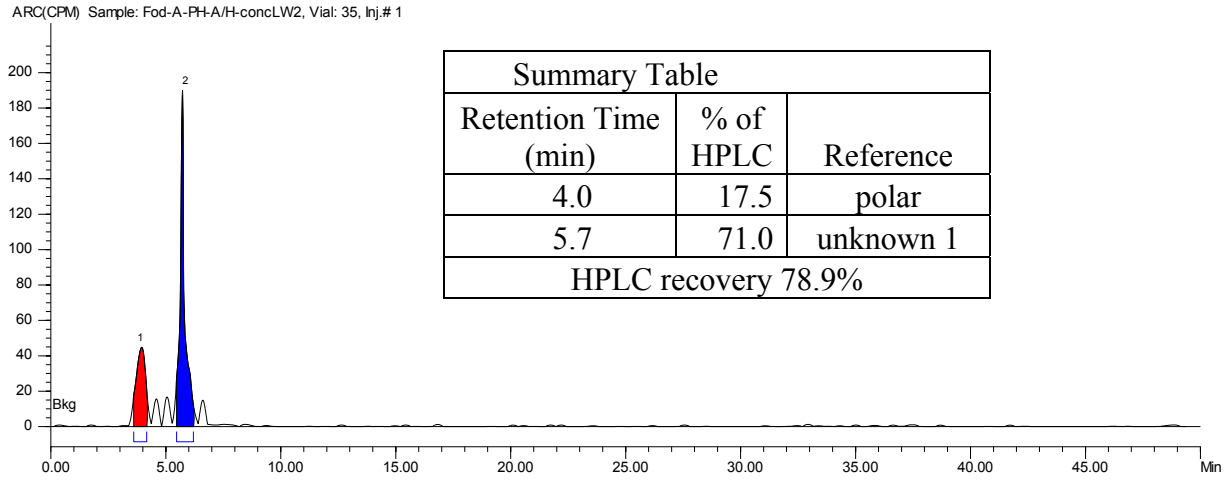


Summary Table		
Retention Time (min)	% of HPLC	Reference
3.2	6.5	polar
11.0	2.8	
11.7	11.8	unknown 2
14.1	2.8	
14.5	5.6	
15.0	10.1	unknown 3
16.0	9.7	
16.4	3.5	
18.6	4.6	
24.0	3.8	Quiz-acid
29.2	2.1	QPEE
HPLC recovery 83.9%		

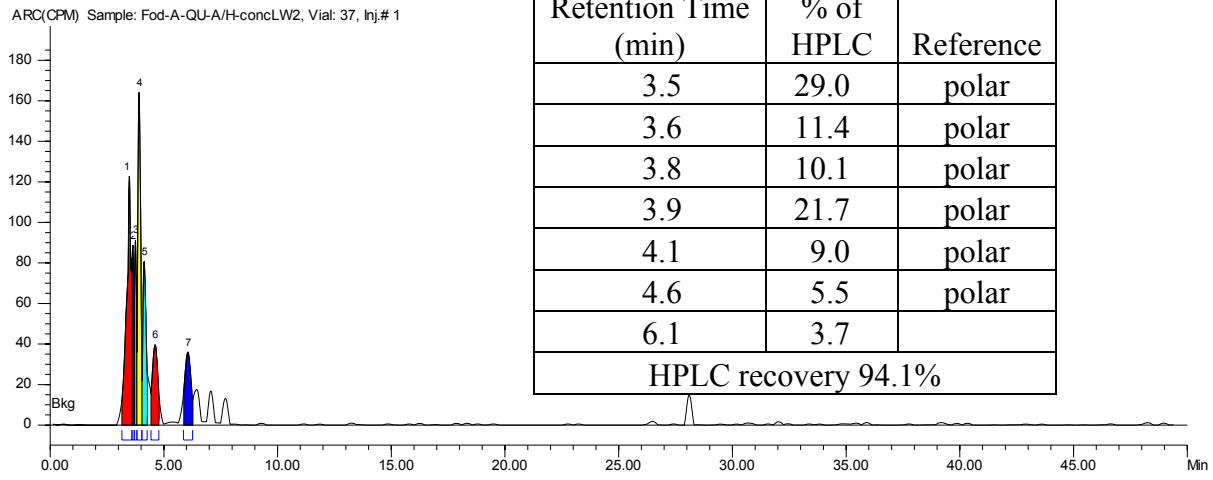


Summary Table			
Peak #	Retention Time (min)	% of HPLC	Reference
1	2.9	4.4	polar
7	9.9	3.6	
8	10.2	3.0	
12	11.5	9.5	unknown 2
13	11.9	3.2	
17	13.0	3.4	
18	13.2	3.4	
19	13.8	4.6	
20	14.1	4.3	
21	14.2	3.4	
23	14.9	10.6	unknown 3
24	15.5	3.6	
26	15.8	4.6	
27	16.2	3.8	
quiza-P	24.0	0.8	Quiz-acid
QPEE	29.1	1.1	QPEE
HPLC recovery 81.8% (Peaks <3% HPLC not included in Table)			

Figure 10. HPLC Chromatograms of the Fodder Neutral Organic Extracts, Concentrated SPE Load/Wash, Top – PH-label; Bottom – QU-label (both Replicate A)

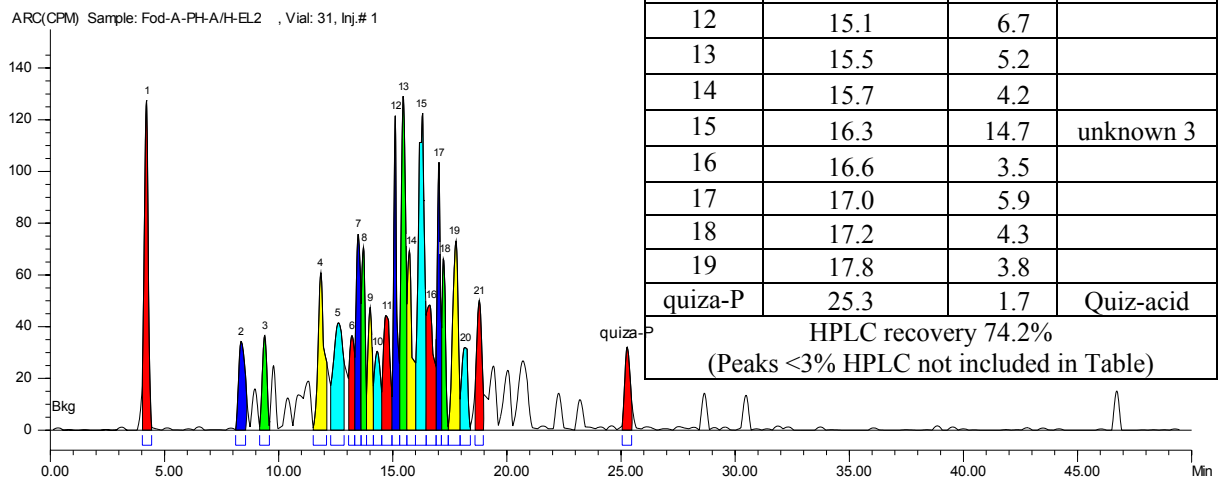


Summary Table		
Retention Time (min)	% of HPLC	Reference
4.0	17.5	polar
5.7	71.0	unknown 1
HPLC recovery 78.9%		

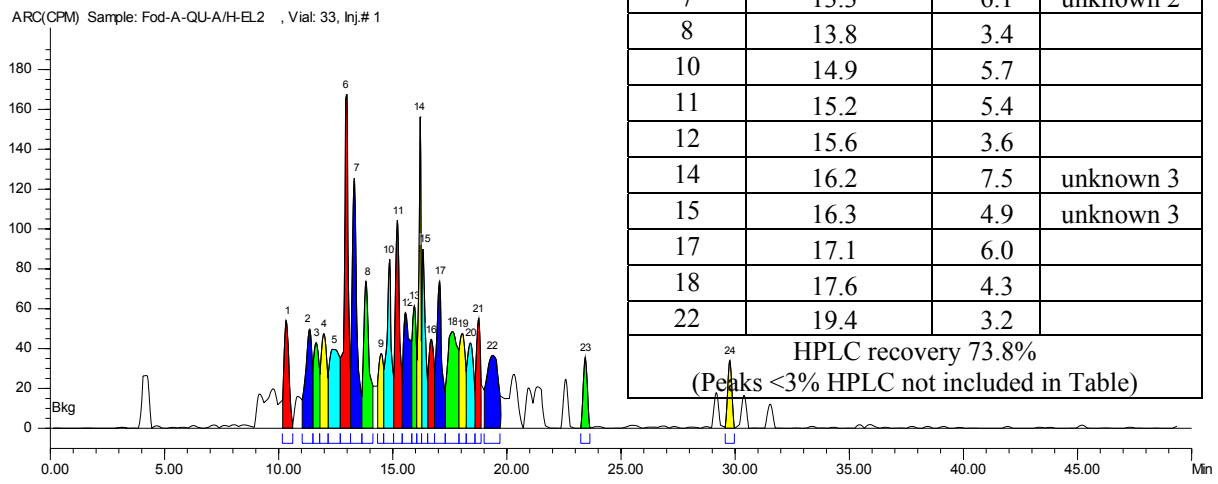


Summary Table		
Retention Time (min)	% of HPLC	Reference
3.5	29.0	polar
3.6	11.4	polar
3.8	10.1	polar
3.9	21.7	polar
4.1	9.0	polar
4.6	5.5	polar
6.1	3.7	
HPLC recovery 94.1%		

Figure 11. HPLC Chromatograms of the Fodder Neutral Organic Extracts, Concentrated SPE Eluents, Top – PH-label; Bottom – QU-label (both Replicate A)

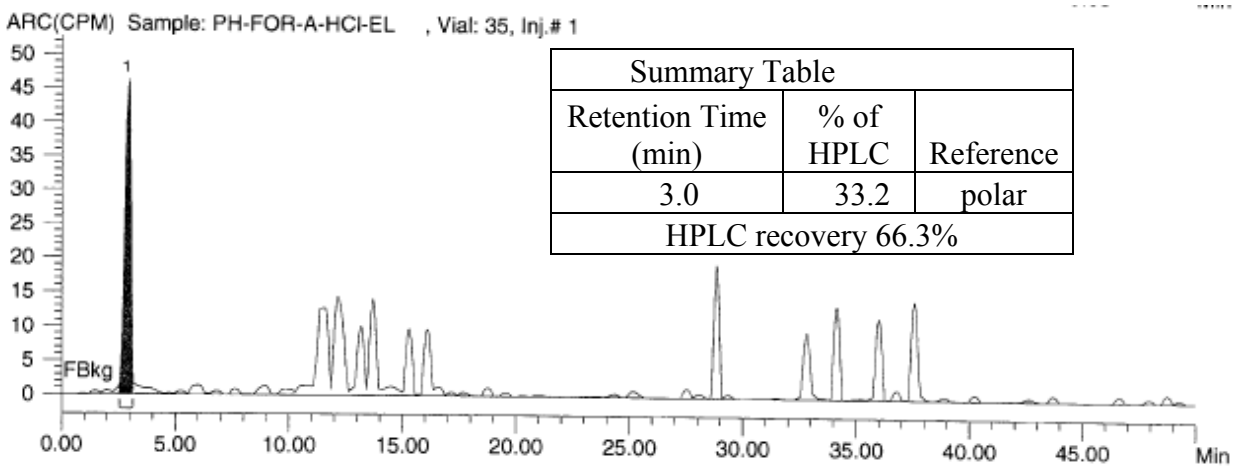


Summary Table			
Peak #	Retention Time (min)	% of HPLC	Reference
1	4.2	6.0	polar
4	11.9	3.8	
5	12.6	4.8	unknown 2
7	13.5	3.6	
8	13.7	3.2	
12	15.1	6.7	
13	15.5	5.2	
14	15.7	4.2	
15	16.3	14.7	unknown 3
16	16.6	3.5	
17	17.0	5.9	
18	17.2	4.3	
19	17.8	3.8	
quiza-P	25.3	1.7	Quiz-acid
HPLC recovery 74.2% (Peaks <3% HPLC not included in Table)			

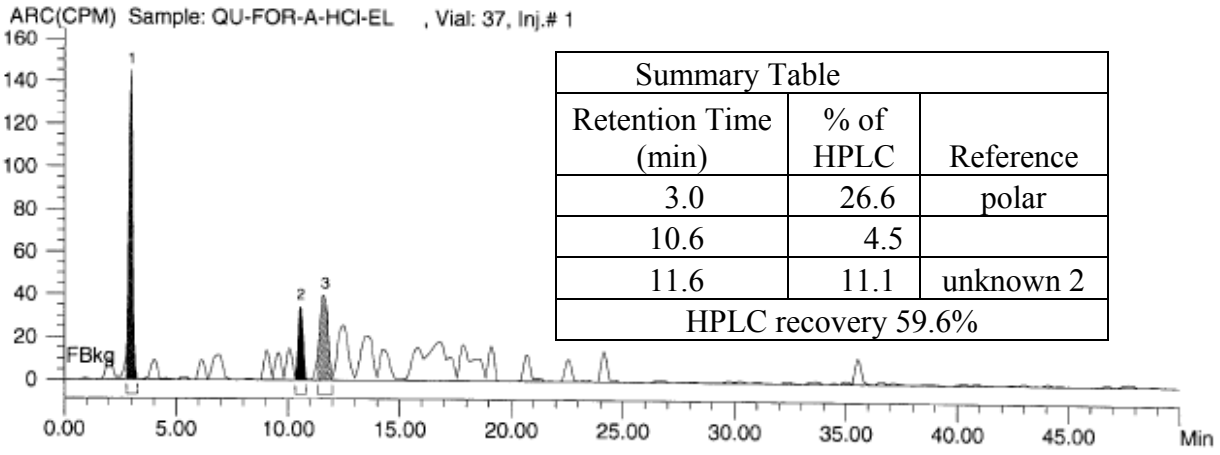


Summary Table			
Peak #	Retention Time (min)	% of HPLC	Reference
1	10.3	3.0	
6	13.0	12.9	unknown 2
7	13.3	6.1	unknown 2
8	13.8	3.4	
10	14.9	5.7	
11	15.2	5.4	
12	15.6	3.6	
14	16.2	7.5	unknown 3
15	16.3	4.9	unknown 3
17	17.1	6.0	
18	17.6	4.3	
22	19.4	3.2	
HPLC recovery 73.8% (Peaks <3% HPLC not included in Table)			

Figure 12. HPLC Chromatograms of the Forage Acid Extracts, Concentrated SPE Eluents, Top – PH-label; Bottom – QU-label (both Replicate A)

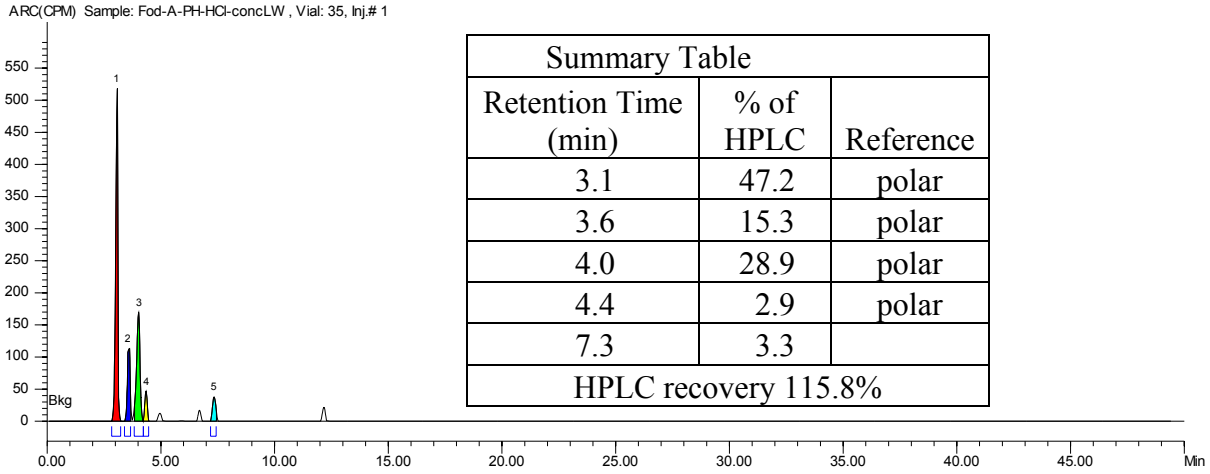


Summary Table		
Retention Time (min)	% of HPLC	Reference
3.0	33.2	polar
HPLC recovery 66.3%		

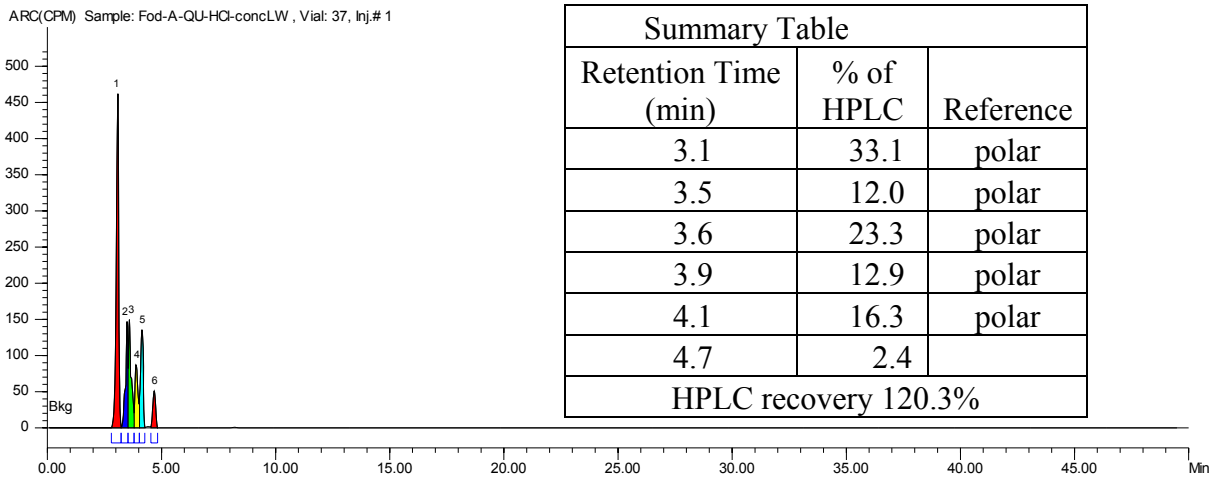


Summary Table		
Retention Time (min)	% of HPLC	Reference
3.0	26.6	polar
10.6	4.5	
11.6	11.1	unknown 2
HPLC recovery 59.6%		

Figure 13. HPLC Chromatograms of the Fodder Acid Extracts, Concentrated SPE Load/Wash, Top – PH-label; Bottom – QU-label (both Replicate A)

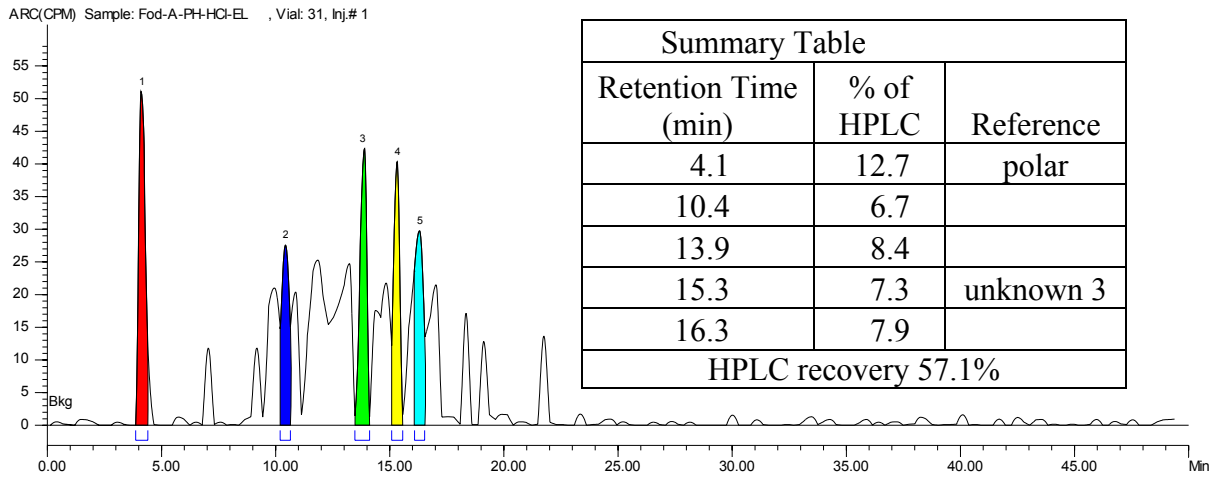


Summary Table		
Retention Time (min)	% of HPLC	Reference
3.1	47.2	polar
3.6	15.3	polar
4.0	28.9	polar
4.4	2.9	polar
7.3	3.3	
HPLC recovery 115.8%		

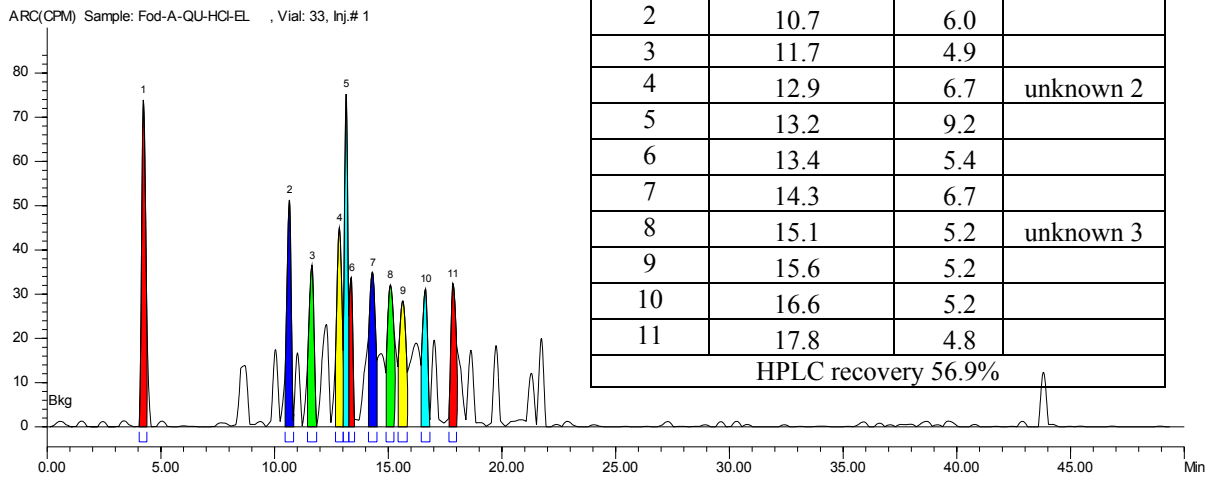


Summary Table		
Retention Time (min)	% of HPLC	Reference
3.1	33.1	polar
3.5	12.0	polar
3.6	23.3	polar
3.9	12.9	polar
4.1	16.3	polar
4.7	2.4	
HPLC recovery 120.3%		

Figure 14. HPLC Chromatograms of the Fodder Acid Extracts, Concentrated SPE Eluents, Top – PH-label; Bottom – QU-label (both Replicate A)

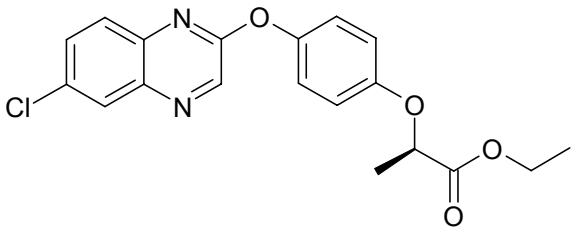


Summary Table		
Retention Time (min)	% of HPLC	Reference
4.1	12.7	polar
10.4	6.7	
13.9	8.4	
15.3	7.3	unknown 3
16.3	7.9	
HPLC recovery 57.1%		

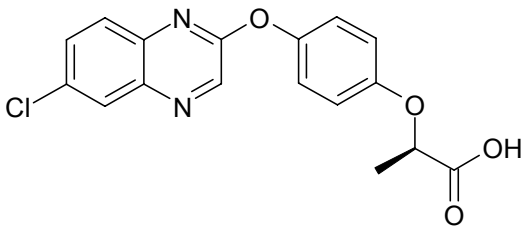


Summary Table			
Peak #	Retention Time (min)	% of HPLC	Reference
1	4.2	9.5	polar
2	10.7	6.0	
3	11.7	4.9	
4	12.9	6.7	unknown 2
5	13.2	9.2	
6	13.4	5.4	
7	14.3	6.7	
8	15.1	5.2	unknown 3
9	15.6	5.2	
10	16.6	5.2	
11	17.8	4.8	
HPLC recovery 56.9%			

Figure 15. Proposed Metabolic Pathway for Quizalofop-P-Ethyl Ester in AAD-1 Corn



quizalofop-ethyl



quizalofop acid

multiple low-level components
and natural incorporation

Appendix A—In-Life Report

(Research For Hire)

IN-LIFE PHASE FINAL REPORT

STUDY TITLE

A Nature of the Residue Study with ^{14}C-Quizalofop-P ethyl ester applied to AAD-1Maize 2008

SPONSOR

Dow AgroSciences, LLC
9330 Zionsville Road
Indianapolis, IN 46268

DATA REQUIREMENT

EPA (OPPTS 860.1300), OECD 501 Metabolism in Crops (8 January 2007) and European Annex II and III 96/68/EEC Lundehn (7028/VI/95 EN rev 3 (7/22/97)).

IN-LIFE PHASE TEST SITE

Research For Hire
1696 South Leggett Street
Porterville, CA 93257

STUDY DIRECTOR AND TESTING FACILITY

Sandra L. Rotondaro
Dow AgroSciences, LLC
9330 Zionsville Road
Indianapolis, IN 46268

IN-LIFE PHASE COMPLETION DATE

September 3, 2008

STUDY IDENTIFICATION

Dow AgroSciences: Protocol No. 080057 Project No.10001126-5051-3
Research For Hire: Study Number R050802

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

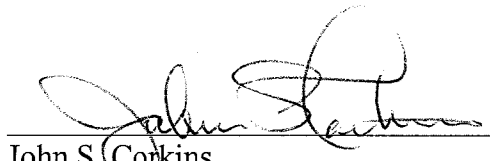
No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d) (1) (A), (B), or (C).

These data are the property of Dow AgroSciences, LLC and as such are considered to be confidential for all purposes other than compliance with FIFRA Section 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality, which may exist under any other statute or in any other country.

Company: Research For Hire

Company Agent: John S. Corkins

Title: General Manager



John S. Corkins
General Manager

1-7-09
Date

REGULATORY COMPLIANCE STATEMENT

A Nature of the Residue Study with ^{14}C-Quizalofop-P ethyl ester applied to AAD-1Maize 2008

I was directly involved with the conduct and supervision of the above-captioned study and do herby certify that, with the following exceptions, this study was conducted in accordance with the Environmental Protection Agency's Good Laboratory Practice Regulations (40 CFR 110) with the following exceptions:

1. Weather Data

2. Equipment used for plot maintenance is calibrated prior to use but not GLP verified.

Blaine Turner
Principal Field Investigator

1/8/09
Date

QUALITY ASSURANCE STATEMENT

The in-life phase of this study was monitored by the Quality Assurance Unit in accordance with the GLP Standards set forth in 40 CFR 110. The following list describes the inspections made and the dates that the findings were reported.

Summary of Inspections

Date of Inspection or Audit	Phase Inspected	Date Findings Reported to Study Director and Test Facility Management
7/02/08	First Application	7/11/08
8/07/08	Forage Sampling	8/12/08
1/01/09	Raw Data and In-Life Phase Report	1/08/09

Mary Jones
Quality Assurance

1/8/09
Date

CERTIFICATION OF AVAILABILITY OF RAW DATA

Original study specific raw data will be sent to Dow AgroSciences, LLC. Research For Hire will maintain certified copies.

Research For Hire
1696 South Leggett Street
Porterville, CA 93257

Mary Jones
Quality Assurance

11/8/09
Date

PROJECT PERSONNEL

RESEARCH FOR HIRE

Personnel	Position
John Corkins	General Manager, Contract Research
Blaine Turner	Principal Field Investigator
Emily Dement	Research Assistant
Griselda Mena	Research Assistant
Thomas Sukut	Technician I
Joshua Tilton	Technician I
Stephanie Phipps	Office Coordinator

DOW AGROSCIENCES

Personnel	Position
Sandra L. Rotondaro	Study Director

REPORT APPROVAL

Sandra L. Rotondaro
Study Director
Dow AgroSciences, LLC

01 March 2010
Date

John S. Corkins
General Manager
Research For Hire

1/7/09
Date

Blaine Turner
Principal Field Investigator
Research For Hire

1/8/09
Date

STUDY IDENTIFICATION PAGE

In-Life Phase Study Site:	Research For Hire 1696 South Leggett Street Porterville, CA 93257
Sponsor:	Dow AgroSciences, LLC 9330 Zionsville Road Indianapolis, IN 46268
Sponsor Representative:	D. Fonseca Dow AgroSciences, LLC 9330 Zionsville Road Indianapolis, IN 46268
Testing Facility:	Dow AgroSciences, LLC 9330 Zionsville Road Indianapolis, IN 46268
Study Director:	Sandra L. Rotondaro Dow AgroSciences, LLC 9330 Zionsville Road Indianapolis, IN 46268
Principal Field Investigator:	Blaine Turner Research For Hire 1696 S. Leggett Street Porterville, CA. 93257
Study Initiation Date:	March 25, 2008
RFH Experimental Start Date:	May 28, 2008
RFH Experimental End Date:	September 3, 2008

Research for Hire shall send all original study specific raw data to Dow AgroSciences LLC at the Sponsor's request.

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INTRODUCTION

In this study, the radiolabeled test substance, ^{14}C-quizalofop-P was applied to maize plants, which were grown in a sandy clay loam soil.

Dow AgroSciences, LLC sponsored the study. The testing facility was Dow AgroSciences, LLC (DAS), and the DAS protocol number was 080057. The in-life phase was contracted to Research For Hire (RFH), and the RFH study number was R050802.

OBJECTIVE

To determine the nature, amount and distribution of residues in the forage, fodder, cobs, and grain of maize plants following a single application of [^{14}C]-labeled quizalofop-P ethyl ester to the plants. All phases of this study were conducted to meet the standards of Good Laboratory Practices (GLP).

CONDUCT OF THE STUDY

The in-life phase of this study was conducted at Research For Hire according to Dow AgroSciences, LLC protocol, "A Nature of the Residue Study with [^{14}C]-Quizalofop-P ethyl ester applied to AAD-1 Maize 2008" (Protocol no. 080057) and amendments. The study was also conducted in accordance with U.S. EPA, OECD 501 guidelines of the testing of chemicals for Metabolism in Crops (Issued 8 January 2007), OPPTS 860.1300 Nature of the Residue – Plants, livestock and European Annex II and III 96/68/EEC Lundehn (7028/VI/95 EN rev 3 (7/22/97)). The study also adhered to the Good Laboratory Practices Standards (GLP) (40 CFR Part 160) with exceptions noted on the compliance statement.

MATERIALS AND METHODS - IN-LIFE STUDY

EXPERIMENTAL FACILITIES

Research For Hire, 1696 S. Leggett Street, Porterville, California 93257 conducted the in-life phase of the study from March 25, 2008 to September 3, 2008. Two [^{14}C]-quizalofop-P treated boxes, one per radiolabel, were secured in an outdoor area that was enclosed with a wire mesh security fence with a locked gate and was marked with a weatherproof radioactive materials placard. The area was accessible to authorized personnel only.

APPARATUS

Appendix A lists the analytical and field instruments used in the study.

TEST MATERIAL

PH-Labeled Test Material

- ^{14}C-PH-Quizalofop-P ethyl ester

Chemical name:	(R)-2-[4-(6-Chloro-quinoxalin-2-yloxy)-phenoxy] -propionic acid ethyl ester-Ph-UL-^{14}C
Common name:	Quizalofop-Ethyl PH-UL -^{14}C
Lot or Identification Nos.:	^{14}C-PH-Quizalofop-P ethyl ester
Stated specific activity:	34.3 µCi/mg
Radiopurity:	98.2%
Expiration date:	April, 2009

QU-Labeled Test Material

- ^{14}C-QU-Quizalofop-P ethyl ester

Chemical name:	(R)-2-[4-(6-Chloro-quinoxalin-2-yloxy-2-^{14}C) -phenoxy]-propionic acid ethyl ester
Common name:	Quizalofop-Ethyl Quinoxaline label -^{14}C
Lot or Identification Nos.:	^{14}C-QU-Quizalofop-P ethyl ester
Stated specific activity:	45.2 µCi/mg
Radiopurity:	97.2%
Expiration date:	April, 2009

TEST MATERIAL RECEIPT AND DISTRIBUTION

All radioactive materials were handled in accordance with Nuclear Regulatory Commission regulations and with RFH Standard Operating Procedures (SOP's). For research involving the use of radioactive materials, RFH operates under NRC License No. 1433-54.

All test materials received at Research For Hire were logged in per RFH SOP's. The test materials for the applications were received in good condition. The packing material was monitored with a survey meter, and radiation levels were at background levels and documented into the raw data. One vial each of ^{14}C-PH-quizalofop and ^{14}C-QU-quizalofop, containing a total of 1.11 mCi, were received.

Upon receipt, 0.120 g of blank formulation (E2469-23) was added to each vial. The test substances were stored in the RFH Lab Refrigerator (EQP 28-4) with a set temperature between 1.67 °C to 8.89 °C (35 to 48 °F). Refer to Table 1 - Test Material Receipt and Distribution.

TEST SITE

The test site was located at Research For Hire, 1696 S. Leggett Street, Porterville, Tulare County, California.

A total of three boxes were used to conduct this study, each with inside dimensions of 1.5 m long x 0.91 m wide x 0.46 m deep (5 feet x 3 feet x 1.5 feet), were double-lined with 6-mil plastic and filled with sandy loam soil to within approximately 5 cm (2 inches) from the top. One box was used for the control plot, one box was used for ^{14}C-PH-quizalofop treated plot, and one box was used for ^{14}C-QU-quizalofop treated plot.

The two treated boxes were placed inside a secured fence, with a locked gate that was marked with a radioactive materials weatherproof placard. A plastic drape was installed around the treated boxes and was raised at the time of the test material application to prevent cross contamination. The test plots (boxes) were identified with a placard bearing the study number, project number, test material ID (control, PH-label, or QU-label), date of application, transgenic ID and the name of the Research For Hire (RFH) study coordinator. Placards were placed on their respective box/plot.

The control box was maintained in the same manner as the treated boxes. The untreated box was enclosed with fencing and was located upwind approximately 67 feet from the treated plots.

The maps for the test site and plot diagrams are documented in the raw data.

SOIL HISTORY AND CHARACTERIZATION

The soil used for characterization was collected from all boxes. This soil came from an area at Research For Hire facility where no radiolabeled substances of any kind had previously been applied. The absence of any radioactivity in the soil was confirmed by combustion analysis of a representative aliquot.

Soil for characterization was collected on May 28, 2008. A stainless steel 1 inch diameter soil probe with acetate liner that had been cleaned with a 50% solution of IPA and water was used to take soil to a depth of approximately 0-15 cm (0-6 inches). The soil was composited and placed into an Agvise bag. The Agvise bag was shipped under ambient conditions to Agvise Laboratories on May 28, 2008. Table 2 summarizes the soil characteristics.

TEST CROPS AND PLOT MAINTENANCE

TEST CROP GROUP CLASSIFICATION/VARIETY

The treated boxes were planted with variety pDAS 1740-474 (from DAS). The corn seeds were received from Dow AgroSciences, LLC, on May 15, 2008.

PLANTING OF CROP

On May 28, 2008, the corn was planted into the boxes in two rows, row spacing of 24 inches and a plant spacing of 4 inches.

FERTILIZATION

The test boxes were fertilized on June 19, 2008 and July 19, 2008 with Miracle Gro at a rate of 5 table spoons per 5 gallons of water.

IRRIGATION

Irrigation water was applied by hand using a spray wand. The water was carefully added to the soil in order to prevent washing the test substance off of the treated leaves and to minimally disturb the soil. RFH well water was applied as needed to grow a healthy crop.

CLIMATIC DATA

Climatic data were collected from the California Irrigation Management Information System Weather Station number 169. The station was approximately 5 miles Southwest

of the test site. On-site rainfall was monitored by a Tru-Check rain gauge during the study. Table 3 summarizes the climatic data.

TEST MATERIAL PREPARATION AND APPLICATION

GENERAL PREPARATION

The vials containing the test substance aliquots for applications were received at Research For Hire on June 17, 2008. As noted previously, upon receipt at RFH 0.120 g of blank formulation (E2469-23) was added to each vial. The test substances were stored refrigerated pending application.

APPLICATION PH-QUIZALOFOP AND QU-QUIZALOFOP

The application solutions for this study were prepared on July 2, 2008. The 14C-PH-quizalofop-P ethyl ester and 14C-QU-quizalofop-P ethyl ester test substances were removed from the refrigerator and allowed to come to room temperature. The following procedure was followed and performed for each of the two dosing solutions. The test substance was sonicated for 1 minute to insure the test substance was in solution. Distilled water (75 mL) was added to a glass beaker. Quantitatively, the application solution was transferred to the glass beaker. Then the vial was rinsed with three sequential 5 mL portions of distilled water and was added to the beaker. Then 55 mL of distilled water was added to the beaker to bring the final volume to 150 mL.

Three (3) 100 µL aliquots were removed from the application solution to clean LSC vials. 4.90 mL of acetonitrile was added to each of the three aliquots, bringing their final volume to 5 mL. Each vial was capped and mixed thoroughly by inversion. Three (3) 100 µL aliquots were removed from each of the three dilutions to a pre-counted LSC vial containing 10 mL of Ready–Solv. The vials were counted in the Beckman LS6500 for one minute and five minute counts.

Two (2) 0.25 mL aliquots (pre-aliquots) were taken of the application solution and were dispensed into separate 20 mL glass scintillation vials. These vials were stored frozen in the RFH walk-in freezer EPQ 28-2. Two (2) 0.25 mL aliquots (post-aliquots) were taken of the application solution and were dispensed into separate 20 mL glass scintillation vials. These aliquots were transported to the field along with the spray solution.

The spray solution was transferred to a spray container wrapped with aluminum foil to protect against photodegradation. The spray solution was applied to the plot. After application, the post-application retention aliquots were stored in the RFH walk-in freezer EQP 28-2.

TIME AND RATE OF APPLICATION

On July 2, 2008 the application was made to the treated QU-label and PH-label boxes. The corn plants were at the V6 growth stage at the time of application. Application was made with an R&D sprayer, model GS (EQP 11-4), with a single aluminum spray wand outfitted with a flat fan 8002 nozzle. The system was pressurized with CO_2 at 20 psi. The R&D sprayer was connected to the spray vessel with a flexible hose approximately three feet in length. The air supply hose was approximately 30-feet long so that the CO_2 tank and regulator could remain outside the treated area. Each radiolabeled treatment solution contained 150 mL of final spray solution and the volume actually applied to the plot was 148.7 mL. The spray solution was applied evenly in two passes per row. Following the application the empty spray container was rinsed with 30 mL of distilled water and sprayed on the plot similar to the solution.

SAMPLE COLLECTION

Table 4 summarizes the sample collection dates, sample weights, and the sample shipment dates.

IMMATURE FORAGE CORN SAMPLES

On August 7, 2008 harvest of the immature corn forage occurred. The untreated plot was sampled first followed by the PH-label & QU-label treatments. The corn plants were at R4 (milky inner fluid in kernels) growth stage. Two entire plants per treatment were cut 2 inch above the soil surface. The plants were cut into sections approximately 8 inches long to fit into ziplock bags. Each plant was placed into a pre-labeled, tared plastic ziplock bags. After weighing on scales EQP 13-1 and EQP 42-2, the samples were placed into labeled cloth residue bags and then into the RFH walk-in freezer (EQP 28-2) until shipment to ABC Laboratories. The clippers used for harvesting were cleaned before and after harvest with a 50% solution of isopropyl alcohol and water. Samplers wore disposable gloves, which were changed between each sample collection, and lab coats.

CORN MATURE HARVEST

On September 3, 2008, the mature corn samples were harvested. The corn plants were harvested at growth stage BBCH 89 (fully ripe: kernels hard and shiny, about 65% dry matter). The untreated plot was harvested first followed by the PH-label and QU-label treatments. The cobs were removed from stalks; stalks were cut approximately three inches above the soil surface. The plants were then cut into sections to fit into ziplock bags. The grain and cobs were separated by hand and placed into tared and labeled ziplock bags, weighed, and then placed into labeled cloth residue bags. The weights were

recorded in the trial notebook, and the samples were stored in the RFH walk in freezer (EQP 28-2) until shipment to ABC Laboratories. The clippers used for harvesting were cleaned before and after harvest with a 50% solution of isopropyl alcohol and water. Samplers wore disposable gloves, changed between each treatment and lab coats at all times. The same procedure was followed for the PH and QU treated samples.

SAMPLE HANDLING

All treated and control samples were handled and stored with adequate separation to prevent cross contamination. Table 4 summarizes the dates of sample shipment.

SAMPLE SHIPMENTS

All plant samples were shipped in coolers containing approximately twenty-five pounds of dry ice via Federal Express to the following address:

Sheila Hecht, RSO
Tom Sanders
Martha Pezold
ABC Laboratories
7200 East ABC Lane
Columbia, MO 65202

All pre- and post-application retention aliquots were shipped in coolers containing approximately twenty-five pounds of dry ice via Federal Express to the following address.

Attention: Sandra Rotondaro, DAS/RSO
Dow AgroSciences, LLC
Regulatory Science and Government Affairs
9330 Zionsville Road
Indianapolis, IN 46268

RESULTS AND DISCUSSION

As shown in Table 5, LSC analysis of the aliquots taken from the spray solution for the application events showed the solution to be within the desired range of radioactivity and homogeneous. This served as confirmation that the targeted amount of the ^{14}C test material was applied to the test boxes.

The treated crops showed no signs of phytotoxicity during the course of the study.

Photographs were taken on the day of applications and sampling events, and stored in the raw data. Foliar-applied corn forage, mature corn grain, cobs, and fodder were harvested from the untreated and treated plots to enable determination of the nature of the residue of ^{14}C-PH-quizalafop and ^{14}C-QU-quizalafop.

Table 1 - Test Material Receipt and Distribution

Material	Date	Distribution	Purpose	Quantity
^{14}C-PH-quizalofop	6-17-08	Receipt	Received	0.481 mCi
	7-2-08	Application	1st Application	0.481 mCi
^{14}C-QU-quizalofop	6-17-08	Receipt	Received	0.629 mCi
	7-2-08	Application	1st Application	0.629 mCi

TABLE 2 - SOIL ANALYSIS RESULTS

pH	7.4
Bulk Density, disturbed (gm/cc)	1.09
Field Water Holding Capacity (% @ 1/3 bar)	24.6
Cation Exchange Capacity (meq/100 g)	26.3
Organic Matter (%) Walkley Black	4.0
Texture	49% sand-20% silt- 31% clay (sandy clay loam)

TABLE 3 - CLIMATIC DATA

Date Range	Minimum Temperature (°F)[2]	Maximum Temperature (°F)[2]	Minimum Humidity (%)[2]	Maximum Humidity (%)[2]	Precipitation (inches)[1]
05/01/08-05/31/08	50	81	28	81	0.28
06/01/08-06/30/08	56	92	20	76	0.00
07/01/08-07/31/08	63	95	26	80	0.00
08/01/08-08/31/08	61	96	23	80	0.00
09/01/08-09/30/08	54	91	24	81	0.00

[1] Information obtained from RFH Station Tru-Check Raingauge.
[2] Information obtained from Porterville CIMIS Weather Station# 169 ~6.9 miles Southwest.

TABLE 4 - SAMPLING, SHIPPING DATES AND WEIGHTS OF CONTROL AND TREATED SAMPLES

Sample Number	Sample Description	Plot ID	Sample Weight (g)	Date Sampled	Date Shipped
R050802-Soil Char.	Soil Characterization	Untreated	N/A	05/28/08	05/28/08
R050802-1	Pre-applic retention (a) foliar applic 1	PH-label quizalofop-P ethyl ester	N/A	07/02/08	07/07/08
R050802-2	Pre-applic retention (b) foliar applic 1	PH-label quizalofop-P ethyl ester	N/A	07/02/08	07/07/08
R050802-3	Post-applic retention (a) foliar applic 1	PH-label quizalofop-P ethyl ester	N/A	07/02/08	07/07/08
R050802-4	Post-applic retention (b) foliar applic 1	PH-label quizalofop-P ethyl ester	N/A	07/02/08	07/07/08
R050802-5	Pre-applic retention (a) foliar applic 1	QU-label quizalofop-P ethyl ester	N/A	07/02/08	07/07/08
R050802-6	Pre-applic retention (b) foliar applic 1	QU-label quizalofop-P ethyl ester	N/A	07/02/08	07/07/08
R050802-7	Post-applic retention (a) foliar applic 1	QU-label quizalofop-P ethyl ester	N/A	07/02/08	07/07/08
R050802-8	Post-applic retention (b) foliar applic 1	QU-label quizalofop-P ethyl ester	N/A	07/02/08	07/07/08
R050802-9	Forage Plants	Control	2258	08/07/08	08/11/08
R050802-10	Forage Plants	PH-label quizalofop-P ethyl ester	2338	08/07/08	08/11/08
R050802-11	Forage Plants	QU-label quizalofop-P ethyl ester	2578	08/07/08	08/11/08
R050802-12	Mature Fodder	Control	8548	09/03/08	09/09/08
R050802-13	Mature Cobs	Control	1389	09/03/08	09/09/08
R050802-14	Mature Grain	Control	1506	09/03/08	09/09/08
R050802-15	Mature Fodder	PH-label quizalofop-P ethyl ester	5203	09/03/08	09/09/08
R050802-16	Mature Cobs	PH-label quizalofop-P ethyl ester	1097	09/03/08	09/09/08
R050802-17	Mature Grain	PH-label quizalofop-P ethyl ester	2515	09/03/08	09/09/08
R050802-18	Mature Fodder	QU-label quizalofop-P ethyl ester	4578	09/03/08	09/09/08
R050802-19	Mature Cobs	QU-label quizalofop-P ethyl ester	1066	09/03/08	09/09/08
R050802-20	Mature Grain	QU-label quizalofop-P ethyl ester	2158	09/03/08	09/09/08

TABLE 5 - TEST MATERIAL FORMULATED SPRAY SOLUTION VERIFICATION RESULTS (DPM'S)

Plot ID	App #	Sub-Sample No.	dpm/100 µL of App. Solution Dilution [a]	Mean dpm/100µL of App. Solution Dilution	% of Theoretical
^{14}C-PH-Quizalofop	1	1	13155.87	12958.11	91.07
		2	12948.05		
		3	12993.33		
		4	13194.89		
		5	13139.47		
		6	13124.77		
		7	12801.93		
		8	12746.60		
		9	12518.05		
^{14}C-QU-Quizalofop	1	1	18353.20	19117.00	102.60
		2	18159.38		
		3	18062.08		
		4	19743.87		
		5	19625.04		
		6	19714.09		
		7	19459.17		
		8	19485.12		
		9	19451.03		

[a] Values shown in this column represent the five minute counts from the LSC analysis.

APPENDIX A: LIST OF EQUIPMENT USED FOR GENERATING IN-LIFE PHASE RAW DATA

Liquid Scintillation Counter - Model LS 6500 (EQP 30-2)
Mfg: Beckman Instruments, Inc., 3500 Harbor Blvd., Fullerton, California 92134-3100 (714) 871-4848

Psychro-Dyne (Psychrometer)- (EQP 27-15)
Mfg: Environmental Tectonics Corporation, County Line Industrial Park, Southampton, Pennsylvania 18911

Top Loading Balance - Model AB-87 (EQP 13- 1)
Mfg: Abbeon Cal, Inc., 123-21T Gray Avenue, Santa Barbara, California 93101-1895

Wind Speed Indicator/Turbo Meter – Model 271 (EQP 47-2)
Mfg: Davis Instruments, 3415 Diablo Avenue, Hayward, California 94545

Survey Monitor – Model AB-87 (EQP 14-1 and 2)
Mfg: Technical Associates, 7051 Eton Avenue, Canoga Park, CA 91303

Todd Windshield Thermometer – (EQP 35-34)
Mfg: Todd Windshield Thermometer, 1221 W. Ontario St., Corona, CA 91720

Lindberg Sola Basic Oxidizer - Model 55035 (EQP 15-1)
Mfg: Lindberg, 2450 W. Hubbard Street, Chicago, Illinois 10112

Appendix B—Milling and TRR Determination Report

(ABC Laboratories)

SAMPLE PROCESSING REPORT FOR

STUDY TITLE

A Nature of the Residue Study with 14C-Quizalofop-P ethyl ester applied to AAD-1 Maize 2008

DATA REQUIREMENT

OECD Guidance Document 501 for Metabolism in Crops (Issued 08 January 2007)
Environmental Protection Agency (OPPTS 860.1300)
European Annex II and III 96/68/EEC Lundehn (7028/VI/95 EN rev 3 (7/22/97)

AUTHOR

Clark Chickering

STUDY INITIATION DATE

25 March 2008

STUDY COMPLETION DATE

29 December 2008

SPONSOR

Dow AgroSciences, LLC
9330 Zionsville Road
Indianapolis, Indiana 46268

PERFORMING LABORATORY

ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, Missouri 65202

STUDY IDENTIFICATION

ABC Study No. 63896
Dow Study No. 080057

ABC Study No. 63896
DAS Study No. 080057

STATEMENT OF GLP COMPLIANCE

Compound: 14C-Quizalofop-P ethyl ester

Study Title: A Nature of the Residue Study with 14C-Quizalofop-P ethyl ester applied to AAD-1 Maize 2008

The sample processing portion of this study, described in this report, was conducted in compliance with the following Good Laboratory Practice Standards:

United States Environmental Protection Agency, (EPA-FIFRA)
Title 40 of the US Code of Federal Regulations Part 160
(August 17, 1989)

The original raw data and the protocol were provided to Dow AgroSciences, LLC with the final sample processing report. Copies of all data in support of this report were retained at ABC Laboratories, Inc. along with original facility records and a copy of the final sample processing report and the study plan.

Clark D Chickering 29Dec08

Clark Chickering Date
Senior Chemist/Group Leader
Residue Chemistry & Field Programs
ABC Laboratories, Inc.

Jon E. Rhodes 29Dec08

Jon E. Rhodes, MS Date
Director
Chemical Services
ABC Laboratories, Inc.

Sponsor:

S. L. Rotondaro Date
Study Director
Dow AgroSciences, LLC

Submitter:

D. Fonseco Date
Regulatory Manager
Dow AgroSciences, LLC

ABC Study No. 63896
DAS Study No. 080057

QUALITY ASSURANCE STATEMENT

ABC's Quality Assurance Unit reviewed the Study No. 63896 entitled "A Nature of the Residue Study with 14C-Quizalofop-P ethyl ester applied to AAD-1 Maize 2008" for Dow AgroSciences, LLC. The following inspections/audits were conducted on this study.

Date of Study Based Inspection	Phase Inspected	Date Reported to Principal Investigator	Date Reported to ABC Management	Date Reported to Study Director/Study Director Management
14 Aug 08	Procedure: Sample Preparation	15 Aug 08	15 Aug 08	21 Aug 08
26 Sep 08	Procedure: Sample Combustion	06 Oct 08	22 Oct 08	28 Oct 08
03 Oct 08	Procedure: Sampling Weighing	06 Oct 08	22 Oct 08	28 Oct 08
01 Dec 08	Raw Data & Draft Sample Processing Report	01 Dec 08	17 Dec 08	15 Dec 08
23 Dec 08	Final Sample Processing Report	23 Dec 08	24 Dec 08	29 Dec 08

These audits indicate that the report is an accurate reflection of the study as it was conducted by ABC Laboratories, Inc.

Chris Hughes 29 Dec 2008

Chris Hughes
Quality Assurance Manager, Chemical Services
ABC Laboratories, Inc.

Date

ABC Study No. 63896
DAS Study No. 080057

SIGNATURE PAGE

Prepared by: ABC Laboratories, Inc.
7200 E. ABC Lane
Columbia, Missouri 65202

Prepared by: Clark D Chickering 29 Dec 08
Clark Chickering, B.A. Date
Senior Chemist/Group Leader
Residue Chemistry & Field Programs
ABC Laboratories, Inc.

Approved by: Jon E. Rhodes 29 Dec 08
Jon E. Rhodes, M.S. Date
Director, Chemical Services
ABC Laboratories, Inc.

ABC Study No. 63896
DAS Study No. 080057

SAMPLE PROCESSING SUMMARY REPORT

Study Sponsor:	Dow AgroSciences, LLC
Study Title:	A Nature of the Residue Study with 14C-Quizalofop-P ethyl ester applied to AAD-1 Maize 2008
Study Director:	Sandra Rotondaro
Location of Study:	ABC Laboratories, Inc. 7200 East ABC Lane Columbia, Missouri 65202

SAMPLE RECEIPT

Corn forage, fodder, cob, and grain samples shipped frozen on dry ice by FedEx were received from Research for Hire (RFH), Porterville, California as stated in Table 1. Upon receipt, all samples received were verified against the RFH shipping transmittal document and placed into frozen storage pending sample milling.

SAMPLE PROCESSING

Homogenization

All samples were homogenized as per ABC SOP CD.EQ.1.40. In general, the samples were removed from frozen storage, pre-weighed, broken down as needed and milled (entire sample) with dry ice, to maintain frozen state during milling, returned to frozen storage to allow for sublimation of the dry ice (typically 2 days), post weighed, and returned to frozen storage pending combustion and total radioactive residue (TRR) analysis. Weighing and milling procedures were documented using ABC Laboratories' "Daily Sample Preparation Log" form.

Sample Nos. R050802-13 through 14, 16, 17, 19, and 20 were milled using the Straub grinding mill and Sample Nos. R050802-9 through 12 the Robot Coupe cutter/mixer. Sample Nos. R050802-15 and 18 were initially broken down using both the Robot Coupe cutter/mixer and milling was completed with the Straub grinding mill. The Straub grinding mill (Model #4E) was equipped with grinding plates that could be adjusted in a horizontal plane relative to each other. The closer the plates were set to each other, the finer the samples were ground. In order to obtain a good homogeneous sample, the grinding plates were adjusted as close together as possible. Dry ice was passed through the Straub mill to chill the machine prior to milling the samples. Frozen samples, which had been broken down with a hammer, were then passed through the mill a minimum of three times, along with enough dry ice to maintain a frozen state throughout the milling process. As the sample/dry ice mixture was being passed through the mill it was captured in a stainless steel pan, with continuous stirring (using a plastic spatula) of the sample during the milling process. At the completion of the milling process, the homogenized sample/dry ice mixture was transferred to labeled container(s), loosely capped, then placed in a holding freezer to allow sublimation of the dry ice to occur. Depending upon the mass of the sample received, multiple bottles may have been required.

The Robot Coupe cutter/mixer (Model #RSI 23) was equipped with a 24-quart stainless steel bowl with lid which housed a "crescent-shaped" 3-blade assembly to chop and mix the samples during operation. Prior to the addition of the frozen sample, dry ice was added to the bowl and the machine was turned on to chill the bowl and blade assembly. Once the bowl and blade assembly were chilled, the frozen sample and additional dry ice were added to the bowl, the lid was closed and the machine was run long enough to sufficiently break down the sample. After chopping, the machine was stopped, and the contents of the bowl were stirred using a plastic spatula. After stirring, the lid was closed and the machine was run a second time to sufficiently produce a finely ground homogeneous sample. At the completion of the cutter/mixing process, the homogenized sample/dry ice mixture was transferred to labeled container(s), loosely capped, and placed in a holding freezer for sublimation of the dry ice to occur. Depending upon the mass of the sample received, multiple bottles may have been required.

The homogenization equipment was cleaned after each sample was processed. Control samples were homogenized before treated samples.

Sample homogeneity resulting from the sample processing procedure above was assessed using the results obtained from the combustion and TRR analysis of five 0.2-g aliquots of each sample (used to determine TRR, below). In the event that the results of the TRR analysis indicated greater than 15% variance between the aliquots for any of the sample, the entire sample was re-milled using the Straub grinding mill and the combustion and TRR analysis repeated. TRR analyses of samples resulting in less than 15% variance were readied for shipment to Dow AgroSciences. TRR data for Sample Nos. R050802-9, -10, and -11 were not reported due to an apparent error in the recording of the weights combusted for these samples. Otherwise, combustion results indicated that treated samples were homogeneous with CVs of no greater than 11%.

MEASUREMENT OF RADIOACTIVITY

Total radioactive residue (TRR) and combustion results are found in Table 2.

Oxidation analyses (combustion) of plant tissues were performed on a Harvey OX 500 (R.J. Harvey Instruments Corporation, Tappan, NJ). Oxidized samples were counted in a mixture of CarboSorb® E and Permafluor® E (Packard BioScience, Meriden, CT), or Carbon-14 cocktail (R.J. Harvey Instruments Corporation, Tappan, NJ).

The homogenized corn fractions (control and treated) were maintained on dry ice while aliquots were weighed for combustion. Five, 0.2-g aliquots of each homogenized sample were combusted to determine the total ^{14}C residues (2-minute burn time). Evolved $^{14}CO_2$ was collected and the radioactivity determined by LSC. Total ^{14}C-residues in the samples were reported as dpm/g.

Prior to and after use of the oxidizer for all sample analyses, the oxidizer efficiency was determined by combusting known levels of ^{14}C-benzoic acid standard spiked on cellulose and determining the amount of ^{14}C-activity recovered versus the amount applied. The efficiency of the oxidizer was determined to be within 95 and 105% prior to and after use, indicating the oxidizer was functioning properly during sample analysis.

Radioactivity measurements were made with a Beckman Model 6000 Liquid Scintillation Counting System. The quench curve was obtained by counting a set of Beckman quenched carbon-14 liquid scintillation quench standards. The amount of quench in a sample was

determined by analyzing the position of its Compton spectrum. In this LSC system, the defining parameter was the H-number. The value of the H-number was equal to the difference between the inflection points of the Compton spectrum of the unquenched standard and the sample. As quench increases, so does the H-number. Each combustion sample was counted for 5.0 minutes. The single-label dpm program was designed to establish the quench curve and to resolve the sample count to dpm by the relationship:

$$\text{dpm} = \frac{(\text{cpm} - \text{background cpm})}{\text{counting efficiency}}$$

Sample dpm/g = sample dpm/aliquot size in g

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Table 1 Sample Information for Corn Fractions from Plots Treated with 14C-Quizalofop-P ethyl ester

Sample ID	Matrix	Plot	Sampling Time Point	Date Received at ABC	Date Prepared	Date of TRR Determination	Date Shipped to DAS
R050802-9	plants	Control	Forage	12 Aug 08	14 Aug 08	20 Aug 08[a]	02 Sep 08
R050802-10	plants	PH-label quizalofop-P ethyl ester	Forage	12 Aug 08	14 Aug 08	20 Aug 08[a]	02 Sep 08
R050802-11	plants	QU-label quizalofop-P ethyl ester	Forage	12 Aug 08	15 Aug 08	20 Aug 08[a]	02 Sep 08
R050802-12	fodder	Control	Mature	11 Sep 08	16 Sep 08	06 Oct 08	08 Oct 08
R050802-13	cobs	Control	Mature	11 Sep 08	15 Sep 08	06 Oct 08	08 Oct 08
R050802-14	grain	Control	Mature	11 Sep 08	15 Sep 08	06 Oct 08	08 Oct 08
R050802-15	fodder	PH-label quizalofop-P ethyl ester	Mature	11 Sep 08	25 Sep 08	06 Oct 08	08 Oct 08
R050802-16	cobs	PH-label quizalofop-P ethyl ester	Mature	11 Sep 08	17 Sep 08	06 Oct 08	08 Oct 08
R050802-17	grain	PH-label quizalofop-P ethyl ester	Mature	11 Sep 08	18 Sep 08	06 Oct 08	08 Oct 08
R050802-18	fodder	QU-label quizalofop-P ethyl ester	Mature	11 Sep 08	01 Oct 08	06 Oct 08	08 Oct 08
R050802-19	cobs	QU-label quizalofop-P ethyl ester	Mature	11 Sep 08	17 Sep 08	06 Oct 08	08 Oct 08
R050802-20	grain	QU-label quizalofop-P ethyl ester	Mature	11 Sep 08	18 Sep 08	06 Oct 08	08 Oct 08

[a] Due to an apparent recording error of the sample weight combusted, the TRR data will not be reported.

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Table 2 Combustion Results in Corn Fraction Samples for Corn Matrices from Plots Treated with 14C-Quizalofop-P ethyl ester

Sample ID	Sample Type	Analysis Date	Rep #	Mean Oxidizer Efficiency (%)	Sample Weight Combusted (g)	dpm Found	Mean Efficiency Blank dpm Found	Net Sample dpm Found[a]	dpm/g[b]	Mean dpm/g
R050802-12	Control Corn Fodder	06 Oct 08	1	97.2	0.202	58.81	55.71	3.10	15.78	3.16
		06 Oct 08	2		0.202	54.76		0.00	0.00	
		06 Oct 08	3		0.200	52.57		0.00	0.00	
		06 Oct 08	4		0.203	53.39		0.00	0.00	
		06 Oct 08	5		0.204	54.05		0.00	0.00	
R050802-13	Control Corn Cob	06 Oct 08	1	97.2	0.203	54.27	55.71	0.00	0.00	3.71
		06 Oct 08	2		0.206	50.21		0.00	0.00	
		06 Oct 08	3		0.206	50.24		0.00	0.00	
		06 Oct 08	4		0.202	59.35		3.64	18.53	
		06 Oct 08	5		0.203	51.68		0.00	0.00	
R050802-14	Control Corn Grain	06 Oct 08	1	97.2	0.202	63.43	55.71	7.72	39.31	7.86
		06 Oct 08	2		0.202	50.13		0.00	0.00	
		06 Oct 08	3		0.202	53.42		0.00	0.00	
		06 Oct 08	4		0.203	49.33		0.00	0.00	
		06 Oct 08	5		0.202	48.90		0.00	0.00	
R050802-15	PH-label Treated Corn Fodder	06 Oct 08	1	97.2	0.210	6212.03	55.71	6156.32	30167.90	31652.65
		06 Oct 08	2		0.210	6798.04		6742.33	33039.53	
		06 Oct 08	3		0.204	6184.00		6128.29	30913.79	
		06 Oct 08	4		0.201	6083.49		6027.78	30860.61	
		06 Oct 08	5		0.207	6750.38		6694.67	33281.43	
R050802-16	PH-label Treated Corn Cobs	06 Oct 08	1	97.2	0.208	151.75	55.71	96.04	475.14	502.17
		06 Oct 08	2		0.204	153.24		97.53	491.97	
		06 Oct 08	3		0.206	174.90		119.19	595.39	
		06 Oct 08	4		0.206	156.37		100.66	502.83	
		06 Oct 08	5		0.206	144.90		89.19	445.53	

[a] Net dpm/aliquot combusted = sample dpm found – mean Efficiency Blank dpm found.
[b] dpm/g in aliquot combusted = 100 x (net dpm/aliquot combusted) ÷ (oxidizer efficiency) ÷ (aliquot weight)

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Table 2 Combustion Results in Corn Fraction Samples for Corn Matrices from Plots Treated with 14C-Quizalofop-P ethyl ester (continued)

Sample ID	Sample Type	Analysis Date	Rep #	Mean Oxidizer Efficiency (%)	Sample Weight Combusted (g)	dpm Found	Mean Efficiency Blank dpm Found	Net Sample dpm Found[a]	dpm/g[b]	Mean dpm/g
R050802-17	PH-label Treated Corn Grain	06 Oct 08	1	97.2	0.209	190.19	55.71	134.48	662.13	701.55
		06 Oct 08	2		0.208	218.16		162.45	803.69	
		06 Oct 08	3		0.205	188.78		133.07	667.97	
		06 Oct 08	4		0.208	200.56		144.85	716.62	
		06 Oct 08	5		0.209	189.22		133.51	657.36	
R050802-18	QU-label Treated Corn Fodder	06 Oct 08	1	97.2	0.201	9115.89	55.71	9060.18	46385.69	45027.55
		06 Oct 08	2		0.207	8607.20		8551.49	42512.31	
		06 Oct 08	3		0.207	9004.38		8948.67	44486.82	
		06 Oct 08	4		0.208	9287.43		9231.72	45673.32	
		06 Oct 08	5		0.208	9369.55		9313.84	46079.60	
R050802-19	QU-label Treated Corn Cobs	06 Oct 08	1	97.2	0.209	174.50	55.71	118.79	584.88	621.97
		06 Oct 08	2		0.208	201.08		145.37	719.19	
		06 Oct 08	3		0.209	179.38		123.67	608.91	
		06 Oct 08	4		0.205	175.34		119.63	600.51	
		06 Oct 08	5		0.208	176.25		120.54	596.35	
R050802-20	QU-label Treated Corn Grain	06 Oct 08	1	97.2	0.203	284.24	55.71	228.53	1158.47	1087.75
		06 Oct 08	2		0.202	270.05		214.34	1091.91	
		06 Oct 08	3		0.201	250.81		195.10	998.84	
		06 Oct 08	4		0.208	273.06		217.35	1075.31	
		06 Oct 08	5		0.202	274.43		218.72	1114.23	

[a] Net dpm/aliquot combusted = sample dpm found – mean Efficiency Blank dpm found.
[b] dpm/g in aliquot combusted = 100 x (net dpm/aliquot combusted) ÷ (oxidizer efficiency) ÷ (aliquot weight)

Appendix C—Sample Calculations

Specific Activity Determinations

The specific activity is the amount of radioactivity per unit of mass of quizalofop in the test substance. First, the total amount of radioactive quizalofop was determined (dpm and µg). Then the specific activity was calculated as the sum of the radioactivity divided by the sum of the mass.

$$\text{Total Radioactivity}_{(\text{dpm})} = \left(\frac{\text{average dpm}}{\text{original aliquot}_{\text{mL}}}\right) \times \left(\frac{\text{dilution volume}_{\text{mL}}}{\text{dilution aliquot}_{\text{mL}}}\right) \times \text{original volume}_{\text{mL}}$$

$$\text{Total Radioactive Quizalofop}_{(\mu\text{g})} = \frac{\text{Total Radioactivity}_{\text{dpm}}}{\text{Original Specific Activity}_{\text{dpm}/\mu\text{g}}}$$

$$\text{New Specific Activity}_{(\text{dpm}/\mu\text{g})} = \frac{\text{total radioactivity}_{\text{dpm}}}{\text{total mass quizalofop (radioactive + non - radioactive)}_{\mu\text{g}}}$$

Example for ^{14}C-PH-quizalofop:
Where the radiolabeled test substance (nominally 0.5 mCi) was diluted to 5.0 mL and 0.025 mL aliquots were diluted to 10.0 mL, and 0.025 mL aliquots were taken for LSC. The average dpm/aliquot was 14,336 dpm/0.025 mL (diluted). The original specific activity was 151,847 dpm/µg. A 4.925 mL portion was mixed with 0.75 mL of a 10.0 mg/mL solution of non-radiolabeled quizalofop (99.0% purity resulting in 7.4 mg or 7,351 µg).

$$\text{Total Radioactivity}_{(\text{dpm})} = \left(\frac{14{,}336\ \text{dpm}}{0.025\ \text{mL}}\right) \times \left(\frac{10\ \text{mL}}{0.025\ \text{mL}}\right) \times 4.925\ \text{mL} = 1.13 \times 10^{9}\ \text{dpm}$$

$$\text{Total Radioactive } ^{14}\text{C-PH-quizalofop} = \frac{1.13 \times 10^{9}\ \text{dpm}}{151{,}847\ \text{dpm}/\mu\text{g}} = 7{,}440\ \mu\text{g}$$

$$\text{Specific Activity } ^{14}\text{C-PH-quizalofop}_{(\text{dpm}/\mu\text{g})} = \frac{1.13 \times 10^{9}\ \text{dpm}}{7{,}440\ \mu\text{g} + 7{,}351\ \mu\text{g}} = 76{,}145\ \text{dpm}/\mu\text{g}$$

(rounding difference noted)

Oxidative Combustion Calculations

All oxidative combustion results were corrected for oxidizer recovery (determined on the day of use) and background dpm values.

$$\text{Net dpm/g value} = \frac{\text{net combustion dpm value}}{\text{combustion recovery} \times \text{aliquot weight}_{g}}$$

Example combustion calculation for PH-label forage TRR determination at DAS:
Where oxidizer recovery = 96.03%, combustion value #1 = 1074 dpm (background subtracted by LSC), and aliquot weight = 0.2005 g

$$\text{Net dpm value} = \frac{1074\ \text{dpm}}{0.9603 \times 0.2005\ \text{g}} = 5{,}578\ \text{dpm/g}$$

Calculation of TRR Levels

a) *ABC Labs determined dpm/g, see* Appendix B

b) *Converting dpm/g to µg/g (or mg/kg)*

To determine the total radioactive residue level in each sample, the average dpm/g value for the sample was converted to mg/g (equivalent to ppm) by dividing the dpm/g value by the specific activity value of the applied ^{14}C-quizalofop (76,145 or 100,384 dpm/µg for the PH-label and QU-label, respectively) and multiplying by the conversion factor 0.925 (mw a.i./mw acid; 372.81/344.76).

For example, the PH-label forage contained an average of 5720 dpm/g. This was converted to a µg/g (or mg/kg) value as follows:

$$\frac{5720\ \text{dpm/g}}{76{,}145\ \text{dpm/µg}} \times 0.925 = 0.069\ \text{mg a.e./kg (or a.e. ppm)}$$

c) *TRR Distribution among Fractions Generated by the Extraction of the Samples*

For Table 8, the percentage distribution of the total radioactive residues in the samples among the fractions generated by the extraction procedure was calculated in three steps, described below:

$$\text{Amount Extracted (dpm)} = \frac{\text{dpm}}{\text{aliquot (mL)}} \times \text{extract volume (mL)}$$

$$\text{Extraction Recovery (\% TRR)} = \frac{\text{Amount Extracted (dpm)}}{\text{extracted tissue weight (g) x TRR (dpm/g)}}$$

Extracted mg/kg = % TRR x TRR (mg/kg)

An example for the Neutral Organic Extract of the PH-label forage (replicate A):

$$\text{Amount Extracted (dpm)} = \frac{369\ \text{dpm}}{1.0\ \text{mL}} \times 203\ \text{mL} = 74{,}839\ \text{dpm (rounding difference noted)}$$

$$\text{Extraction Recovery} = \frac{74{,}839\ \text{dpm}}{20.05\ \text{g x}\ 5720\ \text{dpm/g}} = 65.3\%$$

Extracted mg/kg = 0.653 x 0.069 mg ae/kg = 0.045 mg ae/kg

d) *TRR Distribution among ^{14}C-PH-quizalofop and Its Metabolites Following HPLC Analysis*

The percentage distribution of the TRR among ^{14}C-PH-quizalofop and its metabolites following HPLC analysis of the sample extracts was calculated as follows:

% of TRR = (% of TRR in the Extract Being Assayed) x (% Distribution of Radioactivity in the Extract among the Fractions of Interest as Determined by the HPLC Analysis)

For example in Table 11, the percent of the TRR accounted for as quizalofop acid in the Neutral Organic extract of the PH-label forage (replicate A), where 65.3% was extracted (above), 65.2% was in the SPE eluent fraction, and 3.8% eluted off the HPLC with quizalofop – see Figure 9:

% of TRR = 65.3% x 0.652 x 0.038
= 1.6% of the TRR (average of duplicates reported in Table 11)

To convert the total percentage distribution value for each component of the residue profile to a mg/kg value, the TRR value the sample of interest (expressed as mg/kg of quizalofop acid equivalents) was multiplied by the percentage value at which the component of interest was present.

For the quizalofop acid in the above sample the calculation:
quizalofop acid = 1.6% of the TRR x 0.069 mg ae/kg (TRR – see Table 7)
= 0.001 mg a.e./kg (rounding difference noted)